

BCMA CAR-T CELLS WITH ENHANCED ACTIVITIES**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] The present application claims the benefit of priority to U.S. Provisional Application No. 62/980,914, filed on February 24, 2020; U.S. Provisional Application No. 63/020,713, filed on May 6, 2020; U.S. Provisional Application No. 63/053,409, filed on July 17, 2020; and U.S. Provisional Application No. 63/092,681, filed on October 16, 2020, the contents of all of which are hereby incorporated by reference in their entireties.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 2, 2021, is named AT-034-05WO_SL.txt and is 312,069 bytes in size.

FIELD

[0003] The instant disclosure relates to CAR-T cells, especially BCMA specific CAR-T cells with enhanced activities, and methods of making and uses thereof.

BACKGROUND

[0004] Adoptive transfer of immune cells (e.g. T cells) genetically modified to recognize malignancy-associated antigens is showing promise as a new approach to treating cancer. For example, T cells can be genetically modified to express chimeric antigen receptors (CARs), which are fusion proteins comprised of an antigen recognition moiety and T cell activation domains.

[0005] One CAR-T cell that would be useful is one that recognizes B cell maturation antigen (BCMA, CD269, or TNFRSF17). BCMA is a member of the tumor necrosis factor receptor (TNFR) superfamily and is involved in pro-survival signaling. BCMA was identified in a malignant human T cell lymphoma containing a t(4;16) translocation. BCMA is expressed at high levels on normal and malignant plasma cells at all stages of multiple myeloma (MM) and some other plasma cell malignancies (e.g., diffused large B-cell lymphoma, DLBCL). BCMA is also expressed on most or all myeloma cells, and expression is absent from non-B cell lineages. Exemplary BCMA CARs are disclosed and described in United States Patent No. 10,294,304, the entirety of which is hereby

incorporated by reference herein. BCMA CAR-T cells may be useful in treating a variety of diseases, including multiple myeloma (MM), a malignancy characterized by an accumulation of clonal plasma cells. MM largely remains incurable, and most subjects develop resistance over time.

5 [0006] T cell proliferation, cytotoxic potency and persistence is driven by signal transduction pathways. Conventional CAR designs provide two signals – CD3zeta activation (Signal 1) and co-stimulation (Signal 2, e.g. via 4-1BB, OX40, and/or CD28 expression). In some contexts, a third signal (Signal 3), cytokine-induced cytokine receptor signaling (e.g. cytokine support for immune potentiation), may be desirable. Approaches to
10 provide Signal 3 have however been met with significant limitations.

[0007] In general, one approach to provide cytokine support includes combining CAR-T cell therapy with systemic infusions of recombinant cytokines/cytokine mimetics, and engineering CAR-T cells to secrete/express cytokines extracellularly. As cytokines have pleiotropic effects and can also impact the function of other cell types, the systemic
15 administration or production of immune-potentiating cytokines by CAR-T cells have at least two major drawbacks: (i) these approaches can cause systemic toxicity in humans, and (ii) in the 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
20 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
25 CAR activation), Signal 3 would not be transduced.

[0008] Needed are solutions to circumvent these drawbacks by targeting cytokine signals specifically to BCMA CAR-T cells in a tunable way, thus allowing for an improved safety profile and therapeutic efficacy.

SUMMARY

30 [0009] The present disclosure provides BCMA CAR immune cells, such as BCMA CAR-T cells, that contain constitutively active chimeric cytokine receptors (CACCRs). When present on BCMA chimeric antigen receptor (CAR)-bearing immune cells (CAR-I cells,

e.g. BCMA CAR-T cells), such CACCRs allow for increased immune cell activation, proliferation, persistence, and/or potency. The enhanced activities of BCMA CAR-T cells are dependent on the presence of the target BCMA. Also provided are methods of making and using the CACCRs described herein in BCMA CAR immune cells, such as BCMA
5 CAR-T cells.

[0010] Accordingly, in one aspect, provided herein is a BCMA CAR-T cell, e.g. a BCMA CAR-T cell that contains a 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.

10 [0011] In some embodiments, the CACCR's transmembrane domain and/or JAK-binding domain is derived from the TPOR/MPLR receptor. In some embodiments, 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
15 embodiments, the TPOR/MPLR receptor comprises one or more of the amino acid substitutions selected from H499L, S505N, W515K, and G509N. In some embodiments, the TPOR/MPLR receptor comprises the H499L, S505N and W515K substitutions, or the S505N and W515K substitutions.

[0012] In some embodiments, the recruiting domain is a STAT-recruiting domain. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from
20 IL7Ra, for example, IL7Ra(316-459). In some embodiments, 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), or IL2Rb(333-551, Y364S, Y381S, Y384S, Y387S). In some
25 embodiments, the recruiting domain comprises the STAT-recruiting domain from IL12Rb1, for example, IL12Rb1(622-662). In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL12Rb2, for example, IL12Rb2(714-862) or IL12Rb2(775-825). In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL21R, for example, IL21R(322-538).

[0013] In a related aspect provided herein is a polynucleotide encoding any one of the
30 CACCRs of the disclosure, and an expression vector comprising such polynucleotide. In some embodiments, the polynucleotide further encodes for a chimeric antigen receptor

(CAR), wherein the CAR binds to BCMA (a “BCMA CAR” or a “BCMA specific CAR”) (e.g., human BCMA, Uniprot accession number: Q02223-2).

[0014] In another aspect, provided herein is an engineered immune cell comprising at least one BCMA chimeric antigen receptor and at least one CACCR of the disclosure. In some embodiments the immune cell is a T cell. In some embodiments the immune cell is an allogeneic immune cell. In other embodiments, 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 aspect, provided herein is a pharmaceutical composition comprising any of the engineered immune cells of the disclosure, and a kit comprising such a pharmaceutical composition. In a related aspect, the engineered immune cell comprises one or more polynucleotides that encode a BCMA CAR and a CACCR of the disclosure. In a related aspect, the engineered immune cell comprises a bicistronic or multicistronic polynucleotide that encodes a BCMA CAR and one or more than one CACCR of the disclosure.

[0015] In another aspect, 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.

[0016] In another aspect, a method of making an engineered immune cell is provided comprising providing a cell, for example, an immune cell, a T cell, dendritic cell, killer dendritic cell, mast cell, NK-cell, macrophage, monocyte, B-cell and an immune cell derived from a stem cell, and introducing into the cell at least one polynucleotide that encodes a BCMA CAR and at least one polynucleotide that encodes a CACCR of the disclosure. In some embodiments, the cell is introduced with one or more polynucleotide molecules that encode a BCMA CAR and a CACCR. In some embodiments, a first vector comprises a BCMA CAR polynucleotide and a second vector comprises a CACCR polynucleotide. In some embodiments, one vector comprises both a BCMA CAR polynucleotide and a CACCR polynucleotide. In some embodiments, the vector is a viral vector such as a lentiviral vector. In some embodiments, the cell is an autologous cell, which can mean that it is derived from the same person who will be treated with the engineered immune cell prepared from that cell. In other embodiments, the cell is an allogeneic cell, which can mean that it is derived from a person other than the person who

will be treated with the engineered immune cell, e.g., from a healthy donor. In some embodiments, the allogeneic engineered immune cell further comprises one or more genetic modifications at the TCR alpha constant (TRAC) locus to reduce or negate the expression of the endogenous TCR alpha receptor. In some embodiments, the engineered immune cell

5 further comprises one or more genetic modifications at the CD52 gene to reduce or negate the expression of CD52. In various embodiments, the BCMA CAR polynucleotide and/or the CACCR polynucleotide, and any vector that may comprise either or both polynucleotides is driven by the EF1alpha promoter. In some embodiments, at least one of the BCMA CAR polynucleotide, the CACCR polynucleotide, and the vector or vectors that

10 comprise either or both polynucleotides, further encodes at least one selectable marker that facilitates and/or enables the identification of cells that contain the BCMA CAR polynucleotide and/or the CACCR polynucleotide. In various embodiments, the BCMA CAR polynucleotide and/or the CACCR polynucleotide, and any vector that may comprise either or both polynucleotides further comprises a wild type or mutant WPRE. In some

15 embodiments, the vector further comprises a mutant WPRE. In various embodiments, the BCMA CAR polynucleotide and/or the CACCR polynucleotide, and any vector that may comprise either or both polynucleotides, may be introduced into the cell by methods known in the art, including but not limited to various methods of electroporation, transfection and viral transduction. In some embodiments, a patient in need of treatment with the engineered

20 cell is a person who has a disease or condition that can be treated with the engineered cell or who might benefit from treatment with the engineered cell. In some embodiments, the condition is multiple myeloma. In some embodiments, the disease or condition is relapsed/refractory multiple myeloma. In some embodiments, the disease is relapsed multiple myeloma. In some embodiments, the disease is a plasma cell malignancy, for

25 example, diffused large B-cell lymphoma (DLBCL). In certain embodiments, the patient who has received the engineered immune cells is administered an effective amount of rituximab or dasatinib to reduce or eliminate the engineered immune cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] **FIG. 1** shows a schematic of an exemplary engineered CACCR of the disclosure.

30 [0018] **FIG. 2A** shows schematic representations of exemplary recruiting domains of the CACCR of the disclosure that comprise one or more polypeptide subregions of the IL2Rb intracellular domain. **FIGs. 2B-2D** show schematics of exemplary vectors that can be used

to co-express certain combinations of a CACCR and BCMA CAR of the disclosure. FIG. 2B and FIG. 2C each shows a vector that expresses from a promoter a CD8 signal sequence, a CACCR, a P2A peptide, a CD8 signal sequence, and a BCMA-CAR, with or without a mutant WPRE (Woodchuck hepatitis virus Posttranscriptional Regulatory Element), the CACCR depicted comprising TpoR(478-582; H499L; S505N; W515K) and IL2Rb (339-379,393-433,518-551). The schematic displays a BCMA CAR containing the scFv of clone P5A2, with two copies of rituximab mimotopes (R2), 41BB and CD3 zeta. FIG. 2D shows a vector that expresses from a promoter a CD8 signal sequence, a CACCR, a P2A peptide, a CD8 signal sequence, a BCMA CAR, and a mutant WPRE, the CACCR comprising TpoR(478-582; H499L; S505N; W515K) and IL2Rb (393-433,518-551).

[0019] **FIGs. 3A-3C** show that CACCR CAR-T cells bearing truncated IL2Rb cytotails more closely mimic IL-15, rather than IL-2, signaling.

[0020] **FIG. 4** shows that CACCRs improved the cytotoxic activity of TRAC/CD52 dKO CAR-T cells directed towards a liquid tumor target BCMA.

[0021] **FIGs. 5A-5E** show that CACCRs improved short term and long term in vivo anti-tumor activity and persistence of BCMA CAR-T cells against orthotopic multiple myeloma.

[0022] **FIGs. 6A-6E** show potency of CACCR-BCMA TRAC/CD52 dKO CAR T cells on BCMA-expressing target cell lines in vitro.

[0023] **FIGs 7A-7B** show potency of CACCR-BCMA TRAC/CD52 dKO CAR T cells in vivo.

[0024] **FIGs 8A-8C** show anti-tumor activities of two IL2Rb-derived CACCR-BCMA CAR T cells relative to the unmodified BCMA CAR T cells at a dose of either 1×10^6 or 3×10^6 .

[0025] **FIGs. 9A-9C** show CAR+ percentage and yield of T cells bearing CACCR-BCMA CAR as compared to unmodified BCMA CAR, both transduced to T cells in a lentiviral construct.

[0026] **FIGs. 10A-10E** show results of manufacturability and function analyses of CACCR-BCMA CAR T cells bearing the wildtype or mutant WPRE. **FIG. 10F** shows similar results in CAR T cells derived from PBMC.

[0027] **FIGs. 11A-11H** show results of analysis of any effects of WPRE removal from the lentiviral constructs.

[0028] **FIGs. 12A-12D** show results of analysis of the effects of the full-length EF1a and the EF1a short promoters.

[0029] **FIGs. 13A-13G** show results of analysis of the effects of the full-length EF1a and the MND promoters. The data of EF1a promoter-driven BCMA CAR T without a CACCR
5 (open circle) overlapped with the data of MND promoter-driven BCMA CAR T and CACCR (filled square) (FIG. 13F).

[0030] **FIG. 14** shows proliferation profile of CACCR-BCMA CAR T cells upon repeated target exposure as compared to unmodified BCMA CAR T cells.

[0031] **FIGs. 15A-15B** show the sensitivity of CAR T cells to activation-induced cell
10 death (AICD) with increasing exposure to target cells: mWPRE, mutant WPRE; restim, restimulation.

[0032] **FIGs. 16A-16B** show cytokine expression profiles of CAR T cells based on intracellular cytokine staining of Day 7 of the assay.

[0033] **FIG. 17** shows memory phenotype of CAR T cells on Day 15 of the assay.

15 [0034] **FIGs. 18A-18B** show expansion and persistence of BCMA CAR T cells in Molp8-Luc-GFP-bearing mice that had received 5×10^6 CAR T cells.

[0035] **FIGs. 19A-19B** show the overall survival of mice that had received either 1×10^6 or 5×10^6 CAR T cells up to Day 60 (FIG. 19A) and passing Day 100 (FIG. 19B).

20 [0036] **FIG. 20** shows the tumor burden of individual mouse that had received 5×10^6 CAR T cells as shown in FIG. 19.

[0037] **FIG. 21A** depicts data showing target-dependent cytotoxicity of CACCR-BCMA CAR T cells and unmodified BCMA CAR T cells. **FIG. 21B** depicts data showing target-dependent cytokine secretion profiles of CACCR-BCMA CAR T cells and unmodified BCMA CAR T cells.

