

NOVEL CONSTRUCTS FOR CHIMERIC ANTIGEN RECEPTORS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application Nos. 63/034,873, filed June 4, 2020; 63/044,934, filed June 26, 2020; and 63/082,584, filed September 24, 2020; the contents of which are incorporated herein by reference in their entirety.

BACKGROUND

[0002] Although immunotherapies have been investigated for many diseases and disorders, including cancer, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), systemic amyloidosis, prion disease, cardiovascular disease, atherosclerosis, fibrosis, functional limitations that have been encountered still need to be addressed.

[0003] Therefore, a need exists for the development of new therapeutic modalities optimized to target specific antigens.

SUMMARY OF THE INVENTION

[0004] In one aspect, the disclosure provides modified immune cells comprising a chimeric antigen receptor (CAR) comprising: (a) an extracellular domain, (b) a transmembrane domain, and (c) an intracellular domain, wherein the immune cell comprises a macrophage, monocyte, or dendritic cell. While a variety of CARs have been developed in multiple cells types and for treatment of multiple indications, the present disclosure encompasses the recognition that maximizing CAR and/or effector activity in monocytes, macrophages and/or dendritic cells requires the use of customized CAR components. Accordingly, the present disclosure provides several examples of such CARs and supporting agents, moieties, and adjunct compositions that are particularly useful in treating any of a variety of conditions including, for example, various

cancers, diseases or disorders of the central nervous system (e.g. Alzheimer's Disease, Parkinson's Disease, and/or ALS), cardiovascular disease, fibrosis, and others.

[0005] In another aspect, the disclosure provides methods of modifying a macrophage, monocyte or dendritic cell comprising: delivering to the macrophage, monocyte or dendritic cell a viral vector comprising one or more nucleic acid sequences encoding: (a) an extracellular domain, (b) a transmembrane domain, and (c) an intracellular domain, wherein the viral vector is packaged with at least one Vpx protein, wherein the modified macrophage, monocyte or dendritic cell comprises a chimeric engineered receptor (CAR) comprising (a) through (c), and wherein the modified macrophage, monocyte or dendritic cell exhibits increased CAR expression relative to a modified macrophage, monocyte or dendritic cell comprising a CAR that was delivered a viral vector encoding the CAR that was not packaged with at least one Vpx protein.

[0006] In some ~~embodiment~~examples, a viral vector is or comprises a lentiviral vector. In some ~~embodiment~~examples, a viral vector is delivered at a multiplicity of infection (MOI) of about 1 to about 50. In some ~~embodiment~~examples, a viral vector is delivered at a MOI of about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10.

[0007] In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell exhibits increased CAR expression of about 20%, about 30%, about 40%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or greater relative to a modified macrophage, monocyte or dendritic cell comprising a CAR that was delivered a viral vector encoding the CAR that was not packaged with at least one Vpx protein.

[0008] In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell exhibits CAR expression for an extended time period relative to an unmodified macrophage, monocyte or dendritic cell. In some ~~embodiment~~examples, an extended time period of CAR expression is for at least 5 days, at least 10 days, at least 15 days, at least 20 days, at least 25 days, at least 30 days, or longer. In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell exhibits increased effector activity relative to an unmodified macrophage, monocyte or dendritic cell. In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell exhibits

increased tumor killing ability or phagocytosis relative to an unmodified macrophage, monocyte or dendritic cell.

[0009] In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell does not exhibit increased polarization to a M1 phenotype relative to a macrophage, monocyte or dendritic cell comprising a CAR that was delivered a viral vector encoding the CAR that was not packaged with at least one Vpx protein (e.g., an adeno-associated viral vector (e.g., Ad5 vector, e.g., Ad5f35)). In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell does not exhibit increased expression of one or more markers of M1 phenotype relative to a macrophage, monocyte or dendritic cell comprising a CAR that was delivered a viral vector encoding the CAR that was not packaged with at least one Vpx protein (e.g., an adeno-associated viral vector (e.g., Ad5 vector, e.g., Ad5f35)). In some ~~embodiment~~examples, one or more markers of M1 phenotype comprise or are one, two, three, four, five, six, seven, eight, nine, 10, 11, 12, 13, 14, 15, 16, 17, or 18 of CD86, CD80, MHC II, IL-1R, TLR2, TLR4, iNOS, SOCS3, CD83, PD-L1, CD69, MHC I, CD64, CD32, CD16, IL1R, a IFIT family member, or an ISG family member.

[0010] In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell exhibits increased production of one or more inflammatory cytokines relative to an unmodified macrophage, monocyte or dendritic cell. In some ~~embodiment~~examples, one or more inflammatory cytokines comprise or are one, two, three, four, five, six, seven, eight, nine, 10, 11, or 12 of TNF α , IL-6, IL-1 α , IL-1 β , IL-12, IL-18, IL-8, IL-2, IL-23, IFN α , IFN β , IFN γ , IL-2, IL-8, IL33, CCL3, CXCL12, CCL22, CCL4, CXCL10, or CCL2.

