context of allogeneic CAR-T-cell therapy, these approaches may cause bystander host immune-activation that could accelerate the rejection of allogeneic CAR-T-cells, thereby compromising therapeutic efficacy. Another approach to provide cytokine support was based on introducing a constitutively activated dimerized cytokine receptor, an IL-7Ra – this limits the nature (IL-7 signaling only) and magnitude of signaling output. Yet another approach to provide cytokine support involved incorporating Signal 3 directly into the CAR molecule (Nat Med. 2018 Mar;24(3):352-359.). A limitation of this approach is that the strength of Signal 3 depends on the strength of CAR activation. In the absence of target (and CAR activation), Signal 3 would not be transduced.

[0006] Needed are solutions to circumvent these drawbacks by targeting cytokine signals specifically to CAR-T cells in a tunable way, thus allowing for an improved safety profile and therapeutic efficacy. Provided herein are compositions and methods that address this need.

SUMMARY

[0007] The present disclosure provides constitutively active chimeric cytokine receptors (CACCRs). When present on chimeric antigen receptor (CAR)-bearing immune cells (CAR-I cells, e.g. CAR-T-cells), such CACCRs allow for increased immune cell activation, proliferation, persistence, and/or potency. Also provided are methods of making and using the CACCRs described herein.

[0008] Accordingly, in one aspect, the present disclosure refers to a constitutively active chimeric cytokine receptor (CACCR) composed of two monomers, each monomer comprising: (a) a transmembrane domain; (b) a Janus Kinase (JAK)-binding domain; and (c) a recruiting domain, wherein the monomers are constitutively dimerized. In some examples, the CACCR does not comprise an extracellular domain ligand binding domain.

[0009] In some examples, the transmembrane domain and/or the JAK-binding domain is derived from the TPOR/MPLR receptor. In some examples, the transmembrane domain and/or the JAK binding domain is derived from amino acids 478 – 582 of the naturally occurring TPOR/MPLR receptor of SEQ ID NO: 6. In some examples, the TPOR/MPLR receptor comprises one or more of the amino acid substitutions selected from H499L, S505N, W515K, and G509N. In some examples, the TPOR/MPLR receptor

comprises the H499L, S505N and W515K substitutions, or the S505N and W515K substitutions. In some examples, the recruiting domain is a STAT-recruiting domain. In some examples, the recruiting domain comprises the STAT-recruiting domain from IL7Ra, for example, IL7Ra(316-459). In some examples, the recruiting domain comprises the STAT-recruiting domain from IL2Rb, for example, IL2Rb (333-551), IL2Rb(393-433, 518-551), IL2Rb(339-379, 393-433, 518-551), IL2Rb(333-551, Y381S, Y384S, Y387S), IL2Rb(333-551, Y364S, Y381S, Y384S, Y387S). In some examples, the recruiting domain comprises the STAT-recruiting domain from IL12Rb1, for example, IL12Rb1(622-662). In some examples, the recruiting domain comprises the STAT-recruiting domain from IL12Rb2, for example, IL12Rb2(714-862) or IL12Rb2(775-825). In some examples, the recruiting domain comprises the STAT-recruiting domain from IL21R, for example, IL21R(322-538).

[0010] In a related example provided herein is a polynucleotide encoding any one of the CACCRs of the disclosure, and an expression vector comprising such polynucleotide. In some examples, the polynucleotide further encodes for a chimeric antigen receptor (CAR), wherein the CAR binds to BCMA, EGFRvIII, Flt-3, WT-1, CD20, CD23, CD30, CD38, CD70, CD33, CD133, LeY, NKG2D, CS1, CD44v6, ROR1, CD19, Claudin-18.2 (Claudin-18A2, or Claudin18 isoform 2), DLL3 (Delta-like protein 3, Drosophila Delta homolog 3, Delta3), Muc17 (Mucin17, Muc3, Muc3), FAP alpha (Fibroblast Activation Protein alpha), Ly6G6D (Lymphocyte antigen 6 complex locus protein G6d, c6orf23, G6D, MEGT1, NG25), and/or RNF43 (E3 ubiquitin-protein ligase RNF43, RING finger protein 43).