25 [0038] **FIGs. 22A-22B** show lack of expansion of CACCR-BCMA CAR T cells in the absence of target or exogenous cytokine IL-15 (FIG. 22A) or IL-2 (FIG. 22B). **FIG. 22C** shows results of Ki-67 staining of CAR T cell culture in the absence of target, while **FIG. 22D** shows that the CAR T cells were able to expand in the presence of irradiated target cells (iTarget) and IL2. The data presented are mean $\pm$ SD from three donors.

[0039] **FIG. 23A** shows the expansion profiles of recently activated BCMA CAR T cells that were either continually stimulated with target cells, or had targets withdrawn. **FIGs. 23B-23D** depict data demonstrating that CACCR BCMA CAR T cells could not persist long term in vivo in the absence of target and exerted no negative effects on non-tumor bearing mice. **FIGs. 23E-23F** show results demonstrating that in vivo expansion of CACCR BCMA CAR T cells was target-dependent, and that they returned to quiescence following target clearance. The weight gain of mice receiving BCMA CAR or CACCR BCMA CAR T cells was comparable (**FIG. 23G**).

[0040] **FIGs. 24A-24D** show results of rituximab-mediated depletion assay of BCMA CAR or CACCR BCMA CAR T cells in vitro (**FIGs. 24A-24B**) or in vivo (**FIGs. 24C-24D**).

[0041] **FIGs. 25A-25B** show the effects of the tyrosine kinase inhibitor dasatinib on the activities of BCMA CAR T cells.

[0042] **FIGs. 26A-26C** show that active expansion of CACCR BCMA CAR T cells was target-dependent.

[0043] **FIGs. 27A-27D** show that CACCR improved anti-tumor efficacy and expansion of BCMA CAR T cells in an orthotopic model of multiple myeloma.

DETAILED DESCRIPTION

[0044] The present disclosure provides the combination of a BCMA CAR and constitutively active chimeric cytokine receptors (CACCRs). The BCMA CARs and CACCRs disclosed herein are polypeptides that each comprises domains of different proteins and amino acid sequences, as further detailed herein. The presence of a constitutively active, tunable chimeric cytokine receptor allows for the immune potentiation of Signal 3 to meet the need for immune potentiation. Accordingly, when present on BCMA chimeric antigen receptor (CAR)-bearing immune cells (CAR-I cells, e.g. CAR-T cells) as disclosed herein, such CACCRs allow for increased immune cell activation, proliferation, persistence, and/or potency. Also provided herein are methods of making and using the CACCRs described herein in combination with BCMA specific CARs.

[0045] The CACCRs for use as disclosed herein are tunable, and have flexible cytokine signaling outputs for the enhancement of BCMA CAR-T cell activity, persistence, and the like. The components, methods of making and use are described in turn below.

[0046] I. Constitutively Active Chimeric Cytokine Receptors (CACCRs)

[0047] The CACCRs of the disclosure are composed of two monomers, each monomer comprising: (a) a transmembrane domain; (b) a JAK-binding domain; and (c) a recruiting domain, wherein the monomers are constitutively dimerized. In some embodiments, the
5 CACCR of the disclosure does not comprise an extracellular ligand-binding domain.

[0048] In some embodiments, the monomers are identical, giving rise to a constitutively active homodimer. In such embodiments, the number of proteins that need to be expressed in a vector are reduced. In some embodiments, the monomers are not identical, giving rise to a constitutively active heterodimer, which may be desirable under certain circumstances.

[0049] The monomers of the CACCRs of the disclosure are capable of spontaneously dimerizing, and can activate signaling in the absence of any exogenous stimulation or ligand (ligand-independent dimerization). The level of activity can be controlled by mutations introduced into the transmembrane domain of the CACCRs. A skilled artisan will appreciate that the monomers of the CACCRs are not dimerized 100% of the time, and may
15 exist as a monomer.

A. Transmembrane Domains

[0050] The CACCRs of the disclosure comprise transmembrane domains. The transmembrane domains of the disclosure contain sequences such that they allow for constitutive dimerization of two monomers, thus allowing constitutive JAK activation on
20 the intracellular portion, and constitutive recruitment and phosphorylation of, for example, STAT on the cytoplasmic region of the receptor.

[0051] The transmembrane domains are on the N-terminus and are coupled to intracellular/cytoplasmic domains on the C-terminus. In some embodiments, the coupling is achieved optionally through a linker.

[0052] As used herein, the transmembrane domains are capable of insertion into the membrane of a cell in which it is expressed. In some embodiments, the CACCR transmembrane domains of the disclosure span a cellular membrane, and comprise an extracellular portion, and/or an intracellular portion. In some embodiments, the CACCR transmembrane domains of the disclosure span a cellular membrane, comprise an
30 intracellular portion, and do not comprise an extracellular ligand binding portion.

[0053] In some embodiments, the transmembrane domains of the disclosure are engineered (synthetic) and do not resemble any naturally occurring transmembrane domain, e.g. they are non-naturally occurring.

5 [0054] In other embodiments, the transmembrane domains of the disclosure are derived from naturally occurring receptors.

[0055] In some embodiments, the transmembrane domains and/or JAK-activating domains of the disclosure are derived from, for example, one or more of the following receptors: erythropoietin receptor (EpoR), Interleukin 6 signal transducer (GP130 or IL6ST), prolactin receptor (PrIR), growth hormone receptor (GHR), granulocyte colony-stimulating factor receptor (GCSFR), and thrombopoietin receptor/ myeloproliferative leukemia protein receptor (TPOR/MPLR). When derived from naturally occurring receptors, the entire receptor, or the entire transmembrane sequence of the receptor may not be necessary to effectuate constitutive activation and constitutive JAK binding/activation on the intracellular portion. Accordingly fragments of naturally occurring receptors may be
10 utilized. Furthermore, certain mutations may be introduced into the transmembrane domains derived from naturally occurring receptors, to further tune the downstream signaling.
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[0056] In some embodiments, the transmembrane domain and/or JAK-activating domain of the disclosure is derived from the naturally occurring EpoR receptor.

20 [0057] In some embodiments, the transmembrane domain and/or JAK-activating domain of the disclosure is derived from the naturally occurring GP130 receptor.

[0058] In some embodiments, the transmembrane domain and/or JAK-activating domain of the disclosure is derived from the naturally occurring PrIR receptor.

25 [0059] In some embodiments, the transmembrane domain and/or JAK-activating domain of the disclosure is derived from the naturally occurring GHR receptor.

[0060] In some embodiments, the transmembrane domain and/or JAK-activating domain of the disclosure is derived from the naturally occurring GCSF receptor.

[0061] In some embodiments, the transmembrane domain and/or JAK-activating domain of the disclosure is derived from the naturally occurring TPOR receptor.

30 [0062] Table 1a provides exemplary full-length sequences of naturally occurring receptors provided in the disclosure, from which the transmembrane proteins are derived.

The sequences provided in Table 1a are reference sequences, in relation to which later mutations are expressed, for example in Tables 1b and 1c.

Table 1a: Exemplary Naturally Occurring Receptors

Naturally Occurring Receptor Name	SEQ ID NO:
>AAI12154.1 Erythropoietin receptor [Homo sapiens] MDHLGASLWPQVGSCLLLAGAAWAPPPNLPDPKFESKAALLAARGPEELLCT ERLEDLVCFWEEAASAGVGPNGYSFSYQLEDEPWKLCRLHQAPTARGAVRWC SLPTADTSSFVPLELRVTAASGAPRYHRVIHINEVVLLDAPVGLVARLADESGHV VLRWLPPPETPMTSHIRYEVDSVAGNGAGSVQRVEILEGRTECVLSNLRGRTRYT FAVRARMAEPSFGGFWSAWSEPVSLTSPDLPLILTLSSLVVLVLLTVLALLSH RRALKQKIWP GIPSPSEFEGLFTTHKGNFQLWLYQNDGCLWWSPCTPFTEDPPA SLEVLSERCWGTMQAVEPGTDDEGPLLEPVGSEHAQDTYLVLDKWLLPRNPPSE DLPGP GGSVDIVAMDEGSEASSCSSALASKPSPEGASAA SF EYTILDPSSQLLRPW TLCPELPPTPHLKYLYLVVSDSGISTDYSSGDSQGAQGGLSDGPYSNPYENSLIP AAEPLPPSYVACS	1
>AAI17403.1 Interleukin 6 signal transducer (GP130, oncostatin M receptor) [Homo sapiens] MLTLQTLWLVLQALFILTTESTGELLDPGCGYISPEPVVQLHSNFTAVCVLKEKCM DYFHVNANYIVWKTNHFTIPKEQYTIINRTASSVTFTDIASLNIQLTCNLTFGQLE QNVYGITIISGLPPEKPKNLSCIVNEGKKMRCEWDRGRETHLETNFTLKSEWATH KFADCKAKRDTPTSCTVDYSTVYFVNIEVWVEAENALGKVTSDHINFDPVYKVK PNPPHNLSVINSEELSSILKLTWTNPSIKSVIILKYNIQYRTKDASTWSQIPPEDTAS TRSSFTVQDLKPFTEYVFRIRCMKEDGKGYWSDWSEEASGITYEDRPSKAPSFY KIDPSHTQGYRTVQLVWKTLPPEFANGKILDYEVTLTRWKSHLQNYTVNATKLT VNLTNDRYVATLTVRNLVGKSDAAVLTPACDFQATHPVMDLKAFPKDNMLWV EWTTPRESVKKYILEWCVLSDKAPCITDWQQEDGTVHRTYLRGNLAESKCYLIT VTPVYADGPGSPESIKAYLKQAPPSKGPTVRTKKVGKNEAVLEWDQLPVDVQNG FIRNYTIFYRTIIGNETA VNVDSSTHEYTLSSLTSDTLYMVRMAAYTDEGGKDGPE FTFTTPKFAQGEIEAIVVPVCLAFLLTTLGLVLCFNKRDLIKKHIWPNVPDPKSH IAQWSPHTPPRHNFSKQDQMYSDGNFTDVSVEIEANDKKFPEDLKSLLDFKKE KINTEGHSSGIGGSSCMSSSRPSISSDENESSQNTSSTVQYSTVVHSGYRHQVPSV QVFSRSESTQPLLDSERPEDLQLVDHVDGGDGLPRQQYFKQNCQHESSPDISH FERSKQVSSVNEEDFVRLKQQISDHISQSCGSGQMCMFQEVSAADAFGPGTEGQ VERFETVGMEAATDEGMPKSYLPQTVRQGGYMPQ	2
>XP_011512371.1 prolactin receptor isoform X2 [Homo sapiens] MKENVASATVFTLLFLNTCLLNGQLPPGKPEIFKCRSPNKETFTCWWRPGTDGG LPTNYSLTYHREGETLMHECPDYITGGPNSCHFQYTSMTWRTYIMMVNATNQ MGSSFSDELYVDVTYIVQDPPELAVEVKQPEDRKPYLWIKWSPPTLIDLKTGW FTLLYEIRLKPEKAAEWEIHFAQQTEFKILSLHPGQKYLQVRCKPDHGYWSA WSPATFIQIPSDFTMNDTTVWISVAVLSAVICLIIVWAVALKGYSMVTCIFPPVPGP KIKGFD AHLLEK GKSEELLSALGCQDFPPTS DYEDLLVEYLEVDDSEDQHLMSVH SKEHPSQGMKPTYLDPDTSGRGSCDSPSLLSEKCEEPQANPSTFYDPEVIEKPN PETHTTWDPPQCISMEGKIPYFHAGGSKCSTWPLPQPSQHNPRSSYHNITDVCELA VGPAGAPATLLNEAGKDALKSSQTIKSREEGKATQQREVESFHSETDQDTPWLLP QEKTPFGSAKPLDYVEIHKVNKDGA LSLLPKQRENSGKPKKPGTPENNKEYAKV SGVMDNNILVLVPDPHAKNVACFEESAKEAPPSLEQNQA EKALANFTATSSKCR LQLGGLDYLDPA CFTHSFH	3