[0011] In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell exhibits reduced SIRP α activity relative to an unmodified macrophage, monocyte or dendritic cell. In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell comprises deletion of SIRP α using one or more endonucleases. In some ~~embodiment~~examples, one or more endonucleases comprise or are one or more of a CRISPR/Cas system, zinc finger nuclease (ZFN), transcription activator-like effector-based nuclease (TALEN), or meganuclease. In some ~~embodiment~~examples, a CRISPR/Cas system comprises Cas9, Cas12a, or C2c2. In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell has been treated with one or more anti-SIRP α

antibodies. In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell comprises one or more anti-SIRP α antibodies. In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell comprises one or more siRNAs that downregulate SIRP α .

[0012] In some ~~embodiment~~examples, an extracellular domain of a CAR comprises one or more antigen binding domains and an FcR extracellular domain, and/or a transmembrane domain of a CAR comprises an FcR transmembrane domain, and/or an intracellular domain of a CAR comprises an FcR intracellular domain. In some ~~embodiment~~examples, a CAR comprises, from N-terminus to C-terminus, one or more extracellular binding domains, an FcR extracellular domain, an FcR transmembrane domain, and an FcR intracellular domain. In some ~~embodiment~~examples, one or more of a FcR extracellular domain, a FcR transmembrane domain and a FcR intracellular domain is or comprises a human FcR domain. In some ~~embodiment~~examples, an FcR extracellular domain, a FcR transmembrane domain and a FcR intracellular domain together comprise a full-length FcR. In some ~~embodiment~~examples, an FcR extracellular domain comprises a CD64 (Fc γ RI), CD32a (Fc γ RIIa), CD32b (Fc γ RIIb), CD32c, CD16a (Fc γ RIIIa), CD16b (Fc γ RIIIb), Fc ϵ RI, Fc ϵ RII, or Fc α RI (CD89) domain. In some ~~embodiment~~examples, an FcR transmembrane domain comprises a CD64 (Fc γ RI), CD32a (Fc γ RIIa), CD32b (Fc γ RIIb), CD32c, CD16a (Fc γ RIIIa), CD16b (Fc γ RIIIb), Fc ϵ RI, Fc ϵ RII, or Fc α RI (CD89) domain. In some ~~embodiment~~examples, a FcR intracellular domain comprises a CD64 (Fc γ RI), CD32a (Fc γ RIIa), CD32b (Fc γ RIIb), CD32c, CD16a (Fc γ RIIIa), CD16b (Fc γ RIIIb), Fc ϵ RI, Fc ϵ RII, or Fc α RI (CD89) domain.

[0013] In some ~~embodiment~~examples, an extracellular domain of a CAR comprises one or more antigen binding domains and a toll-like receptor (TLR) extracellular domain and/or a transmembrane domain of a CAR comprises a TLR transmembrane domain and/or an intracellular domain of a CAR comprises a TLR intracellular domain. In some ~~embodiment~~examples, a CAR comprises, from N-terminus to C-terminus, one or more extracellular binding domains, a TLR extracellular domain, a TLR transmembrane domain, and a TLR intracellular domain. In some ~~embodiment~~examples, one or more of a TLR extracellular domain, a TLR transmembrane domain and a TLR intracellular domain is or comprises a human TLR domain. In some ~~embodiment~~examples, a TLR extracellular domain, a TLR transmembrane domain and a TLR intracellular domain together comprise a full-length TLR. In some ~~embodiment~~examples, a TLR extracellular domain comprises a

TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, or TLR9 domain. In some ~~embodiment~~examples, a TLR transmembrane domain comprises a TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, or TLR9 domain. In some ~~embodiment~~examples, a TLR intracellular domain comprises a TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, or TLR9 domain.

[0014] In some ~~embodiment~~examples, a chimeric antigen receptor further comprises one or more of: (d) one or more extracellular leader domains, (e) one or more extracellular hinge domains, and (f) one or more intracellular co-stimulatory domains. In some ~~embodiment~~examples, one or more extracellular leader domains comprise a CD8 extracellular leader domain.

[0015] In some ~~embodiment~~examples, one or more extracellular antigen binding domains comprise a scFv, centyrin, or darpin.

[0016] In some ~~embodiment~~examples, one or more extracellular hinge domains comprise a CD28 extracellular hinge domain, CD8a extracellular hinge domain, or an IgG4 extracellular hinge domain.

[0017] In some ~~embodiment~~examples, a transmembrane domain comprises a CD28, CD8a, CD64, CD32a, CD32c, CD16a, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, or TLR9 transmembrane domain.