[0010a] In another aspect, the present disclosure refers to a polynucleotide encoding any one of the CACCRs as disclosed herein.

[0010b] In another aspect, the present disclosure refers to an expression vector comprising the polynucleotide as disclosed herein.

[0010c] In another aspect, the present disclosure refers to an engineered immune cell comprising the expression vector as disclosed herein.

[0011] In another example, provided herein is an engineered immune cells comprising at least one chimeric antigen receptor (CAR) and at least one CACCR of the disclosure. In some examples the immune cell is a T-cell. In some examples the immune cell is an allogeneic immune cell. In other examples, the immune cell is an autologous immune cell. The immune cell may be selected from the group consisting of: T-cell, dendritic cell, killer dendritic cell, mast cell, NK-cell, macrophage, monocyte, B-cell and an immune cell derived from a stem cell. In a related example, provided herein is a pharmaceutical composition comprising any of the engineered immune cells of the disclosure, and a kit comprising such a pharmaceutical composition.

[0011a] In another aspect, the present disclosure refers to an engineered immune cell comprising a chimeric antigen receptor (CAR) and at least one CACCR as disclosed herein.

[0011b] In another aspect, the present disclosure refers to a method of preparing an engineered immune cell, the method comprising introducing the polynucleotide as disclosed herein or an expression vector as disclosed herein into an immune cell.

[0011c] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the engineered immune cells as disclosed herein.

[0011d] In another aspect, the present disclosure refers to a kit comprising the engineered immune cells as disclosed herein or the pharmaceutical composition as disclosed herein.

[0012] In another example, provided herein is a method of treating a cancer in a subject, comprising administering to the subject a therapeutically effective amount of any of the engineered immune cells described herein.

[0012a] In another aspect, the present disclosure refers to use of the engineered immune cells as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a cancer in a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] **FIG. 1** shows a schematic of an engineered CACCR of the disclosure.

[0014] **FIG. 2** shows a schematic of a vector of the disclosure that can be used to co-express the CACCR and CAR of the disclosure. One or more cytotails may be joined in tandem to mimic signaling from one or more cytokines. A schematic diagram of the vector expressing the control BFP (blue fluorescent protein) CAR is also shown.

[0015] **FIGs. 3A-3B** show the identification of TpoR transmembrane (TM) mutants that constitutively activate cytokine receptor signaling.

[0016] **FIGs. 4A-4C** show results for the expansion of CAR-T-cells coexpressing a constitutively active chimeric cytokine receptor.

[0017] **FIG. 5** shows differentiation and the memory T-cell subset distribution in the CAR-T-cell product, under different IL-2 conditions.

[0018] **FIGs. 6A-6B** show the extent of constitutive cytokine signaling mediated by each TpoR TM variant.

[0019] **FIGs. 7A-7D** show the cytotoxic activity of TpoR TM mutants, indicating that constitutive cytokine receptor signaling enhances CAR-T-cell potency.

[0020] **FIGs. 8A-8B** show the cytotoxic activity and durability of TpoR TM mutants.

[0021] **FIG. 9** shows the enrichment of CAR-T-cells over time in a growth factor-independent assay.

[0022] **FIGs. 10A-10B** show the fold expansion of CAR-T-cells over time in a growth factor-independent assay.

[0023] **FIG. 11** shows the memory T-cell subset distribution among CAR+ T-cells over time in a growth factor-independent assay.

WHAT IS CLAIMED IS:

1. A constitutively active chimeric cytokine receptor (CACCR) composed of two monomers, each monomer comprising:

a. a transmembrane domain;

b. a Janus Kinase (JAK)-binding domain; and

c. a recruiting domain,

wherein the monomers are constitutively dimerized.

2. The CACCR of claim 1, wherein the JAK-binding domain comprises a JAK1-binding domain.

3. The CACCR of claim 1, wherein the JAK-binding domain comprises a JAK2-binding domain.

4. The CACCR of claim 1, wherein the JAK-binding domain comprises a JAK3-binding domain.

5. The CACCR of claim 1, wherein the JAK-binding domain comprises a TYK2-binding domain.

6. The CACCR of any one of claims 1 to 5, wherein the JAK-binding domain comprises one of the transmembrane amino acid sequences presented in Table 1b.