Naturally Occurring Receptor Name	SEQ ID NO:
>NP_000154.1 growth hormone receptor isoform 1 precursor [Homo sapiens] MDLWQLLLTLALAGSSDAFSGSEATAAILS RAPWSLQSVNPGLKTNSSKEPKFTK CRSPERETF SCHWTDEVHHGTKNLGPIQLFYTRRNTQEW TQEWKECPDYVSAGE NSCYFNSSFTSIWIPYCIKLT SNGGTVDEKCF SVDEIVQDPPIALNWTLLNVSLTG IHADIQVRWEAPRNADIQKGWMVLEYELQYKEVNETKWKMMDPILTTSPVYS LKVDKEYEVRVR SKQRNSGNYGEFSEVLYVTLPQMSQFTCEEDFYFPWLLIIIFGI FGLTVMLFVFLFSKQQR IKMLILPPVPVKIKGIDPDLLKEGKLEEVNTILAIHDSY KPEFHSDDSWVEFIELDIDEPDEKTEESDTRLLSSDHEKSHSNLGVKDGDSGRTS CCEPDILETDFNANDIHEGTSEVAQPQRLKGEADLLCLDQKNQNNSPYHDACPAT QQPSVIAEKNKPQPLTEGAESTHQA AHIQLSNPSSLSNIDFYAQV SDITPAGSVVLSPGQKNKAGMSQCDMHP EMVSLCQENFLMDNAYFCEADAKKC IPVAPHIKVESHIQPSLNQEDIYITTESLT TAAGRPGTGEHVP GSEMPVPDYTSIHIV QSPQGLILNATALPLPDKEFLSSCGYVSTDQLNKIMP	4
>XP_016855859.1 granulocyte colony-stimulating factor receptor isoform X1 [Homo sapiens] MARLGNCSLTWAALIILLPGSLEECGHISVSAPIVHLGDPITASCIHKQNC SHLDPE PQILWRLGAELQPGGRQQR LSDGTQESIITLPHLNHTQAF LSCCLNWGNSLQILDQ VELRAGYPPAIPHNLSCLMNLT TSSLICQWEPGPETHLPTSFTLKSFKSRGNCQTQ GDSILDGCVPKHLLQSHCCIPRKHLLLYQNMGIWVQAENALGTSMSPQLCLDPM DV VKLEPPMLRTMDPSPEAAPPQAGCLQLCWEPWQPGLHINQKCEL RHKPQRGEAS WALVGPLPLEALQYELCGLLPATAYTLQIRCIRWPLPGHWSDWSPSLELR TTERA PTVRLDTWWRQRQLDPRTVQLFWKVPVPLEEDSGRIQGYV VSWRPSGQAGAILPL CNTTEL SCTFHLPSEAQEVALVAYNSAGTSRTPVVFSES RGPALTRLHAMARDP HSLWVGWEPPNPWPQGYVIEWGLGPPSASNSNKTWRMEQNGRATGFLLKENIR PFQLYEIIVTPLYQDTMGPSQH VYAYSQEMAPSHAPELHLKHIGKTWAQLEWVP EPPELGKSPLTHYTIFWTNAQNQSFSAILNASSRGFVLHGLEPASLYYHIHMAASQ AGATNSTVLTMLTLP EGSELHIILGLFGLLLLLTCLCGTAWLCCSPNRKNPLWPS VPDPAHSSLGSWVP TIMEELPGPRQGQWLGTSEMSRALTPHPCVQDAFQLPGL GTPPITKLTVLEEDEKKVPWESHNSSETCGLPTLVQTYVLQGDPRAVSTQPQSQS GTSDQVLYGQLLGSPTSPGPGHYLRCDSTQPLL AGLTPSPKSYENLWFQASPLGT LVTAPSQEDDCVFGPLLNFPLLQGIRVHGMEALGSF	5
>NP_005364.1 thrombopoietin receptor precursor [Homo sapiens] MPSWALFMVTSCLLAPQNLAQVSSQDVSL LASDSEPLKCF SRTFEDLTCFWDEE EAAPSGTYQLLYAYPREKPRACPLSSQSMPHFGTRYVCQFPDQEEVRLFFPLHLW VKNVFLNQTRTQRVLFVDSVGLPAPPSIIKAMGGSQPGELQISWEEPAP EISDFLR YELRYGPRDPKNSTGPTVIQLIATETCCPALQRPHSASALDQSPCAQPTMPWQDG PKQTSPSREASALTAEGGSCLISGLQPGNSYWLQLRSEPDGISLGGSWGWSL PVT VDLPGDAVALGLQCFTLDLKNVTCQWQQQDHASSQGFFYHSRARC CPRDRYPI WENCEEEEEKTNPGLQTPQFSRCHF KSRNDSIIHILVEVTTAPGTVHSYLGSPFWIH QAVRLPTPNLHWREISSGHLELEWQHPSWAAQETCYQLRYTGEGHQDWKVLE PPLGARGGTLELRPRSRYRLQLRARLNGPTYQGPWSSWSDPTRVETATETAWISL VTALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTA ALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLCSSQAQMDYRRLQPSCLGT MPL SVCPPMAESGSCCTTHIANHSYLPLSYWQQP	6

[0063] In some embodiments, the transmembrane domain of the disclosure is derived from a truncated version of the naturally occurring TPOR/MPLR (myeloproliferative leukemia protein) receptor show in Table 1a.

[0064] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor of Table 1a.

[0065] Table 1b provides exemplary transmembrane domain amino acid sequences of the disclosure, wherein the transmembrane domain is derived from the naturally occurring TPOR receptor. In the sequences listed in Table 1b, the TPOR receptor transmembrane amino acid sequence either comprises the SEQ ID NO: 7 (“naturally occurring transmembrane sequence”) or differs from SEQ ID NO: 7 in comprising one or more amino acid substitutions of SEQ ID NO: 7 (“modified transmembrane sequence”).

Table 1b: Exemplary transmembrane domain amino acid sequences

Transmembrane Domain	Amino acid sequence	SEQ ID NO:
TPOR/MPLR(478-582)	SDPTRVETATETAWISLVTALHLVLGLSAVLGGLL LLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PL	7
TPOR/MPLR(478-582; H499L,S505N)	SDPTRVETATETAWISLVTAL <u>LL</u> VLGL <u>N</u> AVLGGLL LLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PL	8
TPOR/MPLR(478- 582,S505N)	SDPTRVETATETAWISLVTALHLVLGL <u>N</u> AVLGGLL LLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PL	9
TPOR/MPLR(478- 582,H499L,W515K)	SDPTRVETATETAWISLVTAL <u>LL</u> VLGLSAVLGGLL LLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PL	10
TPOR/MPLR(478- 582,W515K)	SDPTRVETATETAWISLVTALHLVLGLSAVLGGLL LLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PL	11
TPOR/MPLR(478- 582,H499L,S505N,W515 K)	SDPTRVETATETAWISLVTAL <u>LL</u> VLGL <u>N</u> AVLGGLL LLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PL	12
TPOR/MPLR(478- 582,S505N,W515K)	SDPTRVETATETAWISLVTALHLVLGL <u>N</u> AVLGGLL LLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PL	13
TPOR/MPLR(478- 582,H499L,G509N)	SDPTRVETATETAWISLVTAL <u>LL</u> VLGLSAVL <u>N</u> LL LLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PL	14

[0066] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and an amino acid substitution at least at H499. In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and the amino acid substitution H499L.

5 [0067] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and an amino acid substitution at least at S505. In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and the amino acid substitution S505N.

10 [0068] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and an amino acid substitution at least at G509. In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and the amino acid substitution G509N.

[0069] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and an amino acid substitution at least at W515.
15 In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and the amino acid substitution W515K.

[0070] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and an amino acid substitution at H499 and S505 (sequence provided in Table 1b).

20 [0071] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and an amino acid substitution at H499 and W515 (sequence provided in Table 1b).

[0072] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and an amino acid substitution at H499, S505,
25 and W515 (sequence provided in Table 1b).

[0073] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and an amino acid substitution at S505 and W515 (sequence provided in Table 1b).

30 [0074] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and an amino acid substitution at H499 and G509 (sequence provided in Table 1b).

[0075] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and the amino acid substitutions H499L and S505N (sequence provided in Table 1b).

5 [0076] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and the amino acid substitutions H499L and W515K (sequence provided in Table 1b).

[0077] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and the amino acid substitutions H499L and G509N (sequence provided in Table 1b).

10 [0078] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and the amino acid substitutions S505N and W515K (sequence provided in Table 1b).

[0079] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and the amino acid substitutions H499L, S505N, and W515K (sequence provided in Table 1b).

[0080] In some embodiments, the transmembrane domain of the CACCR comprises amino acids 478-582 of the TPOR receptor, and an amino acid substitution at H499 and S505 (sequence provided in Table 1b).

20 [0081] The CACCRs of the disclosure are tunable, to achieve the level of Signal 3/immune potentiation required in a BCMA CAR-bearing immune cell (e.g. BCMA CAR-T cell) and desired in a particular context or condition.

[0082] In some embodiments, a low level of STAT5 activation is desired in a BCMA CAR-bearing immune cell (e.g. BCMA CAR-T cell). By way of example, in such embodiments, the transmembrane domain of the CACCR comprising amino acids 478-582 of the TPOR receptor, and the amino acid substitution S505N, W515K, or H499L/G509N may be introduced.

30 [0083] In some embodiments, an increased level of STAT5 activation is desired in a BCMA CAR-bearing immune cell (e.g. BCMA CAR-T cell). By way of example, in such embodiments, the transmembrane domain of the CACCR comprising amino acids 478-582 of the TPOR receptor, and the amino acid substitutions H499L, S505N, and W515K may be introduced. By way of another example, in such embodiments, the transmembrane domain

of the CACCR comprising amino acids 478-582 of the TPOR receptor, and the amino acid substitutions S505N and W515K may be introduced.

[0084] In some embodiments, increased differentiation into memory T cells is desired in a BCMA CAR-bearing immune cell (e.g. BCMA CAR-T cell). By way of example, in such 5 embodiments, the transmembrane domain of the CACCR comprising amino acids 478-582 of the TPOR receptor, and the amino acid substitutions W515K, or H499L/G509N may be introduced.

[0085] In some embodiments, increased differentiation into memory T cells is desired in a BCMA CAR-bearing immune cell (e.g. BCMA CAR-T cell). By way of example, in such 10 embodiments, the transmembrane domain of the CACCR comprising amino acids 478-582 of the TPOR receptor, and the amino acid substitutions S505N/W515K and H499L/S505N/W515K may be introduced.

[0086] Also substitutions to increase cytotoxic potency, durability of response, and increased persistence are provided herein, for example S505N/W515K and 15 H499L/S505N/W515K substitutions.

Table 1c: Exemplary Transmembrane + JAK2 Binding Domain Sequences

Transmembrane and JAK2 binding domain	Amino acid sequence	SEQ ID NO:
GCSFR(614-710)	LTLMTLTPEGSELHIILGLFGLLLLLTCLCGTAWL CCSPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDA FQLPGLGTPPITKLTVLEEDEKKPVPWE	15
GP130(609-700)	TTPKFAQGEIEAIVVPVCLAFLLTTLLGVLFCEFNK RDLIKKHIWPNVPDPSKSHIAQWSPHTPPRHNFN SKDQMYSDGNFTDVSVEIEAND	16
TPOR/MPLR(478-582)	SDPTRVETATETAWISLVTALHLVLGLSAVLGLL LLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PL	17
TPOR/MPLR(N-1)	SDPTRVETATETWISLVTALHLVLGLSAVLGLLL LRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRD TAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLP L	18
TPOR/MPLR(N-2)	SDPTRVETATETISLVTALHLVLGLSAVLGLLLLR WQFPAHYRRLRHALWPSLPDLHRVLGQYLRDT AALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	19
TPOR/MPLR(N-2+1)	SDPTRVETATETLISLVTALHLVLGLSAVLGLLLL RWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDT AALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	20

Transmembrane and JAK2 binding domain	Amino acid sequence	SEQ ID NO:
TPOR/MPLR(N-3)	SDPTRVETATETSLVTALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	21
TPOR/MPLR(N-4)	SDPTRVETATETLVTALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	22
TPOR/MPLR(N-4+1)	SDPTRVETATETILVTALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	23
TPOR/MPLR(N-5)	SDPTRVETATETVTALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	24
TPOR/MPLR(N-6)	SDPTRVETATETTALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	25
TPOR/MPLR(N-7)	SDPTRVETATETALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	26
TPOR/MPLR(N-8)	SDPTRVETATETLHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	27
TPOR/MPLR(N-9)	SDPTRVETATETHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	28
TPOR/MPLR(N-10)	SDPTRVETATETLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	29
TPOR/MPLR(N-11)	SDPTRVETATETVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	30
TPOR/MPLR(N-12)	SDPTRVETATETLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	31
TPOR/MPLR(N-13)	SDPTRVETATETGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	32
TPOR/MPLR(N-14)	SDPTRVETATETLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	33
TPOR/MPLR(N-15)	SDPTRVETATETSAVLGLLLLRWQFPAHYRRLRALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	34
TPOR/MPLR(N-16)	SDPTRVETATETAVLGLLLLRWQFPAHYRRLRALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	35

Transmembrane and JAK2 binding domain	Amino acid sequence	SEQ ID NO:
TPOR/MPLR(N-17)	SDPTRVETATETVLGLLLLRWQFPAHYRRLRHALLWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	36
TPOR/MPLR(N-18)	SDPTRVETATETLGLLLLRWQFPAHYRRLRHALLWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	37
TPOR/MPLR(N+1)	SDPTRVETATETAWLISLVTALHLVLGLSAVLGLLLLLRWQFPAHYRRLRHALLWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	38
TPOR/MPLR(N+2)	SDPTRVETATETAWVLISLVTALHLVLGLSAVLGLLLLLRWQFPAHYRRLRHALLWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	39
TPOR/MPLR(N+3)	SDPTRVETATETAWLVLISLVTALHLVLGLSAVLGLLLLLRWQFPAHYRRLRHALLWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	40
TPOR/MPLR(N+4)	SDPTRVETATETAWILVLISLVTALHLVLGLSAVLGLLLLLRWQFPAHYRRLRHALLWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	41
TPOR/MPLR(N+5)	SDPTRVETATETAWLILVLISLVTALHLVLGLSAVLGLLLLLRWQFPAHYRRLRHALLWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	42
TPOR/MPLR(N+6)	SDPTRVETATETAWLLILVLISLVTALHLVLGLSAVLGLLLLLRWQFPAHYRRLRHALLWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	43
TPOR/MPLR(N+7)	SDPTRVETATETAWVLLILVLISLVTALHLVLGLSAVLGLLLLLRWQFPAHYRRLRHALLWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	44
TPOR/MPLR(N+8)	SDPTRVETATETAWLVLILVLISLVTALHLVLGLSAVLGLLLLLRWQFPAHYRRLRHALLWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	45

B. Janus Kinase (JAK)-Binding Domains

[0087] The CACCRs of the disclosure comprise intracellular JAK-binding domains. The JAK-binding domain is coupled to the C-terminus of the transmembrane domain, either directly, or via a linker. The JAK-binding domain is coupled to the transmembrane domain on the intracellular side of the chimeric cytokine receptor.

[0088] In some embodiments, the JAK-binding domain is a JAK-1-binding domain, a JAK-2 binding domain, a JAK-3 binding domain, or a TYK2 binding domain.

[0089] In some embodiments, the JAK-binding domains of the CACCRs of the disclosure are naturally occurring, and derived from a naturally occurring receptor.