[0018] In some ~~embodiment~~examples, an intracellular domain comprises one or more intracellular signaling domains. In some ~~embodiment~~examples, one or more intracellular signaling domains comprise a CD3-zeta, FcR γ , CD64, CD32a, CD32c, CD16a, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, ALK, AXL, DDR2, EGFR, EphA1, INSR, cMET, MUSK, PDGFR, PTK7, RET, ROR1, ROS1, RYK, TIE2, TRK, VEGFR, CD40, CD19, CD20, 41BB, CD28, OX40, GITR, TREM-1, TREM-2, DAP12, MR, ICOS, MyD88, V/I/LxYxxL/V, SIRPa, CD45, Siglec-10, PD1, SHP-1, SHP-2, KIR-2DL, KIR-3DL, NKG2A, CD170, CD33, BTLA, CD32b, SIRPb, CD22, PIR-B, LILRB1, 41BBL (TNFSF9), CD27, OX40L, CD32b, CD11b, ITGAM, SLAMF7, CD206, CD163, CD209, Dectin-2, IL1R, IL2R, IL3R, IL4R, IL5R, IL6R, IL7R, IL8R, IL9R, IL10R, IL11R, IL12R, IL13R, IL14R, IL15R, IL17R, IFN α R, IFN γ R, TNFR, CSF1R, CSF2R, Dap10, CD36, Dectin-1, ICOSL and/or Syk intracellular signaling

domain. In some ~~embodiment~~examples, one or more intracellular signaling domains comprises at least one intracellular co-stimulatory domain.

[0019] In another aspect, the disclosure provides pharmaceutical compositions comprising an immune cell of any aspect or ~~embodiment~~example described herein. In some ~~embodiment~~examples, a pharmaceutical composition comprises a pharmaceutically acceptable carrier.

[0020] In another aspect, the disclosure provides nucleic acid constructs (e.g., viral vectors) comprising one or more nucleic acid sequences encoding: (a) an extracellular domain, (b) a transmembrane domain, and (c) an intracellular domain, wherein the nucleic acid construct encodes a chimeric antigen receptor (CAR) comprising (a) through (c). In some ~~embodiment~~examples, a nucleic acid construct further comprises one or more nucleic acid sequences encoding: (e) one or more extracellular leader domains, (f) one or more extracellular hinge domains, (g) one or more intracellular co-stimulatory domains, (h) a cleavage peptide, and (i) one or more peptide agents. In some ~~embodiment~~examples, a cleavage peptide is or comprises a P2A, F2A, E2A or T2A peptide.

[0021] In another aspect, the disclosure provides pharmaceutical compositions comprising a nucleic acid construct of any aspect or ~~embodiment~~example described herein. In some ~~embodiment~~examples, a pharmaceutical composition comprises a pharmaceutically acceptable carrier.

[0022] In another aspect, the disclosure provides pharmaceutical compositions comprising a macrophage, monocyte or dendritic cell modified by a method of any aspect or ~~embodiment~~example described herein. In some ~~embodiment~~examples, a pharmaceutical composition comprises a pharmaceutically acceptable carrier.

[0023] In another aspect, the disclosure provides methods of treating a disease or disorder in a subject, comprising: delivering to the subject a therapeutically effective amount of a pharmaceutical composition of any aspect or ~~embodiment~~example described herein.

[0024] In another aspect, the disclosure provides methods of modifying an immune cell, the method comprising: delivering to the immune cell a nucleic acid construct comprising one or more nucleic acid sequences encoding: (a) an extracellular domain, (b) a transmembrane domain, and (c) an intracellular domain, wherein the modified immune cell comprises a chimeric

engineered receptor comprising (a) through (c), and wherein the modified immune cell comprises a macrophage, monocyte or dendritic cell.

[0025] In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell exhibits reduced SIRP α activity relative to an unmodified macrophage, monocyte or dendritic cell. In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell comprises or expresses at least one CAR comprising one, two, three, or four of an anti-HER2 scFv, CD8 hinge, CD8 transmembrane domain, or CD3-zeta intracellular signaling domain. In some ~~embodiment~~examples, at least one CAR comprises a CD8a signal peptide. In some ~~embodiment~~examples, delivering a nucleic acid construct encoding (a)-(c) to a modified macrophage, monocyte or dendritic cell comprises transduction with a viral vector. In some ~~embodiment~~examples, a viral vector comprises or is an adenoviral vector, an adeno-associated viral vector, or a retroviral vector (e.g., a lentiviral vector or a gammaretroviral vector). In some ~~embodiment~~examples, an adeno-associated viral vector comprises or is an Ad5 vector (e.g., Ad5f35 viral vector).

[0026] In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell comprises deletion of SIRP α using one or more endonucleases. In some ~~embodiment~~examples, one or more endonucleases comprise or are one or more of a CRISPR/Cas system, zinc finger nuclease (ZFN), transcription activator-like effector-based nuclease (TALEN), or meganuclease. In some ~~embodiment~~examples, a CRISPR/Cas system comprises one or more of Cas9, Cas12a, or C2c2. In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell has been treated with one or more anti-SIRP α antibodies. In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell comprises one or more anti-SIRP α antibodies. In some ~~embodiment~~examples, a modified macrophage, monocyte or dendritic cell comprises one or more siRNAs that downregulate SIRP α .

