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 example, 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.

**[0008a]** 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) an intracellular recruiting domain comprising a STAT-activation domain, wherein the CACCR does not comprise an extracellular ligand binding domain, wherein the monomers are constitutively dimerized and wherein the transmembrane domain and JAK-binding domain comprise amino acids 478-582 of SEQ ID NO: 6 comprising amino acid substitutions at S505 and W515.

**[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

## 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. an intracellular recruiting domain comprising a STAT-activation domain,

wherein the CACCR does not comprise an extracellular ligand binding domain, wherein the monomers are constitutively dimerized and wherein the transmembrane domain and JAK-binding domain comprise amino acids 478-582 of SEQ ID NO: 6 comprising amino acid substitutions at S505 and W515.

**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 recruiting domain comprises a STAT-recruiting domain selected from the group consisting of a STAT-1-, a STAT-3-, a STAT-4-, and a STAT-5-recruiting domain.

**7.** The CACCR of any one of claims 1-6, wherein amino acids 478-582 of SEQ ID NO: 6 further comprise an amino acid substitution at H499.

**8.** The CACCR of any one of claims 1-6, wherein amino acids 478-582 of SEQ ID NO: 6 further comprise an amino acid substitution at G509.

**9.** The CACCR of any one of claims 1-6, wherein amino acids 478-582 of SEQ ID NO: 6 further comprise an amino acid substitution at H499 and G509.

**10.** The CACCR of any one of claims 1-9, wherein the amino acid substitutions at S505 and W515 are S505N and W515K.

**11.** The CACCR of claims 7 or 9, wherein the amino acid substitution at H499 is H499L.

**12.** The CACCR of claims 8 or 9, wherein the amino acid substitution at G509 is G509N.

**13.** The CACCR of any one of claims 1 to **Error! Reference source not found.**, wherein the monomers are identical.

**14.** The CACCR of any one of claims 1 to 12, wherein the monomers are different.

**15.** The CACCR of any one of claims 1 to 14, wherein the recruiting domain comprises a STAT-recruiting domain from a cytokine receptor.

**16.** The CACCR of any one of claims 1 to 14, wherein the recruiting domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 67, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, and SEQ ID NO: 86.

**17.** The CACCR of any one of claims 1 to 16, wherein the recruiting domain comprises the STAT-recruiting domain from IL7Ra.

**18.** The CACCR of claim 17, wherein the IL7Ra is IL7Ra(316-459) (SEQ ID NO: 46).

**19.** The CACCR of any one of claims 1-17, wherein the recruiting domain comprises the STAT-recruiting domain from IL2Rb.

**20.** The CACCR of claim 19, wherein the IL2Rb comprises the recruiting domain comprises the STAT-recruiting domain from IL7Ra.

**21.** The CACCR of any one of claims 1-16, wherein the recruiting domain comprises the STAT-recruiting domain from IL12Rb2.

**22.** The CACCR of claim 21, wherein the IL12Rb2 comprises IL12Rb2(714-862) (SEQ ID NO: 86) or IL12Rb2(775-825) (SEQ ID NO: 67).

**23.** The CACCR of any one of claims 1 to 22, wherein the recruiting domain comprises the STAT-recruiting domains from two cytokine receptors.

**24.** The CACCR of claim 23, wherein the two cytokine receptors are selected from the group consisting of IL7Ra, IL2Rb, and IL12Rb2.

**25.** A polynucleotide encoding any one of the CACCRs of any one of claims 1 to 24.

**26.** An expression vector comprising the polynucleotide of claim 25.

**27.** The expression vector of claim 26 comprising the polynucleotide of claim 25 and a polynucleotide expressing a chimeric antigen receptor (CAR).

**28.** The expression vector of claim 27, 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), or RNF43 (E3 ubiquitin-protein ligase RNF43, RING finger protein 43).

**29.** The expression vector of any one of claims 26-28, wherein the vector is a lentiviral vector.

**30.** An engineered immune cell comprising the expression vector of any one of claims 26-29.

**31.** The engineered immune cell of claim 30, wherein the immune cell is a T-cell.

**32.** An engineered immune cell comprising a chimeric antigen receptor (CAR) and at least one CACCR of any one of claims 1 to 24.

**33.** The engineered immune cell of claim 32, wherein the CAR and the CACCR are expressed in stoichiometrically equal amounts.

**34.** The engineered immune cell of any one of claims 30-33, wherein the immune cell is a T-cell.

**35.** The engineered immune cell of any one of claims 32-34, 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), or RNF43 (E3 ubiquitin-protein ligase RNF43, RING finger protein 43).

**36.** The engineered immune cell of any one of claims 32-35, wherein the cell is an allogeneic immune cell.

**37.** The engineered immune cell of any one of claims 32-35, wherein the cell is an autologous immune cell.

**38.** The engineered immune cell of any one of claims 32-37, 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.

**39.** A method of preparing an engineered immune cell, the method comprising introducing the polynucleotide of claim 25 or an expression vector of any one of claims 26-29 into an immune cell.

**40.** The method of claim 39, 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.

**41.** A pharmaceutical composition comprising the engineered immune cells of any one of claims 33-38.

**42.** A kit comprising the engineered immune cell of any one of claims 30-38 or the pharmaceutical composition of claim 41.

**43.** Use of the engineered immune cells of any one of claims 30-38 or the pharmaceutical composition of claim 41 in the manufacture of a medicament for treating a cancer in a subject.

**44.** The use of claim 43, wherein the cancer comprises a solid tumor.

**45.** The use of claim 43, wherein the cancer comprises a liquid tumor.