5 [0090] In some embodiments, the JAK-binding domains of the CACCRs of the disclosure are synthetic.

[0091] Table 1b and Table 1c provide exemplary amino acid sequences for transmembrane and JAK2 binding domains of the disclosure. In some embodiments, the CACCR of the disclosure comprises a transmembrane and JAK2 binding domain
10 comprising an amino acid sequence selected from the sequences in Tables 1b and 1c. An amino acid sequence that comprises both a transmembrane and a JAK2 binding domain is a polypeptide that may be referred to herein as a “TM/JAK polypeptide.” Thus, for example, Table 1c lists exemplary TM/JAK polypeptides such as TPOR/MPLR(478-582), and similarly Table 1b lists variants of TPOR/MPLR(478-582) that are also TM/JAK
15 polypeptides, such as TPOR/MPLR(478-582;H499L,S505N,W515K) and TPOR/MPLR(478-582;S505N,W515K). In some embodiments, the CACCR of the disclosure comprises a transmembrane and JAK2 binding domain comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, 99%, or 100% identical to any one of the sequences in Tables 1b and 1c.

20 C. Recruiting Domains

[0092] The CACCRs of the disclosure comprise cytoplasmic recruiting domains. The recruiting domain can be a STAT-recruiting domain, an API1-recruiting domain, a Myc/Max-recruiting domain, or an NFkB-recruiting domain. In some embodiments, the recruiting domain is a Signal Transducer and Activator of Transcription (STAT)-recruiting
25 (STAT-activating) domains, for example, from receptor tails (cytotails) or from cytokine receptor tails. These intracellular recruiting domains of the CACCRs of the disclosure allow for the propagation of Signal 3 in an immune cell comprising a BCMA CAR and a chimeric cytokine receptor (e.g. a BCMA CAR-T cell with a chimeric cytokine receptor of the disclosure). Cytokine signaling propagated through the STAT-recruiting domain allows
30 for the cytokine-based immune potentiation of the cell. In some embodiments, the immune-potentiation is homeostatic, e.g. signaling gives rise to increase in immune cells bearing the CAR. In some embodiments, the immune-potentiation is inflammatory, e.g. signaling gives

rise to an increase in the potency of the immune cells bearing the CAR. In some embodiments, the immune-potential prevents exhaustion, e.g. signaling maintains the long-term functionality of immune cells bearing the CAR.

[0093] In some embodiments, the recruiting domains of the disclosure are synthetic, and do not resemble any naturally occurring receptor fragment. In some embodiments, the immune-potential prevents exhaustion, e.g. signaling maintains the long-term functionality of immune cells bearing the BCMA CAR.

[0094] In some embodiments, the Stat-recruiting domains of the disclosure are synthetic, and do not resemble any naturally occurring receptor fragment.

[0095] In other embodiments, the Stat-recruiting domains of the disclosure are derived from cytoplasmic tails of naturally occurring receptors, e.g. derived from naturally occurring cytokine receptors. These cytoplasmic tails of naturally occurring receptors may be the regions downstream of the JAK-activating domains of the transmembrane domain of the receptor. The Stat-recruiting domains of the chimeric cytokine receptors comprise at least one STAT-recruiting domain from at least one receptor. In some embodiments, the Stat-recruiting domain comprises at least one STAT1-recruiting domain. In some embodiments, the Stat-recruiting domain comprises at least one STAT2-recruiting domain. In some embodiments, the Stat-recruiting domain comprises at least one STAT3-recruiting domain. In some embodiments, the Stat-recruiting domain comprises at least one STAT4-recruiting domain. In some embodiments, the Stat-recruiting domain comprises at least one STAT5-recruiting domain. In some embodiments, the Stat-recruiting domain comprises at least one STAT6-recruiting domain. In some embodiments, the Stat-recruiting domain comprises at least one STAT7-recruiting domain.

[0096] In some embodiments, the naturally occurring receptor from which the Stat-recruiting domain is derived, is not a cytokine receptor.

[0097] In some embodiments, the naturally occurring receptor from which the Stat-recruiting domain is derived, is a cytokine receptor. Exemplary cytokine receptors through which T cell-immune potentiating cytokines signal include, but are not limited to IL-2 receptor, IL-7 receptor, IL-12 receptor, IL-15 receptor and IL-21 receptor. In alternative embodiments, the receptor from which the Stat-recruiting domain is derived, is not a cytokine receptor. By choosing the Stat-recruiting domain of the CACCR, the receptor can be redirected to signaling of choice.

[0098] In some embodiments, the CACCR of the disclosure comprises a recruiting domain connected to the C-terminus of the transmembrane/JAK2 binding domain, with or without a linker. In some embodiments, the linker comprises one or more amino acid residues.

- 5 [0099] Table 2a provides exemplary receptors from which recruiting domains of the CACCRs of the disclosure are derived. Table 2b provides exemplary amino acid sequences of recruiting domains of the disclosure. In some embodiments, the CACCR of the disclosure comprises a recruiting domain comprising the amino acid sequence selected from one or more of the receptor sequences in Table 2b. In some embodiments, the CACCR of
- 10 the disclosure comprises a recruiting domain comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, 99%, or 100% identical to any one of the sequences in Table 2b.

Table 2a Exemplary Receptors

Source of Recruiting Domains
BLNK
IL2RG
EGFR
EpoR
GHR
IFNAR1
IFNAR2
IFNAR1/2
IFNLR1
IL10R1
IL12Rb1
IL12Rb2
IL21R
IL2Rb
IL2small
IL7R
IL7Ra
IL9R
IL15R
IL21R

Table 2b Recruiting Domain (Cytotail) Sequences

Cytotail sequences	Amino acid sequence	SEQ ID NO:
IL7R(316-459)	ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPN CPSEVVITPESFGRDSSLTCLAGNVSACDAPILS SSRSLDCRESGKNGPHVYQDLLLSLGTNSTLPPP FSLQSGILTLNPVAQGGPILTSLSNQEEAYVTMS SFYQNNQ	46
IL2Rb(333-551)	VTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFF FHLPDALIEACQVYFTYDPYSEEDPDEGVAGAP TGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLL GGPSPSTAPGGSGAGEERMPPSLQERVPRDWD QPLGPPTPGVPDLVDFQPPPELVREAGEEVPDA GPREGVSFPWSRPPGQGEFRALNARLPLNTDAYL SLQELQGQDPHTLV	47
IFNAR1(508-557)	ISTIATVEETNQTDHKKYSSQTSQDSGNYSNE DESESKTSEELQQDFV	48
IFNAR2(310-515)	KKKVWDYNYDDESDSDTEAAPRTSGGGYTMHG LTVRPLGQASATSTESQLIDPESEEEPDLPEDVE LPTMPKDSPPQLELLSGPCERRKSPLQDPFPEEDY SSTEGSGGRITFNVDLNSVFLRVLDDESDDDLEA PLMLSSHLEEMVDPEDPDNVQSNHLLASGEGTQ PTFPSPSSEGLWSEDAPSDQSDTSESVDLGDGYI MR	49
IFNAR1/2(IFNAR1 residues 508-557-IFNAR2 residues 310-515)	ISTIATVEETNQTDHKKYSSQTSQDSGNYSNE DESESKTSEELQQDFVKKKVWDYNYDDESDSDT EAAPRTSGGGYTMHGLTVRPLGQASATSTESQLI DPSEEEEPDLPEVDVELPTMPKDSPPQLELLSGPC ERRKSPLQDPFPEEDYSSTEGSGGRITFNVDLNSV FLRVLDDESDDDLEAPLMLSSHLEEMVDPEDPD NVQSNHLLASGEGTQPTFPSPSSEGLWSEDAPSD QSDTSESVDLGDGYIMR	50
IFNLR1(300-520)	RGVRPTPRVRAPATQQTRWKKDLAEDEEEDEE DTEDGVSFQPYIEPPSFLGQEHQAPGHSEAGGVD SGRPRAPLPVSEGSSAWDSSDRSWASTVDSSWD RAGSSGYLAEKGPQGPGGDGHQESLPPPEFSKD SGFLEELPEDNLSSWATWGTLPEPNLVPGGPPV SLQTLTFCWESSPEEEEEARESEIEDSDAGSWGAE STQRTEDRGRITLGHYMAR	51
IL2RG (335-369)	IPPKGGALGEGPGASPCNQHSPYWAPPCYTLKPE T	52
IL9R(356-521)	TALLTCGPARPWKSVALEEEQEGPGTRLPGNLSS EDVLPAGCTEWRVQTLAYLPQEDWAPTSLTRPA PPDSEGSRSSSSSSSSNNNNYCALGCYGGWHLA LPGNTQSSGPIPALACGLSCDHQGLTQQGVAV VLAGHCQRPLHEDLQGMLLPSVLSKARSWTF	53
IL21R(322-538)	PRSPAKRLQLTELQEPaelVESDGVKPSFWPTA QNSGGSAYSEERDRPYGLVSIDTVTVLDAEGPCT	54

Cytotail sequences	Amino acid sequence	SEQ ID NO:
	WPCSCEDDGYPALDLDAGLEPSPGLEDPLLDAG TTVLSCGCVSAGSPGLGGPLGSLDRLKPPLADG EDWAGGLPWGGRSPGGVSESEAGSPLAGLDMD TFDSGFVGSDCSSPVECDFTSPGDEGPFRSYLRQ WVVIPLPLSSPGPQAS	
GHR(353-638)	PDEKTEESDTRLLSSDHEKSHSNLGVKDGDGSGR TSCCEPDILETDFNANDIHEGTSEVAQPQRLKGE ADLLCLDQKNQNNSPYHDACPATQQPSVIAEK NKPQPLPTEGAESTHQAHIQLSNPSSLSNIDFYA QVSDITPAGSVVLSPGQKNKAGMSQCDMHPM VSLCQENFLMDNAYFCEADAKKCIPVAPHIKVES HIQPSLNQEDIYITTESLTTAAGRPGTGEHVPGE MPVPDYTSIHIVQSPQGLILNATALPLPDKEFLSS CGYVSTDQLNKIMP	55
EpoR(339-508)	WGTMQAVEPGTDDEGPLLEPVGSEHAQDTYLVL DKWLLPRNPPSEDLPGPGGSVDIVAMDEGSEASS CSSALASKPSPEGASAAFEYITLDPSSQLLRPWT LCPELPPTPPHLKYLVLVSDSGISTDYSSGDSQG AQGGLSDGPYSNPYENSLIPAAEPLPPSYVACS	56
murine IL2Rb(337-539)	AVQLLLLQKDSAPLPSPSGHSQASCFTNQGYFFF HLPNALEIESCQVYFTYDPCVEEEVEEDGSRLPE GSPHPPLLPLAGEQDDYCAFPDRDDLLLFSPSLST PNTAYGGSRAPEERSPLSLHEGLPSLASRDLMLG QRPLERMPEGDGEGLSANSSGEQASVPEGNLHG QDQDRGQGPIILTLNTDAYLSLQELQAQDSVHLI	57
murine IL7Ra(316-459)	ARDEVESFLPNDLPAQPEELETQGHRAAVHSAN RSPETSVSPPETVRRESPLRCLARNLSTCNAPPLL SSRSPDYRDGDRNRPPVYQDLLPNSGNTNVPVPV PQPLPFQSGILIPVSQRQPISTSSVLNQEEAYVTMS SFYQNK	58
EGFR(955-1186)	VIQGDERMHLPSPTDNSNFYRALMDEEDMDDVVD ADEYLIPQQGFFSSPSTSRTPLLSSLSATSNNSTVA CIDRNGLQSCPIKEDSFLQRYSSDPTGALTEDSID DTFLPVPEYINQSVPKRPAGSVQNPVYHNQPLNP APSRDPHYQDPHSTAVGNPEYLNTVQPTCVNSTF DSPAHWAQKGSHQISLDNPDYQQDFFPKEAKPN GIFKGSTAENAAYLRVAPQSSEFIGA	59
EGFR(955-1186;Y974F,d1045-1057)	VIQGDERMHLPSPTDNSNFFRALMDEEDMDDVVD ADEYLIPQQGFFSSPSTSRTPLLSSLSATSNNSTVA CIDRNGLQSCPIKEDSFLQRIDDTFLPVPEYINQSV PKRPAGSVQNPVYHNQPLNPAPSRDPHYQDPHS TAVGNPEYLNTVQPTCVNSTFDSPAHWAQKGSH QISLDNPDYQQDFFPKEAKPNGIFKGSTAENAAY LRVAPQSSEFIGA	60
EGFR(955-1009;Y974F)	VIQGDERMHLPSPTDNSNFFRALMDEEDMDDVVD ADEYLIPQQGFFSSPSTSRTP	61