[0026a] In one aspect, the present disclosure refers to a method of modifying a macrophage or monocyte comprising delivering to the macrophage or monocyte: (i) a viral vector comprising one or more nucleic acid sequences encoding a chimeric antigen receptor (CAR) comprising: (a) an extracellular domain, (b) a transmembrane domain, and (c) an

intracellular domain, (ii) at least one Vpx protein, thereby producing a modified macrophage or monocyte that exhibits increased CAR expression relative to a macrophage or monocyte comprising a CAR delivered by a viral vector and not delivered at least one Vpx protein, wherein the modified macrophage or monocyte does not exhibit an M1 phenotype relative to a macrophage or monocyte not delivered at least one Vpx protein.

[0026b] In another aspect, the present disclosure refers to a method of modifying a macrophage or monocyte comprising delivering to the macrophage or monocyte (i) a viral vector comprising one or more nucleic acid sequences encoding a chimeric antigen receptor (CAR) comprising: (a) an extracellular domain, (b) a transmembrane domain, and (c) an intracellular domain, and (ii) at least one Vpx protein, wherein the macrophage or monocyte is one or both of electroporated or transfected with at least one Vpx mRNA prior to transduction with the viral vector, thereby producing a modified macrophage or monocyte that exhibits increased CAR expression relative to a macrophage or monocyte comprising a CAR delivered by a viral vector and not delivered at least one Vpx protein.

BRIEF DESCRIPTION OF THE DRAWING

[0027] The drawings are for illustration purposes only, not for limitation.

CLAIMS

1. An immune cell comprising a chimeric antigen receptor (CAR) comprising:

- ~~(a) an extracellular domain;~~
- ~~(b) a transmembrane domain; and~~
- ~~(c) an intracellular domain;~~

wherein the immune cell comprises a macrophage, monocyte, or dendritic cell.

2. The immune cell of claim 1, wherein the extracellular domain of the CAR comprises one or more antigen binding domains and an FcR extracellular domain, and/or the transmembrane domain of the CAR comprises an FcR transmembrane domain, and/or the intracellular domain of the CAR comprises an FcR intracellular domain.

3. The immune cell of claim 2, wherein the CAR comprises, from N-terminus to C-terminus, one or more extracellular binding domains, an FcR extracellular domain, an FcR transmembrane domain, and an FcR intracellular domain.

4. The immune cell of claim 2 or claim 3, wherein one or more of the FcR extracellular domain, the FcR transmembrane domain and the FcR intracellular domain is or comprises a human FcR domain.

5. The immune cell of any one of claims 2-4, wherein the FcR extracellular domain, the FcR transmembrane domain and the FcR intracellular domain together comprise a full length FcR.

6. The immune cell of any one of claims 2-5, wherein the FcR extracellular domain comprises a CD64 (FcγRI), CD32a (FcγRIIa), CD32b (FcγRIIb), CD32c, CD16a (FcγRIIIa), CD16b (FcγRIIIb), FcεRI, FcεRII, or FcαRI (CD89) domain.

7. The immune cell of any one of claims 2-6, wherein the FcR transmembrane domain comprises a CD64 (FcγRI), CD32a (FcγRIIa), CD32b (FcγRIIb), CD32c, CD16a (FcγRIIIa), CD16b (FcγRIIIb), FcεRI, FcεRII, or FcαRI (CD89) domain.

8. The immune cell of any one of claims 2-7, wherein the FcR intracellular domain comprises a CD64 (FcγRI), CD32a (FcγRIIa), CD32b (FcγRIIb), CD32c, CD16a (FcγRIIIa), CD16b (FcγRIIIb), FcεRI, FcεRII, or FcαRI (CD89) domain.

~~9. The immune cell of claim 1, wherein the extracellular domain of the CAR comprises one or more antigen binding domains and a toll like receptor (TLR) extracellular domain and/or the transmembrane domain of the CAR comprises a TLR transmembrane domain and/or the intracellular domain of the CAR comprises a TLR intracellular domain.~~

~~10. The immune cell of claim 9, wherein the CAR comprises, from N-terminus to C-terminus, one or more extracellular binding domains, a TLR extracellular domain, a TLR transmembrane domain, and a TLR intracellular domain.~~

~~11. The immune cell of claim 9 or claim 10, wherein one or more of the TLR extracellular domain, the TLR transmembrane domain and the TLR intracellular domain is or comprises a human TLR domain.~~

~~12. The immune cell of any one of claims 9-11, wherein the TLR extracellular domain, the TLR transmembrane domain and the TLR intracellular domain together comprise a full length TLR.~~

~~13. The immune cell of any one of claims 9-12, wherein the TLR extracellular domain comprises a TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, or TLR9 domain.~~