7. The CACCR of any one of claims 1 to 6, wherein the recruiting domain comprises a STAT-recruiting domain is selected from a STAT-1, STAT-2, STAT-3, STAT-4, STAT-5, STAT-6, or STAT-7-recruiting domain from at least one receptor.

8. The CACCR of any one of claims 1 to 7, wherein the transmembrane domain and/or the JAK-binding domain is derived from EpoR, GP130, PrlR, GHR, GCSFR, or TPOR/MPLR receptors.

9. The CACCR of any one of claims 1 to 7, wherein the transmembrane domain and/or the JAK-binding domain is derived from the TPOR/MPLR receptor.

10. The CACCR of claim 9, wherein the transmembrane domain and/or the JAK-binding domain is derived from the TPOR/MPLR receptor, and the TPOR/MPLR receptor comprises amino acids 478 – 582 of the naturally occurring TPOR/MPLR receptor of SEQ ID NO: 6.

11. The CACCR of claim 10, wherein the TPOR/MPLR receptor comprises one or more amino acid substitutions at H499, S505, W515, and G509 of SEQ ID NO: 6.

12. The CACCR of claim 11, wherein the TPOR/MPLR receptor comprises one or more of the amino acid substitutions selected from H499L, S505N, W515K, and G509N.

13. The CACCR of claim 11, wherein the TPOR/MPLR receptor comprises the amino acid substitution H499L.

14. The CACCR of claim 11, wherein the TPOR/MPLR receptor comprises the amino acid substitution S505N.

15. The CACCR of claim 11, wherein the TPOR/MPLR receptor comprises the amino acid substitution W515K.

16. The CACCR of claim 11, wherein the TPOR/MPLR receptor comprises the amino acid substitution G509N.

17. The CACCR of claim 11, wherein the TPOR/MPLR receptor comprises the amino acid substitutions H499L and S505N.

18. The CACCR of claim 11, wherein the TPOR/MPLR receptor comprises the amino acid substitutions H499L and W515K.

19. The CACCR of claim 11, wherein the TPOR/MPLR receptor comprises the amino acid substitutions S505N and W515K.

20. The CACCR of claim 11, wherein the TPOR/MPLR receptor comprises the amino acid substitutions H499L and G509N.

21. .The CACCR of claim 11, wherein the TPOR/MPLR receptor comprises the amino acid substitutions H499L and S505N.

22. The CACCR of claim 11, wherein the TPOR/MPLR receptor comprises the amino acid substitutions H499L, S505N, and W515K.

23. The CACCR of any one of claims 1 to 22, wherein the monomers are identical.

24. The CACCR of any one of claims 1 to 22, wherein the monomers are different.

25. The CACCR of any one of claims 1 to 24, wherein the recruiting domain comprises a STAT-recruiting domain from a cytokine receptor.

26. The CACCR of any one of claims 1 to 24, wherein the recruiting domain comprises a STAT-recruiting domain from a receptor selected from receptors presented in Table 2a.

27. The CACCR of any one of claims 1 to 24, wherein the recruiting domain comprises the amino acid sequence of one or more of the receptor sequences presented in Table 2b.

28. The CACCR of any one of claims 1 to 27, wherein the recruiting domain comprises the STAT-recruiting domain from IL7Ra.

29. The CACCR of claim 28, wherein the IL7Ra is IL7Ra(316-459).

30. The CACCR of any one of claims 1-27, wherein the recruiting domain comprises the STAT-recruiting domain from IL2Rb.

31. The CACCR of claim 30, wherein the IL2Rb comprises the recruiting domain comprises the STAT-recruiting domain from IL7Ra.

32. The CACCR of any one of claims 1-27, wherein the recruiting domain comprises the STAT-recruiting domain from IL12Rb2.

33. The CACCR of claim 32, wherein the IL12Rb2 comprises IL12Rb2(714-862) or IL12Rb2(775-825).

34. The CACCR of any one of claims 1 to 33, wherein the recruiting domain comprises the STAT-recruiting domains from two receptors.