Cytotail sequences	Amino acid sequence	SEQ ID NO:
EGFR(1019-1085)	NNSTVACIDRNGLQSCPIKEDSFLQRIDDTFLPVP EYINQSVPKRPAGSVQNPV	62
EGFR(1037- 1103;Y1068/1101F,d1045 -1057)	KEDSFLQRIDDTFLPVPFINQSVPKRPAGSVQNP VYHNQPLNPAPSRDPHFQD	63
EGFR(1066- 1118;Y1068/1086F)	VPEFINQSVPKRPAGSVQNPVFNQPLNPAPSRD PHYQDPHSTAVGNPEYLNTV	64
EGFR(1122-1165)	PEYLNTVQPTCVNSTFDSPAHWAKGSHQISLDN PDYQQDFFPKEAKPNGIFKG	65
EGFR(1133- 1186;Y1148F)	WAQKGSHQISLDNPDFQQDFFPKEAKPNGIFKGS TAENAEYLRVAPQSSEFIGA	66
IL12Rb2(775-825)	SDPKPENPACPWTVLPAGDLPTHGGLPSNIDDL PSHEAPLADSLEELPQ	67
IL7Ra(376-416)	ACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLG TTNSTLP	68
IL7Ra(424-459)	GILTLNPVAQQQPILTSLGSNQEEAYVTMSSFYQ NQ	69
IL7Ra(376-416,424-459)	ACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLG TTNSTLPQQQPILTSLGSNQEEAYVTMSSFYQ	70
IL7Ra(424-459;Y456F)	GILTLNPVAQQQPILTSLGSNQEEAYVTMSSFFQN Q	71
IL7R(376-416,424- 459,Y456F)	ACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLG TTNSTLPQQQPILTSLGSNQEEAYVTMSSFFQNQ	72
IL2Rb _{small} (393-433)	DEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDD LLFSPSGQGEFRALNARLPLNTDAYLSLQELQG QDPHTLV	73
IL2Rb _{small} (518-551)	GQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV	74
IL2Rb _{small} (339-379,393- 433)	QQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDA LEIEACQDEGVAGAPTGSSPQPLQPLSGEDDAYC TFPSRDDLLFSPS	75
IL2Rb _{small} (339-379,518- 551)	QQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDA LEIEACQ GQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV	76
IL2Rb _{small} (393-433,518- 551)	DEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDD LLFSPSGQGEFRALNARLPLNTDAYLSLQELQG QDPHTLV	77
IL2Rb _{small} (339-379,393- 433,518-551)	QQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDA LEIEACQDEGVAGAPTGSSPQPLQPLSGEDDAYC TFPSRDDLLFSPSGQGEFRALNARLPLNTDAYLS LQELQGQDPHTLV	78
IFNAR2 _{small} (310-352)	KKKVWDYNYDDESDDTEAAPRTSGGGYTMHG LTVRPLGQASA	79
IFNAR2 _{small} (486-515)	EGLWSEDAPSDQSDTSESVDLGDGYIMR	80

Cytotail sequences	Amino acid sequence	SEQ ID NO:
IFNAR2small(310-352,486-515)	KKKVWDYNYDDESDSDTEAAPRTSGGGYTMHG LTVRPLGQASA EGLWSEDAPSDQSDTSESVDLGDGYIMR	81
BLNK(53-208)	ASESPADEEEQWSDDFDSDYENPDEHSDSEMYV MPAEENADDSYEPPPVEQETRPVHPALPFARGEY IDNRSSQRHSPPFSTLPSKPSWPSEKARLTSTLP ALTALQKPQVPPKPKGLLEDEADYVVPVEDNDE NYIHPTESSPPPEKAPMVNR	82
BLNK(53-208;Y72F)	ASESPADEEEQWSDDFDSDFENPDEHSDSEMYV MPAEENADDSYEPPPVEQETRPVHPALPFARGEY IDNRSSQRHSPPFSTLPSKPSWPSEKARLTSTLP ALTALQKPQVPPKPKGLLEDEADYVVPVEDNDE NYIHPTESSPPPEKAPMVNR	83
BLNK(53-208;Y72F,Y96F)	ASESPADEEEQWSDDFDSDFENPDEHSDSEMYV MPAEENADDSFEPPPVEQETRPVHPALPFARGEY IDNRSSQRHSPPFSTLPSKPSWPSEKARLTSTLP ALTALQKPQVPPKPKGLLEDEADYVVPVEDNDE NYIHPTESSPPPEKAPMVNR	84
EpoR(339-508)	WGTMQAVEPGTDDDEGPLLEPVGSEHAQDTYLVL DKWLLPRNPPSEDLPGPGGSVDIVAMDEGSEASS CSSALASKPSPEGASAAAFETYILDPSSQLLRPWT LCPELPPTPHLKLYLVVSDSGISTDYSSGDSQG AQGGLSDGPYSNPYENSLIPAAEPLPPSYVACS	85
IL12Rb2(714-862)	VTPVFRHPPCSNWPQREKGIQGHQASEKMMHS ASSPPPPRALQAESRQLVDLYKVLESRGSDPKPE NPACPWTVLPAGDLPTHGYPNIDDLPSHEAP LADSLEELEPQHISLSVFPSSSLHPLTFSCGDKLTL DQLKMRCDSLML	86
IL12Rb1(622-662)	WDKGERTEPLEKTELPEGAPELALDTELSLEDGD RCKAKM	87
IL10R1(304-578)	VSPKLNLDLHGSTDGFGSTKPSLQTEEPQFLLP DHPHQADRTLGNREPPVLGDSCSSGSSNSTDSGIC LQEPSLSPSTGPTWEQQVGSNSRGQDDSGIDL VQ NSEGRAGDTQGGGALGHHSPPEPEVPGEEDPAA VAFQGYLRQTRCAEEKATKTGCLEESPLTDGL GPKFGRCLVDEAGLHPPALAKGYLKQDPLEMTL ASSGAPTQWNQPTTEWSLLALSSCDLGISDWS FAHDLAPLGCVAAPGGLLGSFNSDLVTLPLISSLQ SSE	88
IL2Rb(333-551, Y381S,Y384S,Y387S)	VTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFF FHLPPDALEIEACQVSTSDPSSEEDPDEGVAGAPT GSSPQPLQPLSGEDDAYCTFSPSRDDLFLFSPSLLG GPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPO PLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAG PREGVSFPWSRPPGQGEFRALNARLPLNTDAYLS LQELQGQDPHTLV	106

Cytotail sequences	Amino acid sequence	SEQ ID NO:
IL2Rb(333-551, Y364S,Y381S,Y384S,Y387S)	VTQLLLQQDKVPEPASLSSNHSLTSCFTNQGSFFF HLPDALEIEACQVSFTSDPSSEEDPDEGVAGAPTG SSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGG PSPPTAPGGSGAGEERMPPSLQERVPRDWDQP LGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGP REGVSFPWSRPPGQGEFRALNARLPLNTDAYLSL QELQGQDPHTLV	142

[0100] In some embodiments, the Stat-recruiting domain of a CACCR of the disclosure comprises a STAT-recruiting domain from one receptor.

[0101] In order to generate multiple outputs, two or more STAT-recruiting domains may be joined in tandem to mimic signaling from one or more cytokines.

[0102] In some embodiments, the two or more STAT-recruiting domains may be joined in tandem with or without a linker. In some embodiments, the linker comprises one or more amino acid residues.

[0103] In some embodiments, the STAT-recruiting domain comprises portions of more than one receptor, e.g. comprising more than one STAT-recruiting domain. In such embodiments, a tandem cytokine signaling domain is provided, allowing for enhanced signaling. Accordingly, in some embodiments, the STAT-recruiting domain of a monomer of the CACCR of the disclosure comprises the STAT-recruiting domains from more than one receptor, e.g. comprises the STAT-recruiting domains from two, three, four, five, or even six receptors. For example, in some embodiments, STAT-recruiting domains can be linked in tandem to stimulate multiple pathways (e.g., the IL7R(316-459)-IL12Rb2(775-825) fragment fusion for pro-persistence STAT5 and pro-inflammatory STAT4; IL7R(316-459)-IL2Rbsmall(393-433,518-551) for pro-persistence; IL7R(316-459)-EGFR(1122-1165) for pro-persistence and anti-exhaustion; IL2Rbsmall(393-433,518-551)-EGFR(1122-1165) for pro-persistence and anti-exhaustion).

[0104] When generating multiple outputs, the proximity of individual STAT-recruiting domains to the cell membrane can influence the strength of their respective signaling outputs. Table 2c shows examples of CACCRs with the dual outputs, where each output can be placed either proximal or distal to the cell membrane. In some embodiments, the CACCRs of the disclosure comprise a recruiting domain with dual outputs selected from Table 2c.

Table 2c: Examples of CACCRs with dual outputs

Dual output STAT-recruiting domain	Membrane proximal	Membrane distal
IL2Rbsmall(393-433,518-551)/ IL21R(322-538)	IL2Rbsmall(393-433,518-551)	IL21R(322-538)
IL2Rb(333-551)/ IL21R(322-538)	IL2Rb(333-551)	IL21R(322-538)
IL21R(322-538)/ IL2Rbsmall(393-433,518-551)	IL21R(322-538)	IL2Rbsmall(393-433,518-551)
IL21R(322-538)/ IL2Rb(333-551)	IL21R(322-538)	IL2Rb(333-551)
IL2Rbsmall(339-379,393-433,518-551)/IL21R(322-538)	IL2Rbsmall(339-379,393-433,518-551)	IL21R(322-538)
IL21R(322-538)/ IL2Rbsmall(339-379,393-433,518-551)	IL21R(322-538)	IL2Rbsmall(339-379,393-433,518-551)
IL2Rb(333-551)/ IL12Rb1(622-662)	IL2Rb(333-551)	IL12Rb1(622-662)
IL2Rbsmall(393-433,518-551)/ IL12Rb1(622-662)	IL2Rbsmall(393-433,518-551)	IL12Rb1(622-662)
IL2Rbsmall(339-379,393-433,518-551)/IL12Rb1(622-662)	IL2Rbsmall(339-379,393-433,518-551)	IL12Rb1(622-662)
IL12Rb1(622-662)/ IL2Rb(333-551)	IL12Rb1(622-662)	IL2Rb(333-551)
IL12Rb1(622-662)/IL2Rbsmall(393-433,518-551)	IL12Rb1(622-662)	IL2Rbsmall(393-433,518-551)

Dual output STAT-recruiting domain	Membrane proximal	Membrane distal
IL12Rb1(622-662)/IL2Rbsmall(339-379,393-433,518-551)	IL12Rb1(622-662)	IL2Rbsmall(339-379,393-433,518-551)
IL2Rb(333-551)/ IL12Rb2(714-862)	IL2Rb(333-551)	IL12Rb2(714-862)
IL2Rbsmall(393-433,518-551)/ IL12Rb2(714-862)	IL2Rbsmall(393-433,518-551)	IL12Rb2(714-862)
IL2Rbsmall(339-379,393-433,518-551)/IL12Rb2(714-862)	IL2Rbsmall(339-379,393-433,518-551)	IL12Rb2(714-862)
IL2Rb(333-551)/ IL12Rb2(775-825)	IL2Rb(333-551)	IL12Rb2(775-825)
IL2Rbsmall(393-433,518-551)/ IL12Rb2(775-825)	IL2Rbsmall(393-433,518-551)	IL12Rb2(775-825)
IL2Rbsmall(339-379,393-433,518-551)/IL12Rb2(775-825)	IL2Rbsmall(339-379,393-433,518-551)	IL12Rb2(775-825)
IL12Rb2(714-862)/ IL2Rb(333-551)	IL12Rb2(714-862)	IL2Rb(333-551)
IL12Rb2(714-862)/IL2Rbsmall(393-433,518-551)	IL12Rb2(714-862)	IL2Rbsmall(393-433,518-551)
IL12Rb2(714-862)/IL2Rbsmall(339-379,393-433,518-551)	IL12Rb2(714-862)	IL2Rbsmall(339-379,393-433,518-551)
IL12Rb2(775-825)/ IL2Rb(333-551)	IL12Rb2(775-825)	IL2Rb(333-551)

Dual output STAT-recruiting domain	Membrane proximal	Membrane distal
IL12Rb2(775-825)/IL2Rb2small(393-433,518-551)	IL12Rb2(775-825)	IL2Rb2small(393-433,518-551)
IL12Rb2(775-825)/IL2Rb2small(339-379,393-433,518-551)	IL12Rb2(775-825)	IL2Rb2small(339-379,393-433,518-551)
IL7Ra (316-459)/IL21R(322-538)	IL7Ra (316-459)	IL21R(322-538)
IL7Ra (376-416, 424-459, Y456F)/IL21R(322-538)	IL7Ra (376-416, 424-459, Y456F)	IL21R(322-538)
IL21R(322-538)/IL7Ra (316-459)	IL21R(322-538)	IL7Ra (316-459)
IL21R(322-538)/IL7Ra(376-416, 424-459, Y456F)	IL21R(322-538)	IL7Ra (376-416, 424-459, Y456F)
IL7Ra (316-459)/IL12Rb1(622-662)	IL7Ra (316-459)	IL12Rb1(622-662)
IL7Ra (376-416, 424-459, Y456F)/IL12Rb1(622-662)	IL7Ra (376-416, 424-459, Y456F)	IL12Rb1(622-662)
IL7Ra (316-459)/IL12Rb2(714-862)	IL7Ra (316-459)	IL12Rb2(714-862)
IL7Ra (376-416, 424-459, Y456F)/IL12Rb2(714-862)	IL7Ra (376-416, 424-459, Y456F)	IL12Rb2(714-862)
IL7Ra (316-459)/IL12Rb2(775-825)	IL7Ra (316-459)	IL12Rb2(775-825)
IL7Ra (376-416, 424-459, Y456F)/IL12Rb2(775-825)	IL7Ra (376-416, 424-459, Y456F)	IL12Rb2(775-825)
IL12Rb1(622-662)/ IL7Ra (316-459)	IL12Rb1(622-662)	IL7Ra (316-459)
IL12Rb1(622-662)/IL7Ra (376-416, 424-459, Y456F)	IL12Rb1(622-662)	IL7Ra (376-416, 424-459, Y456F)