~~14. The immune cell of any one of claims 9-13, wherein the TLR transmembrane domain comprises a TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, or TLR9 domain.~~

~~15. The immune cell of any one of claims 9-14, wherein the TLR intracellular domain comprises a TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, or TLR9 domain.~~

~~16. The immune cell of claim 1, wherein the chimeric antigen receptor further comprises one or more of:~~
~~(d) one or more extracellular leader domains,~~
~~(e) one or more extracellular hinge domains, and~~
~~(f) one or more intracellular co-stimulatory domains.~~

~~17. The immune cell of claim 16, wherein the one or more extracellular leader domains comprise a CD8 extracellular leader domain.~~

18. The immune cell of any one of claims 1-17, wherein the one or more extracellular antigen binding domains comprise a scFv, centyrin, or darpin.

19. The immune cell of any one of claims 16-18, wherein the one or more extracellular hinge domains comprise a CD28 extracellular hinge domain, CD8a extracellular hinge domain, or an IgG4 extracellular hinge domain.

20. The immune cell of any one of claims 1-19, wherein the transmembrane domain comprises a CD28, CD8a, CD64, CD32a, CD32e, CD16a, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, or TLR9 transmembrane domain.

21. The immune cell of any one of claims 1-20, wherein the intracellular domain comprises one or more intracellular signaling domains.

22. The immune cell of claim 21, wherein the one or more intracellular signaling domains comprise a CD3-zeta, FcR- γ , CD64, CD32a, CD32e, CD16a, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, ALK, AXL, DDR2, EGFR, EphA1, INSR, cMET, MUSK, PDGFR, PTK7, RET, ROR1, ROS1, RYK, TIE2, TRK, VEGFR, CD40, CD19, CD20, 41BB, CD28, OX40, GITR, TREM-1, TREM-2, DAP12, MR, ICOS, MyD88, V/I/LxYxxL/V, SIRPa, CD45, Siglec-10, PD1, SHP-1, SHP-2, KIR-2DL, KIR-3DL, NKG2A, CD170, CD33, BTLA, CD32b, SIRPb, CD22, PIR-B, LILRB1, 41BBL (TNFSF9), CD27, OX40L, CD32b, CD11b, ITGAM, SLAMF7, CD206, CD163, CD209, Dectin-2, IL1R, IL2R, IL3R, IL4R, IL5R, IL6R, IL7R, IL8R, IL9R, IL10R, IL11R, IL12R, IL13R, IL14R, IL15R, IL17R, IFN α R, IFN γ R, TNFR, CSF1R, CSF2R, Dap10, CD36, Dectin-1, ICOSL and/or Syk intracellular signaling domain.

23. The immune cell of claim 22, when the one or more intracellular signaling domains comprises at least one intracellular co-stimulatory domain.

24. A pharmaceutical composition comprising an immune cell of any one of the previous claims.

25. The pharmaceutical composition of claim 24, wherein the pharmaceutical composition comprises a pharmaceutically acceptable carrier.

26. A nucleic acid construct comprising one or more nucleic acid sequences encoding:

(a) an extracellular domain;
~~(b) a transmembrane domain, and~~
(c) an intracellular domain;
wherein the nucleic acid construct encodes a chimeric antigen receptor (CAR) comprising (a) through (c).

~~27. The nucleic acid construct of claim 26, further comprising one or more nucleic acid sequences encoding:~~

~~(e) one or more extracellular leader domains,~~
~~(f) one or more extracellular hinge domains,~~
~~(g) one or more intracellular co-stimulatory domains,~~
(h) a cleavage peptide, and
(i) one or more peptide agents.

~~28. The nucleic acid construct of claim 27, wherein the cleavage peptide is a P2A, F2A, E2A or T2A peptide.~~

~~29. A pharmaceutical composition comprising the nucleic acid construct of any one of claims 26-28.~~

~~30. The pharmaceutical composition of claim 29, wherein the pharmaceutical composition comprises a pharmaceutically acceptable carrier.~~

~~31. A method of treating a disease or disorder in a subject, the method comprising:~~
~~delivering to the subject a therapeutically effective amount of the pharmaceutical composition of claim~~
~~24, 25, 29, or 30.~~

~~32. A method of modifying an immune cell, the method comprising:~~
~~delivering to the immune cell a nucleic acid construct comprising one or more nucleic acid sequences encoding:~~

(a) an extracellular domain;
~~(b) a transmembrane domain, and~~
(c) an intracellular domain;
wherein the modified immune cell comprises a chimeric engineered receptor comprising (a) through (c);
and
wherein the modified immune cell comprises a macrophage, monocyte or dendritic cell.