35. The CACCR of any one of claims 1 to 34, wherein the recruiting domain comprises the STAT-recruiting domains from two cytokine receptors.

36. The CACCR of claim 35, wherein the two cytokine receptors are selected from the group consisting of IL7Ra, IL2Rb, and IL12Rb2.

37. A polynucleotide encoding any one of the CACCRs of any one of claims 1 to 36.

38. An expression vector comprising the polynucleotide of claim 37.

39. The expression vector of claim 37 comprising the polynucleotide of claim 37 and a polynucleotide expressing a chimeric antigen receptor (CAR).

40. The expression vector of claim39, wherein the CAR binds to BCMA, EGFRvIII, Flt-3, WT-1, CD20, CD23, CD30, CD38, CD70, CD33, CD133, LeY, NKG2D, CS1, CD44v6, ROR1, CD19, Claudin-18.2 (Claudin-18A2, or Claudin18 isoform 2), DLL3 (Delta-like protein 3, Drosophila Delta homolog 3, Delta3), Muc17 (Mucin17, Muc3, Muc3), FAP alpha (Fibroblast Activation Protein alpha), Ly6G6D (Lymphocyte antigen 6 complex locus protein G6d, c6orf23, G6D, MEGT1, NG25), and/or RNF43 (E3 ubiquitin-protein ligase RNF43, RING finger protein 43).

41. The expression vector of any one of claims 38-40, wherein the vector is a lentiviral vector.

42. An engineered immune cell comprising the expression vector of any one of claims 38-41.

43. The engineered immune cell of claim 42, wherein the immune cell is a T-cell.

44. An engineered immune cell comprising a chimeric antigen receptor (CAR) and at least one CACCR of any one of claims 1 to 36.

45. The engineered immune cell of claim 44, wherein the CAR and the CACCR are expressed in stoichiometrically equal amounts.

46. The engineered immune cell of any one of claims 42-45, wherein the immune cell is a T-cell.

47. The engineered immune cell of any one of claims 42-46, wherein the CAR binds to BCMA, EGFRvIII, Flt-3, WT-1, CD20, CD23, CD30, CD38, CD70, CD33, CD133, LeY, NKG2D, CS1, CD44v6, ROR1, CD19, Claudin-18.2 (Claudin-18A2, or Claudin18 isoform 2), DLL3 (Delta-like protein 3, Drosophila Delta homolog 3, Delta3), Muc17 (Mucin17, Muc3, Muc3), FAP alpha (Fibroblast Activation Protein alpha), Ly6G6D (Lymphocyte antigen 6 complex locus protein G6d, c6orf23, G6D, MEGT1, NG25), and/or RNF43 (E3 ubiquitin-protein ligase RNF43, RING finger protein 43).

48. The engineered immune cell of any one of claims 42-47, wherein the cell is an allogeneic immune cell.

49. The engineered immune cell of any one of claims 42-47, wherein the cell is an autologous immune cell.

50. The engineered immune cell of any one of claims 42-49, wherein the immune cell is selected from the group consisting of: T-cell, dendritic cell, killer dendritic cell, mast cell, NK-cell, macrophage, monocyte, B-cell and an immune cell derived from a stem cell.

51. A method of preparing an engineered immune cell, the method comprising introducing the polynucleotide of claim 37 or an expression vector of any one of claims 38-41 into an immune cell.

52. The method of claim 51, wherein the immune cell is selected from the group consisting of: T-cell, dendritic cell, killer dendritic cell, mast cell, NK-cell, macrophage, monocyte, B-cell and an immune cell derived from a stem cell.

53. A pharmaceutical composition comprising the engineered immune cells of any one of claims 42-50.

54. A kit comprising the engineered immune cells of any one of claims 42-50 or the pharmaceutical composition of claim 53.

55. Use of the engineered immune cells of any one of claims 42-50 or the pharmaceutical composition of claim 53 in the manufacture of a medicament for treating a cancer in a subject.

56. The use of claim 55, wherein the cancer comprises a solid tumor.

57. The use of claim55, wherein the cancer comprises a liquid tumor.