Dual output STAT-recruiting domain	Membrane proximal	Membrane distal
IL12Rb2(714-862)/ IL7Ra (316-459)	IL12Rb2(714-862)	IL7Ra (316-459)
IL12Rb2(714-862)/IL7Ra (376-416, 424-459, Y456F)	IL12Rb2(714-862)	IL7Ra (376-416, 424-459, Y456F)
IL12Rb2(775-825)/ IL7Ra (316-459)	IL12Rb2(775-825)	IL7Ra (316-459)
IL12Rb2(775-825)/IL7Ra (376-416, 424-459, Y456F)	IL12Rb2(775-825)	IL7Ra (376-416, 424-459, Y456F)
IL7Ra (316-459)/IL2Rb(333-551)	IL7Ra (316-459)	IL2Rb(333-551)
IL7Ra (376-416, 424-459, Y456F)/IL2Rb(333-551)	IL7Ra (376-416, 424-459, Y456F)	IL2Rb(333-551)
IL7Ra (316-459)/IL2Rbsmall(393-433, 518-551)	IL7Ra (316-459)	IL2Rbsmall(393-433, 518-551)
IL7Ra (376-416, 424-459, Y456F)/IL2Rbsmall(393-433, 518-551)	IL7Ra (376-416, 424-459, Y456F)	IL2Rbsmall(393-433, 518-551)
IL7Ra (316-459)/IL2Rbsmall(339-379, 393-433, 518-551)	IL7Ra (316-459)	IL2Rbsmall(339-379, 393-433, 518-551)
IL7Ra (376-416, 424-459, Y456F)/IL2Rbsmall(339-379, 393-433, 518-551)	IL7Ra (376-416, 424-459, Y456F)	IL2Rbsmall(339-379, 393-433, 518-551)
IL2Rb(333-551)/IL7Ra (316-459)	IL2Rb(333-551)	IL7Ra (316-459)
IL2Rb(333-551)/IL7Ra (376-416, 424-459, Y456F)	IL2Rb(333-551)	IL7Ra (376-416, 424-459, Y456F)
IL2Rbsmall(393-433, 518-551)/IL7Ra (316-459)	IL2Rbsmall(393-433, 518-551)	IL7Ra (316-459)
IL2Rbsmall(393-433, 518-551)/IL7Ra (376-416, 424-459, Y456F)	IL2Rbsmall(393-433, 518-551)	IL7Ra (376-416, 424-459, Y456F)

Dual output STAT-recruiting domain	Membrane proximal	Membrane distal
IL2Rb _{small} (339-379, 393-433, 518-551)/IL7Ra (316-459)	IL2Rb _{small} (339-379, 393-433, 518-551)	IL7Ra (316-459)
IL2Rb _{small} (339-379, 393-433, 518-551)/IL7Ra (376-416, 424-459, Y456F)	IL2Rb _{small} (339-379, 393-433, 518-551)	IL7Ra (376-416, 424-459, Y456F)
IL12Rb1(622-662)/ IL21R (322-538)	IL12Rb1(622-662)	IL21R(322-538)
IL12Rb2(714-862)/ IL21R (322-538)	IL12Rb2(714-862)	IL21R(322-538)
IL12Rb2(775-825)/ IL21R (322-538)	IL12Rb2(775-825)	IL21R(322-538)
IL21R (322-538)/ IL12Rb1(622-662)	IL21R(322-538)	IL12Rb1(622-662)
IL21R (322-538)/ IL12Rb2(714-862)	IL21R(322-538)	IL12Rb2(714-862)
IL21R (322-538)/ IL12Rb2(775-825)	IL21R(322-538)	IL12Rb2(775-825)

[0105] Without being bound to theory or mechanism, in some embodiments, a JAK-protein (JAK1, JAK2, JAK3, or TYK2) is bound to a dimerized CACCR of the disclosure. The two bound JAK-proteins are activated, which are capable of phosphorylating tyrosine residues on the recruiting domain of the CACCR. The phosphorylated recruiting domains are then capable of binding the recruited proteins (e.g. a phosphorylated STAT-recruiting domain binds a STAT-protein), which in turn effectuate transcription events in the nucleus.

D. Exemplary CACCRs

[0106] Table 3 shows exemplary CACCR sequences of the disclosure. The receptors may be expressed with a signal sequence, e.g. a CD8SS of sequence MALPVTALLPLALLHAARP (SEQ ID NO: 89).

[0107] In some embodiments, the CACCR of the disclosure comprises any one of the sequences in Table 3. In some embodiments, the CACCR comprises an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to any one of the amino acid sequences of SEQ ID NO: 90-98, and 107-139. In some

embodiments, the CACCR of the disclosure comprises any one of the amino acid sequences of SEQ ID NO: 90-100, 107-139, 143, and 180-186.

[0108] In some embodiments, the CACCR comprises the transmembrane domain and/or JAK-binding domain derived from the TPOR/MPLR receptor. In some embodiments, the CACCR of the disclosure comprises amino acids 478 – 582 of the naturally occurring TPOR/MPLR receptor of SEQ ID NO: 6. In some embodiments, the CACCR of the disclosure comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the CACCR of the disclosure comprises the amino acid sequence of SEQ ID NO: 17. In some embodiments, the CACCR further comprises a recruiting domain comprising the amino acid sequence of one or more of the receptor sequences presented in Table 2b. In some embodiments, the CACCR further comprises one or more recruiting domains selected from the group consisting of the STAT-recruiting domains from IL7Ra, IL2Rb, IL12Rb1, IL12Rb2, and IL21R. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL7Ra. In some embodiments, the STAT-recruiting domain from IL7Ra comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 46, 68, 69, 70, 71 or 72. In some embodiments, the STAT-recruiting domain from IL7Ra comprises the amino acid sequence of SEQ ID NO: 46, 68, 69, 70, 71 or 72. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL2Rb. In some embodiments, the STAT-recruiting domain from IL2Rb comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 47, 73, 74, 75, 76, 77, 78, 106, or 142. In some embodiments, the STAT-recruiting domain from IL2Rb comprises the amino acid sequence of SEQ ID NO: 47, 73, 74, 75, 76, 77, 78, 106 or 142. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL12Rb1 or IL12Rb2. In some embodiments, the STAT-recruiting domain from IL12Rb1 or IL12Rb2 comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 67, 86, or 87. In some embodiments, the STAT-recruiting domain from IL12Rb1 or IL12Rb2 comprises the amino acid sequence of SEQ ID NO: 67, 86, or 87. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL21R. In some embodiments, the STAT-recruiting domain from IL21R comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%,

96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 54. In some embodiments, the STAT-recruiting domain from IL21R comprises the amino acid sequence of SEQ ID NO: 54. In some embodiments, the CACCR comprises one or more recruiting domains presented in Table 2c. In some embodiments, the recruiting domains comprises the
5 STAT-recruiting domains from IL7Ra and IL2Rb. In some embodiments, the recruiting domain comprises the STAT-recruiting domains from IL7Ra and IL12Rb1. In some embodiments, the recruiting domain comprises the STAT-recruiting domains from IL7Ra and IL12Rb2. In some embodiments, the CACCR comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid
10 sequence of SEQ ID NO: 90 or 119, with or without a signal sequence. In some embodiments, the CACCR comprises the amino acid sequence of SEQ ID NO: 90 or 119, with or without a signal sequence.

[0109] In some embodiments, the CACCR of the disclosure comprises the transmembrane domain and/or JAK-binding domain from a TPOR/MPLR receptor that
15 comprises one or more amino acid substitutions at H499, S505, G509 or W515. In some embodiments, the TPOR/MPLR receptor comprises a H499L substitution. In some embodiments, the TPOR/MPLR receptor comprises a S505N substitution. In some embodiments, the TPOR/MPLR receptor comprises a G509N substitution. In some embodiments, the TPOR/MPLR receptor comprises a W515K substitution. In some
20 embodiments, the CACCR further comprises a recruiting domain comprising the amino acid sequence of one or more of the receptor sequences presented in Table 2b. In some embodiments, the CACCR further comprises one or more recruiting domains selected from the group consisting of the STAT-recruiting domains from IL7Ra, IL2Rb, IL12Rb1, IL12Rb2, and IL21R. In some embodiments, the recruiting domain comprises the STAT-
25 recruiting domain from IL7Ra. In some embodiments, the STAT-recruiting domain from IL7Ra comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 46, 68, 69, 70, 71 or 72. In some embodiments, the STAT-recruiting domain from IL7Ra comprises the amino acid sequence of SEQ ID NO: 46, 68, 69, 70, 71 or 72. In some embodiments, the recruiting
30 domain comprises the STAT-recruiting domain from IL2Rb. In some embodiments, the STAT-recruiting domain from IL2Rb comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 47, 73, 74, 75, 76, 77, 78, 106, or 142. In some embodiments, the STAT-

recruiting domain from IL2Rb comprises the amino acid sequence of SEQ ID NO: 47, 73, 74, 75, 76, 77, 78, 106 or 142. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL12Rb1 or IL12Rb2. In some embodiments, the STAT-recruiting domain from IL12Rb1 or IL12Rb2 comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 67, 86, or 87. In some embodiments, the STAT-recruiting domain from IL12Rb1 or IL12Rb2 comprises the amino acid sequence of SEQ ID NO: 67, 86, or 87. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL21R. In some embodiments, the STAT-recruiting domain from IL21R comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 54. In some embodiments, the STAT-recruiting domain from IL21R comprises the amino acid sequence of SEQ ID NO: 54. In some embodiments, the CACCR comprises one or more recruiting domains presented in Table 2c. In some embodiments, the recruiting domains comprises the STAT-recruiting domains from IL7Ra and IL2Rb. In some embodiments, the recruiting domain comprises the STAT-recruiting domains from IL7Ra and IL12Rb1. In some embodiments, the recruiting domain comprises the STAT-recruiting domains from IL7Ra and IL12Rb2. In some embodiments, the CACCR comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 92, 94, 121, or 123, with or without a signal sequence. In some embodiments, the CACCR comprises the amino acid sequence of SEQ ID NO: 92, 94, 121, or 123, with or without a signal sequence.

[0110] In some embodiments, the CACCR of the disclosure comprises the transmembrane domain and/or JAK-binding domain from a TPOR/MPLR receptor that comprises the H499L and S505N substitutions. In some embodiments, the CACCR further comprises a recruiting domain comprising the amino acid sequence of one or more of the receptor sequences presented in Table 2b. In some embodiments, the CACCR further comprises one or more recruiting domains selected from the group consisting of the STAT-recruiting domains from IL7Ra, IL2Rb, IL12Rb1, IL12Rb2, and IL21R. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL7Ra. In some embodiments, the STAT-recruiting domain from IL7Ra comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 46, 68, 69, 70, 71 or 72. In some embodiments, the

STAT-recruiting domain from IL7Ra comprises the amino acid sequence of SEQ ID NO: 46, 68, 69, 70, 71 or 72. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL2Rb. In some embodiments, the STAT-recruiting domain from IL2Rb comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 47, 73, 74, 75, 76, 77, 78, 106, or 142. In some embodiments, the STAT-recruiting domain from IL2Rb comprises the amino acid sequence of SEQ ID NO: 47, 73, 74, 75, 76, 77, 78, 106 or 142. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL12Rb1 or IL12Rb2. In some embodiments, the STAT-recruiting domain from IL12Rb1 or IL12Rb2 comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 67, 86, or 87. In some embodiments, the STAT-recruiting domain from IL12Rb1 or IL12Rb2 comprises the amino acid sequence of SEQ ID NO: 67, 86, or 87. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL21R. In some embodiments, the STAT-recruiting domain from IL21R comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 54. In some embodiments, the STAT-recruiting domain from IL21R comprises the amino acid sequence of SEQ ID NO: 54. In some embodiments, the CACCR comprises one or more recruiting domains presented in Table 2c. In some embodiments, the recruiting domains comprises the STAT-recruiting domains from IL7Ra and IL2Rb. In some embodiments, the recruiting domain comprises the STAT-recruiting domains from IL7Ra and IL12Rb1. In some embodiments, the recruiting domain comprises the STAT-recruiting domains from IL7Ra and IL12Rb2. In some embodiments, the CACCR comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 91, 98, 120, or 127, with or without a signal sequence. In some embodiments, the CACCR comprises the amino acid sequence of SEQ ID NO: 91, 98, 120, or 127, with or without a signal sequence.

[0111] In some embodiments, the CACCR of the disclosure comprises the transmembrane domain and/or JAK-binding domain from a TPOR/MPLR receptor that comprises the H499L and W515K substitutions or the H499L and G509N substitutions. In some embodiments, the CACCR further comprises a recruiting domain comprising the amino acid sequence of one or more of the receptor sequences presented in Table 2b. In some embodiments, the CACCR further comprises one or more recruiting domains selected

from the group consisting of the STAT-recruiting domains from IL7Ra, IL2Rb, IL12Rb1, IL12Rb2, and IL21R. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL7Ra. In some embodiments, the STAT-recruiting domain from IL7Ra comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 46, 68, 69, 70, 71 or 72. In some embodiments, the STAT-recruiting domain from IL7Ra comprises the amino acid sequence of SEQ ID NO: 46, 68, 69, 70, 71 or 72. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL2Rb. In some embodiments, the STAT-recruiting domain from IL2Rb comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 47, 73, 74, 75, 76, 77, 78, 106, or 142. In some embodiments, the STAT-recruiting domain from IL2Rb comprises the amino acid sequence of SEQ ID NO: 47, 73, 74, 75, 76, 77, 78, 106 or 142. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL12Rb1 or IL12Rb2. In some embodiments, the STAT-recruiting domain from IL12Rb1 or IL12Rb2 comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 67, 86, or 87. In some embodiments, the STAT-recruiting domain from IL12Rb1 or IL12Rb2 comprises the amino acid sequence of SEQ ID NO: 67, 86, or 87. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL21R. In some embodiments, the STAT-recruiting domain from IL21R comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 54. In some embodiments, the STAT-recruiting domain from IL21R comprises the amino acid sequence of SEQ ID NO: 54. In some embodiments, the CACCR comprises one or more recruiting domains presented in Table 2c. In some embodiments, the recruiting domains comprises the STAT-recruiting domains from IL7Ra and IL2Rb. In some embodiments, the recruiting domain comprises the STAT-recruiting domains from IL7Ra and IL12Rb1. In some embodiments, the recruiting domain comprises the STAT-recruiting domains from IL7Ra and IL12Rb2. In some embodiments, the CACCR comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 97, or 126, with or without a signal sequence. In some embodiments, the CACCR comprises the amino acid sequence of SEQ ID NO: 97, or 126, with or without a signal sequence.