331. ___A method of modifying a macrophage or, monocyte ~~or dendritic cell~~ comprising:
delivering to the macrophage, or monocyte; ~~or dendritic cell~~

(i) a viral vector comprising one or more nucleic acid sequences encoding a chimeric antigen receptor (CAR) comprising:

- (a) an extracellular domain,
- _____ (b) a transmembrane domain, and
- (c) an intracellular domain,

~~wherein the viral vector is packaged with (ii) at least one Vpx protein,~~
~~wherein the modified macrophage, monocyte or dendritic cell comprises a chimeric engineered~~
~~receptor (CAR) comprising (a) through (c), and~~
~~wherein thereby producing a the modified macrophage, or monocyte or dendritic cell that exhibits~~
increased CAR expression relative to a ~~modified macrophage or~~, monocyte ~~or dendritic cell~~ comprising a
CAR ~~that was delivered by a viral vector encoding the CAR that was not packaged with and not delivered~~
at least one Vpx protein,

wherein the modified macrophage or monocyte does not exhibit an M1 phenotype relative to a
macrophage or monocyte not delivered at least one Vpx protein.

342. ___The method of claim 331, wherein the viral vector is or comprises a lentiviral vector.

35. ~~The method of claim 33 or 34, wherein the viral vector is delivered at a multiplicity of infection~~
~~(MOI) of about 1 to about 50.~~

36. ~~The method of claim 35, wherein the viral vector is delivered at a MOI of about 1, about 2, about 3,~~
~~about 4, about 5, about 6, about 7, about 8, about 9, or about 10.~~

373. ___The method of ~~any one of claims 33-36~~ claim 1, wherein the modified macrophage, or monocyte
~~or dendritic cell~~ exhibits increased CAR expression of ~~about 20%, about 30%, about 40%, about 40%,~~
~~about 50%, about 60%, about 70%, about 80%, about 90%, or greater~~ relative to a modified macrophage,
or monocyte ~~or dendritic cell~~ comprising a CAR ~~that was delivered a viral vector encoding the CAR that~~
~~was not packaged with~~ not delivered at least one Vpx protein.

~~384. ____ The method of claim 1 any one of claims 33-37, wherein the modified macrophage, or monocyte or dendritic cell exhibits CAR expression for an extended time period of at least 5 days relative to an unmodified macrophage, or monocyte or dendritic cell.~~

~~39. The method of claim 38, wherein the extended time period of CAR expression is for at least 5 days, at least 10 days, at least 15 days, at least 20 days, at least 25 days, at least 30 days, or longer.~~

~~40. The method of any one of claims 33-39, wherein the modified macrophage, monocyte or dendritic cell exhibits increased effector activity relative to an unmodified macrophage, monocyte or dendritic cell.~~

~~41. The method of claim 39, wherein the modified macrophage, monocyte or dendritic cell exhibits increased tumor killing ability or phagocytosis relative to an unmodified macrophage, monocyte or dendritic cell.~~

~~42. The method of any one of claims 33-41, wherein the modified macrophage, monocyte or dendritic cell does not exhibit increased polarization to a M1 phenotype relative to a macrophage, monocyte or dendritic cell comprising a CAR that was delivered a viral vector encoding the CAR that was not packaged with at least one Vpx protein.~~

~~435. ____ The method of claim 42, wherein the modified macrophage, or monocyte or dendritic cell does not exhibit increased expression of one or more markers of M1 phenotype relative to a macrophage, or monocyte or dendritic cell comprising a CAR that was delivered a viral vector encoding the CAR that was not packaged with not delivered at least one Vpx protein.~~

~~446. ____ The method of claim 435, wherein the one or more markers of M1 phenotype comprise or are one or more, two, three, four, five, six, seven, eight, nine, 10, 11, 12, 13, 14, 15, 16, 17, or 18 of CD86, CD80, MHC II, IL-1R, TLR2, TLR4, iNOS, SOCS3, CD83, PD-L1, CD69, MHC I, CD64, CD32, CD16, IL1R, a IFIT family member, or an ISG family member.~~

7. ____ A method of modifying a macrophage or monocyte comprising delivering to the macrophage or monocyte:

(i) a viral vector comprising one or more nucleic acid sequences encoding a chimeric antigen receptor (CAR) comprising:

(a) an extracellular domain,

(b) a transmembrane domain, and

(c) an intracellular domain, and

(ii) at least one Vpx protein, wherein the macrophage or monocyte is one or both of electroporated or transfected with at least one Vpx mRNA prior to transduction with the viral vector, thereby producing a modified macrophage or monocyte that exhibits increased CAR expression relative to a macrophage or monocyte comprising a CAR delivered by a viral vector and not delivered at least one Vpx protein.

8. The method of claim 1, wherein the viral vector is packaged with the at least one Vpx protein.

9. The method of claim 1, wherein the viral vector further comprises at least one cleavage peptide and at least one nucleic acid co-expressed with the CAR.

10. The method of claim 9, wherein the cleavage peptide comprises or is a P2A, F2A, E2A or T2A peptide.

11. The method of claim 9, wherein the at least one nucleic acid comprises or is at least one second CAR.

45. The method of any one of claims 33-44, wherein the modified macrophage, monocyte or dendritic cell exhibits increased production of one or more inflammatory cytokines relative to an unmodified macrophage, monocyte or dendritic cell.