[0112] In some embodiments, the CACCR of the disclosure comprises the transmembrane domain and/or JAK-binding domain from a TPOR/MPLR receptor that comprises the S505N and W515K substitutions. In some embodiments, the CACCR further comprises a recruiting domain comprising the amino acid sequence of one or more of the
5 receptor sequences presented in Table 2b. In some embodiments, the CACCR further comprises one or more recruiting domains selected from the group consisting of the STAT-recruiting domains from IL7Ra, IL2Rb, IL12Rb1, IL12Rb2, and IL21R. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL7Ra. In some embodiments, the STAT-recruiting domain from IL7Ra comprises the amino acid
10 sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 46, 68, 69, 70, 71 or 72. In some embodiments, the STAT-recruiting domain from IL7Ra comprises the amino acid sequence of SEQ ID NO: 46, 68, 69, 70, 71 or 72. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL2Rb. In some embodiments, the STAT-recruiting domain from
15 IL2Rb comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 47, 73, 74, 75, 76, 77, 78, 106, or 142. In some embodiments, the STAT-recruiting domain from IL2Rb comprises the amino acid sequence of SEQ ID NO: 47, 73, 74, 75, 76, 77, 78, 106 or 142. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from
20 IL12Rb1 or IL12Rb2. In some embodiments, the STAT-recruiting domain from IL12Rb1 or IL12Rb2 comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 67, 86, or 87. In some embodiments, the STAT-recruiting domain from IL12Rb1 or IL12Rb2 comprises the amino acid sequence of SEQ ID NO: 67, 86, or 87. In some embodiments, the recruiting
25 domain comprises the STAT-recruiting domain from IL21R. In some embodiments, the STAT-recruiting domain from IL21R comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 54. In some embodiments, the STAT-recruiting domain from IL21R comprises the amino acid sequence of SEQ ID NO: 54. In some embodiments, the CACCR
30 comprises one or more recruiting domains presented in Table 2c. In some embodiments, the recruiting domains comprises the STAT-recruiting domains from IL7Ra and IL2Rb. In some embodiments, the recruiting domain comprises the STAT-recruiting domains from IL7Ra and IL12Rb1. In some embodiments, the recruiting domain comprises the STAT-

recruiting domains from IL7Ra and IL12Rb2. In some embodiments, the CACCR comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 96, 107, 109, 111, 113, 115, 117, 125, 128, 129, 132, 134, 136, or 138, with or without a signal sequence. In some
5 embodiments, the CACCR comprises the amino acid sequence of SEQ ID NO: 96, 107, 109, 111, 113, 115, 117, 125, 128, 129, 132, 134, 136, or 138, with or without a signal sequence.

[0113] In some embodiments, the CACCR of the disclosure comprises the transmembrane domain and/or JAK-binding domain from a TPOR/MPLR receptor that
10 comprises the H499L and W515K substitutions. In some embodiments, the CACCR further comprises a recruiting domain comprising the amino acid sequence of one or more of the receptor sequences presented in Table 2b. In some embodiments, the CACCR further comprises one or more recruiting domains selected from the group consisting of the STAT-recruiting domains from IL7Ra, IL2Rb, IL12Rb1, IL12Rb2, and IL21R. In some
15 embodiments, the recruiting domain comprises the STAT-recruiting domain from IL7Ra. In some embodiments, the STAT-recruiting domain from IL7Ra comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 46, 68, 69, 70, 71 or 72. In some embodiments, the STAT-recruiting domain from IL7Ra comprises the amino acid sequence of SEQ ID NO:
20 46, 68, 69, 70, 71 or 72. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL2Rb. In some embodiments, the STAT-recruiting domain from IL2Rb comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 47, 73, 74, 75, 76, 77, 78, 106, or 142. In some embodiments, the STAT-recruiting domain from IL2Rb
25 comprises the amino acid sequence of SEQ ID NO: 47, 73, 74, 75, 76, 77, 78, 106 or 142. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL12Rb1 or IL12Rb2. In some embodiments, the STAT-recruiting domain from IL12Rb1 or IL12Rb2 comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 67, 86, or 87.
30 In some embodiments, the STAT-recruiting domain from IL12Rb1 or IL12Rb2 comprises the amino acid sequence of SEQ ID NO: 67, 86, or 87. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL21R. In some embodiments, the STAT-recruiting domain from IL21R comprises the amino acid sequence that is at least

about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 54. In some embodiments, the STAT-recruiting domain from IL21R comprises the amino acid sequence of SEQ ID NO: 54. In some embodiments, the CACCR comprises one or more recruiting domains presented in Table 2c. In some embodiments, the recruiting domains comprises the STAT-recruiting domains from IL7Ra and IL2Rb. In some embodiments, the recruiting domain comprises the STAT-recruiting domains from IL7Ra and IL12Rb1. In some embodiments, the recruiting domain comprises the STAT-recruiting domains from IL7Ra and IL12Rb2. In some embodiments, the CACCR comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 93, with or without a signal sequence. In some embodiments, the CACCR comprises the amino acid sequence of SEQ ID NO: 93, with or without a signal sequence.

[0114] In some embodiments, the CACCR of the disclosure comprises the transmembrane domain and/or JAK-binding domain from a TPOR/MPLR receptor that comprises the H499L, S505N and W515K substitutions. In some embodiments, the CACCR further comprises a recruiting domain comprising the amino acid sequence of one or more of the receptor sequences presented in Table 2b. In some embodiments, the CACCR further comprises one or more recruiting domains selected from the group consisting of the STAT-recruiting domains from IL7Ra, IL2Rb, IL12Rb1, IL12Rb2, and IL21R. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL7Ra. In some embodiments, the STAT-recruiting domain from IL7Ra comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 46, 68, 69, 70, 71 or 72. In some embodiments, the STAT-recruiting domain from IL7Ra comprises the amino acid sequence of SEQ ID NO: 46, 68, 69, 70, 71 or 72. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL2Rb. In some embodiments, the STAT-recruiting domain from IL2Rb comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 47, 73, 74, 75, 76, 77, 78, 106, or 142. In some embodiments, the STAT-recruiting domain from IL2Rb comprises the amino acid sequence of SEQ ID NO: 47, 73, 74, 75, 76, 77, 78, 106 or 142. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL12Rb1 or IL12Rb2. In some embodiments, the STAT-recruiting domain from IL12Rb1 or IL12Rb2 comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%,

96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 67, 86, or 87. In some embodiments, the STAT-recruiting domain from IL12Rb1 or IL12Rb2 comprises the amino acid sequence of SEQ ID NO: 67, 86, or 87. In some embodiments, the recruiting domain comprises the STAT-recruiting domain from IL21R. In some embodiments, the

5 STAT-recruiting domain from IL21R comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 54. In some embodiments, the STAT-recruiting domain from IL21R comprises the amino acid sequence of SEQ ID NO: 54. In some embodiments, the CACCR comprises one or more recruiting domains presented in Table 2c. In some embodiments, the

10 recruiting domains comprises the STAT-recruiting domains from IL7Ra and IL2Rb. In some embodiments, the recruiting domain comprises the STAT-recruiting domains from IL7Ra and IL12Rb1. In some embodiments, the recruiting domain comprises the STAT-recruiting domains from IL7Ra and IL12Rb2. In some embodiments, the CACCR comprises the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%,

15 99% or 100% identical to the amino acid sequence of SEQ ID NO: 95, 108, 110, 112, 114, 116, 118, 124, 130, 131, 133, 135, 137, 139, 180, 181, 182, 184, 185, or 186, with or without a signal sequence. In some embodiments, the signal sequence comprises the amino acid sequence of SEQ ID NO: 89. In some embodiments, the CACCR comprises the amino acid sequence of SEQ ID NO: 95, 108, 110, 112, 114, 116, 118, 124, 130, 131, 133, 135,

20 137, 139, 180, 181, 182, 184, 185, or 186, with or without a signal sequence. In some embodiments, the signal sequence comprises the amino acid sequence of SEQ ID NO: 89. In some embodiments, the CACCR comprises an extra-cellular ligand binding domain. In some embodiments, the CACCR does not comprise an extra-cellular ligand binding domain. CACCRs are described in co-pending application US2020/0291090, which is hereby

25 incorporated by reference in its entirety.

Table 3 Exemplary CACCR sequences

Receptor	Amino acid sequence	SEQ ID NO:
TPOR/MPLR(478-582).IL7Ra(316-459)	SDPTRVETATETAWISLVTALHLVLGLSAVLGGLL LLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSP NCPSEDVVITPESFGRDSSLTCLAGNVSACDAPIL SSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPP PFSLQSGILTLNPVAQGQPILTSLGSNQEEAYVTM SSFYQNNQ	90
TPOR/MPLR(478-582; H499L, S505N).IL7Ra(316-459)	SDPTRVETATETAWISLVTAL <u>LL</u> VLGL <u>N</u> AVLGGLL LLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSP NCPSEDVVITPESFGRDSSLTCLAGNVSACDAPIL SSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPP PFSLQSGILTLNPVAQGQPILTSLGSNQEEAYVTM SSFYQNNQ	91
TPOR/MPLR(478-582; S505N).IL7Ra(316-459)	SDPTRVETATETAWISLVTALHLVLGL <u>N</u> AVLGGLL LLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSP NCPSEDVVITPESFGRDSSLTCLAGNVSACDAPIL SSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPP PFSLQSGILTLNPVAQGQPILTSLGSNQEEAYVTM SSFYQNNQ	92
TPOR/MPLR(478-582; H499L, W515K).IL7Ra(316-459)	SDPTRVETATETAWISLVTAL <u>LL</u> VLGLSAVLGGLL LLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSP NCPSEDVVITPESFGRDSSLTCLAGNVSACDAPIL SSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPP PFSLQSGILTLNPVAQGQPILTSLGSNQEEAYVTM SSFYQNNQ	93
TPOR/MPLR(478-582; W515K).IL7Ra(316-459)	SDPTRVETATETAWISLVTALHLVLGLSAVLGGLL LLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSP NCPSEDVVITPESFGRDSSLTCLAGNVSACDAPIL SSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPP PFSLQSGILTLNPVAQGQPILTSLGSNQEEAYVTM SSFYQNNQ	94

Receptor	Amino acid sequence	SEQ ID NO:
TPOR/MPLR(478-582;H499L,S505N,W515K).IL7Ra(316-459)	SDPTRVETATETAWISLVTALLLVLGLNAVLGLL LLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSP NCPSEDVVITPESFGRDSSLTCLAGNVSACDAPIL SSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPP PFSLQSGILTLNPVAQGQPILTSLGSNQEEAYVTM SSFYQNNQ	95
TPOR/MPLR(478-582;S505N,W515K).IL7Ra(316-459)	SDPTRVETATETAWISLVTALHLVLGLNAVLGLL LLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSP NCPSEDVVITPESFGRDSSLTCLAGNVSACDAPIL SSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPP PFSLQSGILTLNPVAQGQPILTSLGSNQEEAYVTM SSFYQNNQ	96
TPOR/MPLR(478-582;H499L,G509N).IL7Ra(316-459)	SDPTRVETATETAWISLVTALLLVLGLSAVLNLL LLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSP NCPSEDVVITPESFGRDSSLTCLAGNVSACDAPIL SSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPP PFSLQSGILTLNPVAQGQPILTSLGSNQEEAYVTM SSFYQNNQ	97
TPOR/MPLR(478-582;H499L,S505N).IL7Ra(316-459)	SDPTRVETATETAWISLVTALLLVLGLNAVLGLL LLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSP NCPSEDVVITPESFGRDSSLTCLAGNVSACDAPIL SSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPP PFSLQSGILTLNPVAQGQPILTSLGSNQEEAYVTM SSFYQNNQ	98
TpoR(478-582;S505N,W515K).IL2Rb(393-433,518-551)	SDPTRVETATETAWISLVTALHLVLGLNAVLGLL LLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLLEDEGVAGAPTGSSPQPLQPLSGEDDAYCTFP SRDDLLLFSPSGQGEFRALNARLPLNTDAYLSLQ ELQGQDPHTLV	99
TpoR(478-582;H499L,S505N,W515K).IL2Rb(339-379,393-433,518-551)	SDPTRVETATETAWISLVTALLLVLGLNAVLGLL LLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLLEQQDKVPEPASLSSNHSLTSCFTNQGYFFFHL PDALEIEACQDEGVAGAPTGSSPQPLQPLSGEDD AYCTFPSRDDLLLFSPSGQGEFRALNARLPLNTD AYLSLQELQGQDPHTLV	100