46. The method of claim 45, wherein the one or more inflammatory cytokines comprise or are one, two, three, four, five, six, seven, eight, nine, 10, 11, or 12 of TNF α , IL-6, IL-1 α , IL-1 β , IL-12, IL-18, IL-8, IL-2, IL-23, IFN α , IFN β , IFN γ , IL-2, IL-8, IL-33, CCL3, CXCL12, CCL22, CCL4, CXCL10, or CCL2.

47. The method of claim 1, wherein the modified macrophage or monocyte or dendritic cell exhibits reduced SIRP α activity relative to an unmodified macrophage or monocyte or dendritic cell.

48. The method of claim 1, wherein the modified macrophage or monocyte or dendritic cell comprises deletion of SIRP α using one or more endonucleases.

~~49~~14. The method of claim ~~13~~48, wherein the one or more endonucleases comprise or are one or more of a CRISPR/Cas system, zinc finger nuclease (ZFN), transcription activator-like effector-based nuclease (TALEN), or meganuclease.

~~50~~. The method of claim 49, wherein the CRISPR/Cas system comprises Cas9, Cas12a, or C2c2.

~~51~~. The method of claim 47, wherein the modified macrophage, monocyte or dendritic cell has been treated with one or more anti-SIRP α antibodies.

~~52~~15. The method of claim ~~47~~12, wherein the modified macrophage, or monocyte or dendritic cell comprises one or more anti-SIRP α antibodies.

~~53~~16. The method of claim ~~47~~12, wherein the modified macrophage, or monocyte or dendritic cell comprises one or more siRNAs that downregulate SIRP α .

~~54~~. The method of any one of claims ~~33-53~~, wherein the extracellular domain of the CAR comprises one or more antigen binding domains and an FcR extracellular domain, and/or the transmembrane domain of the CAR comprises an FcR transmembrane domain, and/or the intracellular domain of the CAR comprises an FcR intracellular domain.

~~55~~. The method of claim 54, wherein the CAR comprises, from N terminus to C terminus, one or more extracellular binding domains, an FcR extracellular domain, an FcR transmembrane domain, and an FcR intracellular domain.

~~56~~. The method of claim 54 or 55, wherein one or more of the FcR extracellular domain, the FcR transmembrane domain and the FcR intracellular domain is or comprises a human FcR domain.

~~57~~. The method of any one of claims ~~54-56~~, wherein the FcR extracellular domain, the FcR transmembrane domain and the FcR intracellular domain together comprise a full-length FcR.

~~58~~. The method of any one of claims ~~54-57~~, wherein the FcR extracellular domain comprises a CD64 (Fc γ RI), CD32a (Fc γ RIIa), CD32b (Fc γ RIIb), CD32c, CD16a (Fc γ RIIIa), CD16b (Fc γ RIIIb), Fc ϵ RI, Fc ϵ RII, or Fc α RI (CD89) domain.

59. The method of any one of claims 54-58, wherein the FeR transmembrane domain comprises a CD64 (FcγRI), CD32a (FcγRIIa), CD32b (FcγRIIb), CD32c, CD16a (FcγRIIIa), CD16b (FcγRIIIb), FcεRI, FcεRII, or FcαRI (CD89) domain.

60. The method of any one of claims 54-59, wherein the FeR intracellular domain comprises a CD64 (FcγRI), CD32a (FcγRIIa), CD32b (FcγRIIb), CD32c, CD16a (FcγRIIIa), CD16b (FcγRIIIb), FcεRI, FcεRII, or FcαRI (CD89) domain.

61. The method of any one of claims 33-53, wherein the extracellular domain of the CAR comprises one or more antigen binding domains and a toll-like receptor (TLR) extracellular domain and/or the transmembrane domain of the CAR comprises a TLR transmembrane domain and/or the intracellular domain of the CAR comprises a TLR intracellular domain.

62. The method of claim 61, wherein the CAR comprises, from N-terminus to C-terminus, one or more extracellular binding domains, a TLR extracellular domain, a TLR transmembrane domain, and a TLR intracellular domain.

63. The method of claim 61 or 62, wherein one or more of the TLR extracellular domain, the TLR transmembrane domain and the TLR intracellular domain is or comprises a human TLR domain.

64. The method of any one of claims 61-63, wherein the TLR extracellular domain, the TLR transmembrane domain and the TLR intracellular domain together comprise a full length TLR.

65. The method of any one of claims 61-64, wherein the TLR extracellular domain comprises a TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, or TLR9 domain.

66. The method of any one of claims 61-65, wherein the TLR transmembrane domain comprises a TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, or TLR9 domain.

67. The method of any one of claims 61-66, wherein the TLR intracellular domain comprises a TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, or TLR9 domain.

68. The method of any one of claims 33-67, wherein the CAR further comprises one or more of:
(d) one or more extracellular leader domains;

- ~~(e) one or more extracellular hinge domains, and~~
~~(f) one or more intracellular co-stimulatory domains.~~

~~69. The method of claim 68, wherein the one or more extracellular leader domains comprise a CD8 extracellular leader domain.~~

~~70. The method of any one of claims 33-69, wherein the one or more extracellular binding domains comprise a scFv, centyrin, or darpin.~~

~~71. The method of any one of claims 68-70, wherein the CAR one or more extracellular hinge domains comprises a CD28 extracellular hinge domain and/or a CD28 transmembrane domain, a CD8a extracellular hinge domain, or an IgG4 extracellular hinge domain.~~

18. The method of claim 17, wherein the modified macrophage or monocyte:

- (i) exhibits increased tumor killing ability relative to a macrophage or monocyte without a CD28 extracellular hinge domain and/or a CD28 transmembrane domain,
(ii) exhibits increased TNF α production relative to a macrophage or monocyte without a CD28 extracellular hinge domain and/or a CD28 transmembrane domain, or
(iii) does not exhibit increased IL6 production relative to a macrophage or monocyte without a CD28 extracellular hinge domain and/or a CD28 transmembrane domain.

~~72. The method of any one of claims 33-71, wherein the transmembrane domain comprises a CD28, CD8a, CD64, CD32a, CD32c, CD16a, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, or TLR9 transmembrane domain.~~

~~73. The method of any one of claims 33-72, wherein the intracellular domain comprises one or more intracellular signaling domains.~~

74. The method of claim 17, wherein the one or more intracellular signaling domains comprises one or more of: a CD3-zeta, FcR γ , CD64, CD32a, CD32c, CD16a, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, ALK, AXL, DDR2, EGFR, EphA1, INSR, cMET, MUSK, PDGFR, PTK7, RET, ROR1, ROS1, RYK, TIE2, TRK, VEGFR, CD40, CD19, CD20, 41BB, CD28, OX40, GITR, TREM-1, TREM-2, DAP12, MR, ICOS, MyD88, V/I/LxYxxL/V, SIRPa, CD45, Siglec-10, PD1, SHP-1, SHP-2,

KIR-2DL, KIR-3DL, NKG2A, CD170, CD33, BTLA, CD32b, SIRPb, CD22, PIR-B, LILRB1, 41BBL (TNFSF9), CD27, OX40L, CD32b, CD11b, ITGAM, SLAMF7, CD206, CD163, CD209, Dectin-2, IL1R, IL2R, IL3R, IL4R, IL5R, IL6R, IL7R, IL8R, IL9R, IL10R, IL11R, IL12R, IL13R, IL14R, IL15R, IL17R, IFNaR, IFNgR, TNFR, CSF1R, CSF2R, Dap10, CD36, Dectin-1, ICOSL, CD2, CD7, CD96, CRTAM, DC- SIGN, NKG2D, NTB-A, CD30 and/or Syk intracellular signaling domain.

20. The method of claim 19, wherein the intracellular domain comprises a CD3-zeta intracellular signaling domain.

21. The method of claim 1, wherein the method further comprises treating the modified macrophage or monocyte with one or more of:

- (i) a CD40 agonist, or
- (ii) a 4-1BB-ligand agonist.

22. The method of claim 21, wherein the modified macrophage or monocyte exhibits increased polarization to M1 phenotype relative to an unmodified macrophage or monocyte.

23. The method of claim 7, wherein the viral vector comprises or is a lentiviral vector.

24. The method of claim 7, wherein the method further comprises treating the modified macrophage or monocyte with one or more of:

- (i) a CD40 agonist, or
- (ii) a 4-1BB-ligand agonist.

25. The method of claim 24, wherein the modified macrophage or monocyte exhibits increased polarization to M1 phenotype relative to an unmodified macrophage or monocyte.

~~75. The method of claim 74, when the one or more intracellular signaling domains comprises at least one intracellular co-stimulatory domain.~~

~~76. The method of any one of claims 33-75, wherein the viral vector further comprises one or more nucleic acid sequences encoding:~~

~~(e) one or more extracellular leader domains,~~

- ~~(f) one or more extracellular hinge domains,~~
- ~~(g) one or more intracellular co-stimulatory domains,~~
- ~~(h) a cleavage peptide, and~~
- ~~(i) one or more peptide agents.~~

~~77. The method of claim 76, wherein the cleavage peptide is a P2A, F2A, E2A or T2A peptide.~~

~~78. A pharmaceutical composition comprising a macrophage, monocyte or dendritic cell modified by the method of any one of claims 33-77.~~

~~79. The pharmaceutical composition of claim 78, wherein the pharmaceutical composition comprises a pharmaceutically acceptable carrier.~~

~~80. A method of treating a disease, disorder, or condition in a subject comprising:
administering to the subject a therapeutically effective amount of the pharmaceutical composition of claim 78 or 79.~~