Receptor	Amino acid sequence	SEQ ID NO:
CD8SS-TPOR/MPLR(478-582;S505N,W515K).IL12Rb2(714-862)	MALPVTALLLPLALLLHAARPSDPTRVETATETA WISLVTALHLVLGLNAVLGLLLLLRKQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATV SDTCEEVEPSLLEILPKSSERTPLPLVTPVFRHPPC SNWPQREKGIQGHQASEKDMMHSAASSPPPPRAL QAESRQLVDLYKVLESRGSDPKPENPACPWTVL PAGDLPTHGDLPSNIDDLPSHEAPLADSLEELEP QHISLSVFPSSSLHPLTFSCGDKLTLDQLKMRCDS LML	107
CD8SS-TPOR/MPLR(478-582;H499L,S505N,W515K).IL12Rb2(714-862)	MALPVTALLLPLALLLHAARPSDPTRVETATETA WISLVTALLVLGLNAVLGLLLLLRKQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATV SDTCEEVEPSLLEILPKSSERTPLPLVTPVFRHPPC SNWPQREKGIQGHQASEKDMMHSAASSPPPPRAL QAESRQLVDLYKVLESRGSDPKPENPACPWTVL PAGDLPTHGDLPSNIDDLPSHEAPLADSLEELEP QHISLSVFPSSSLHPLTFSCGDKLTLDQLKMRCDS LML	108
CD8SS-TPOR/MPLR(478-582;S505N,W515K).IL12Rb2(775-825)	MALPVTALLLPLALLLHAARPSDPTRVETATETA WISLVTALHLVLGLNAVLGLLLLLRKQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATV SDTCEEVEPSLLEILPKSSERTPLPLSDPKPENPAC PWTVLPAGDLPTHGDLPSNIDDLPSHEAPLADS LEELEPQ	109
CD8SS-TPOR/MPLR(478-582;H499L,S505N,W515K).IL12Rb2(775-825)	MALPVTALLLPLALLLHAARPSDPTRVETATETA WISLVTALLVLGLNAVLGLLLLLRKQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATV SDTCEEVEPSLLEILPKSSERTPLPLSDPKPENPAC PWTVLPAGDLPTHGDLPSNIDDLPSHEAPLADS LEELEPQ	110
CD8SS-TPOR/MPLR(478-582;S505N,W515K).IL2Rb(333-551)	MALPVTALLLPLALLLHAARPSDPTRVETATETA WISLVTALHLVLGLNAVLGLLLLLRKQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATV SDTCEEVEPSLLEILPKSSERTPLPLVTQLLLQQD KVPEPASLSSNHSLTSCFTNQGYFFHLPDALEIE ACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQP LSGEDDAYCTFSPSRDDLLLFSPSLLGGPSPPSTAP GGSGAGEERMPPSLQERVPRDWDPPQLGPPTPG VPDLVDFQPPPELVLEAGEEVPDAGPREGVSFP WSRPPGQGEFRALNARLPLNTDAYLSLQELQGQ DPTHLV	111
CD8SS-TPOR/MPLR(478-582;H499L,S505N,W515K).IL2Rb(333-551)	MALPVTALLLPLALLLHAARPSDPTRVETATETA WISLVTALLVLGLNAVLGLLLLLRKQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATV SDTCEEVEPSLLEILPKSSERTPLPLVTQLLLQQD KVPEPASLSSNHSLTSCFTNQGYFFHLPDALEIE ACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQP	112

Receptor	Amino acid sequence	SEQ ID NO:
	LSGEDDAYCTFSPSRDDLLLFSPSLLGGPSPPSTAP GGSGAGEERMPPSLQERVPRDWDQPPLGPPTPG VPDLVDFQPPPELVLRAGEEVPDAGPREGVSFP WSRPPGQGEFRALNARLPLNTDAYLSLQELQGQ DPTHLV	
CD8SS- TPOR/MPLR(478- 582;S505N,W515K).IL2R b(393-433,518-551)	MALPVTALLLPLALLLHAARPSDPTRVETATETA WISLVTALHLVLGLNAVGLLLLLRKQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATV SDTCEEVEPSLLEILPKSSERTPLPLDEGVAGAPT GSSPQPLQPLSGEDDAYCTFSPSRDDLLLFSPSGQG EFRALNARLPLNTDAYLSLQELQGQDPTHLV	113
CD8SS- TPOR/MPLR(478- 582;H499L,S505N,W515 K).IL2Rb(393-433,518- 551)	MALPVTALLLPLALLLHAARPSDPTRVETATETA WISLVTALLVLGLNAVGLLLLLRKQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATV SDTCEEVEPSLLEILPKSSERTPLPLDEGVAGAPT GSSPQPLQPLSGEDDAYCTFSPSRDDLLLFSPSGQG EFRALNARLPLNTDAYLSLQELQGQDPTHLV	114
CD8SS- TPOR/MPLR(478- 582;S505N,W515K). IL2Rb(339-379,393- 433,518-551)	MALPVTALLLPLALLLHAARPSDPTRVETATETA WISLVTALHLVLGLNAVGLLLLLRKQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATV SDTCEEVEPSLLEILPKSSERTPLPLQQDKVPEPAS LSSNHSLTSCFTNQGYFFFHLPDALEIEACQDEGV AGAPTGSSPQPLQPLSGEDDAYCTFSPSRDDLLLF PSGQGEFRALNARLPLNTDAYLSLQELQGQDPTH LV	115
CD8SS- TPOR/MPLR(478- 582;H499L,S505N,W515 K).IL2Rb(339-379,393- 433,518-551)	MALPVTALLLPLALLLHAARPSDPTRVETATETA WISLVTALLVLGLNAVGLLLLLRKQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATV SDTCEEVEPSLLEILPKSSERTPLPLQQDKVPEPAS LSSNHSLTSCFTNQGYFFFHLPDALEIEACQDEGV AGAPTGSSPQPLQPLSGEDDAYCTFSPSRDDLLLF PSGQGEFRALNARLPLNTDAYLSLQELQGQDPTH LV	116
CD8SS- TPOR/MPLR(478- 582;S505N,W515K).IL7R a(316-459).IL12Rb2(775- 825)	MALPVTALLLPLALLLHAARPSDPTRVETATETA WISLVTALHLVLGLNAVGLLLLLRKQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATV SDTCEEVEPSLLEILPKSSERTPLPLARDEVEGFLQ DTFPQQLEESEKQRLGGDVQSPNCPSDEVVITPES FGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGK NGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTLNP VAQGQPILTSLGSNQEEAYVTMSSFYQNPQSDPKP ENPACPWTVLPAGDLPTHGYPNIDDLPSHEA PLADSLEELEPQ	117
CD8SS- TPOR/MPLR(478- 582;H499L,S505N,W515	MALPVTALLLPLALLLHAARPSDPTRVETATETA WISLVTALLVLGLNAVGLLLLLRKQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATV	118

Receptor	Amino acid sequence	SEQ ID NO:
K).IL7Ra(316-459).IL12Rb2(775-825)	SDTCEEVEPSLLEILPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLNPVAQQQPILTSLGSNQEEAYVTMSSFYQNQSDPKPENPACPWTVLPAGDLPTHGYPNIDDLPSHEA PLADSLEELEPQ	
TPOR/MPLR(478-582).IL7Ra(316-459)	SDPTRVETATETAWISLVTALHLVLGLSAVLGILLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLEARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLNPVAQQQPILTSLGSNQEEAYVTMSSFYQNQ	119
TPOR/MPLR(478-582; H499L, S505N).IL7Ra(316-459)	SDPTRVETATETAWISLVTAL ~~LL~~ VLGL ~~NA~~ VLGILLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLEARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLNPVAQQQPILTSLGSNQEEAYVTMSSFYQNQ	120
TPOR/MPLR(478-582; S505N).IL7Ra(316-459)	SDPTRVETATETAWISLVTALHLVLGL ~~NA~~ VLGILLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLEARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLNPVAQQQPILTSLGSNQEEAYVTMSSFYQNQ	121
TPOR/MPLR(478-582; H499L, W515K).IL7Ra(316-459)	SDPTRVETATETAWISLVTAL ~~LL~~ VLGLSAVLGILLRR ~~K~~ QFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLEARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLNPVAQQQPILTSLGSNQEEAYVTMSSFYQNQ	122
TPOR/MPLR(478-582; W515K).IL7Ra(316-459)	SDPTRVETATETAWISLVTALHLVLGLSAVLGILLRR ~~K~~ QFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLEARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLNPVAQQQPILTSLGSNQEEAYVTMSSFYQNQ	123

Receptor	Amino acid sequence	SEQ ID NO:
TPOR/MPLR(478-582;H499L,S505N,W515K).IL7Ra(316-459)	SDPTRVETATETAWISLVTAL ~~LL~~ VLGLN ~~NA~~ VLGGLLLR ~~K~~ QFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL ~~LE~~ ARDEVEGF ~~L~~ QDTFPQQLEESEKQRLGGDVQSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQD ~~LL~~ LSLGTNSTLPPPFSLQSGILTLNPVAQGGQPILTSLSNSNQEEAYVTMSSFYQNQ	124
TPOR/MPLR(478-582;S505N,W515K).IL7Ra(316-459)	SDPTRVETATETAWISLVTALHLVLGLN ~~NA~~ VLGGLLLR ~~K~~ QFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL ~~LE~~ ARDEVEGF ~~L~~ QDTFPQQLEESEKQRLGGDVQSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQD ~~LL~~ LSLGTNSTLPPPFSLQSGILTLNPVAQGGQPILTSLSNSNQEEAYVTMSSFYQNQ	125
TPOR/MPLR(478-582;H499L,G509N).IL7Ra(316-459)	SDPTRVETATETAWISLVTAL ~~LL~~ VLGLSAVLN ~~LL~~ LLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL ~~LE~~ ARDEVEGF ~~L~~ QDTFPQQLEESEKQRLGGDVQSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQD ~~LL~~ LSLGTNSTLPPPFSLQSGILTLNPVAQGGQPILTSLSNSNQEEAYVTMSSFYQNQ	126
TPOR/MPLR(478-582;H499L,S505N).IL7Ra(316-459)	SDPTRVETATETAWISLVTAL ~~LL~~ VLGLN ~~NA~~ VLGGLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL ~~LE~~ ARDEVEGF ~~L~~ QDTFPQQLEESEKQRLGGDVQSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQD ~~LL~~ LSLGTNSTLPPPFSLQSGILTLNPVAQGGQPILTSLSNSNQEEAYVTMSSFYQNQ	127
TPOR/MPLR(478-582;S505N,W515K).IL2Rb(333-551)	SDPTRVETATETAWISLVTALHLVLGLN ~~NA~~ VLGGLLLR ~~K~~ QFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL ~~LE~~ VTQ ~~LL~~ LQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDL ~~LL~~ FSPSLLGGSPSPSTAPGGSGAGEERMPPSLQERVPRDWDQPLGPPTPGVPDLVDFQPPPELVLEAGEEVPDAGPREGVSPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPTHLV	128
TPOR/MPLR(478-582;S505N,W515K).IL12Rb2(714-862)	SDPTRVETATETAWISLVTALHLVLGLN ~~NA~~ VLGGLLLR ~~K~~ QFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL ~~LE~~ VT ~~TP~~ VFRHPPCSNWPQREKGIQGHQASEKDMMSASSPPPPRALQAESRQLVDLYKVLESRGS	129

Receptor	Amino acid sequence	SEQ ID NO:
	DPKPENPACPWTVLPAGDLPTHDGYLPSNIDDLPSHEAPLADSLEELEPQHISLSVFPSSSLHPLTFSCGDKLTLDQLKMRCDSLML	
TPOR/MPLR(478-582;H499L,S505N,W515K).IL2Rb(333-551)	SDPTRVETATETAWISLVTALLLVLGLNAVLGGLLLRQKQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLEVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLSFSPLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDQPPLGPPTPGVPDLVDFQPPPELVREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV	130
TPOR/MPLR(478-582;H499L,S505N,W515K).IL12Rb2(714-862)	SDPTRVETATETAWISLVTALLLVLGLNAVLGGLLLRQKQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLEVTPVFRHPPCSNWPQREKGIQGHQASEKDMMHSASSPPPPRALQAESRQLVDLYKVLESRGSDPKPENPACPWTVLPAGDLPTHDGYLPSNIDDLPSHEAPLADSLEELEPQHISLSVFPSSSLHPLTFSCGDKLTLDQLKMRCDSLML	131
TPOR/MPLR(478-582;S505N,W515K).IL12Rb2(775-825)	SDPTRVETATETAWISLVTALHLVLGLNAVLGGLLLRQKQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLESDPKPENPACPWTVLPAGDLPTHDGYLPSNIDDLPSHEAPLADSLEELEPQ	132
TPOR/MPLR(478-582;H499L,S505N,W515K).IL12Rb2(775-825)	SDPTRVETATETAWISLVTALLLVLGLNAVLGGLLLRQKQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLESDPKPENPACPWTVLPAGDLPTHDGYLPSNIDDLPSHEAPLADSLEELEPQ	133
TPOR/MPLR(478-582;S505N,W515K).IL7Ra(316-459).IL12Rb2(775-825)	SDPTRVETATETAWISLVTALHLVLGLNAVLGGLLLRQKQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLEARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTLNPVAQGPILTSLGSNQEEAYVTMSSFYQNRSDPKPENPACPWTVLPAGDLPTHDGYLPSNIDDLPSHEAPLADSLEELEPQ	134
TPOR/MPLR(478-582;H499L,S505N,W515K).IL7Ra(316-459).IL12Rb2(775-825)	SDPTRVETATETAWISLVTALLLVLGLNAVLGGLLLRQKQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLEARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTLNPVAQGPILTSLGSNQEEAYV	135

Receptor	Amino acid sequence	SEQ ID NO:
	TMSSFYQNQSRSDPKPENPACPWTVLPAGDLPTH DGYLPSNIDDLPSHEAPLADSLEELEPQ	
TPOR/MPLR(478-582;S505N,W515K).IL2Rb(333-551; Y381S, Y384S,Y387S)	SDPTRVETATETAWISLVTALHLVLGLN ~~N~~ AVLGLL LLR ~~K~~ QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLLEVTQLLLQQDKVPEPASLSSNHSLTSCFTNQ GYFFFHLPDALEIEACQV ~~S~~ FTSDPSSEEDPDEGVA GAPTGSSPQPLQPLSGEDDAYCTFSPSRDDLLLFSP SLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRD WDPQPLGPPTPGVPDLVDFQPPPELVLEAGEEV PDAGPREGVSFPWSRPPGQGEFRALNARLPLNTD AYLSLQELQGQDPTHLV	136
TPOR/MPLR(478-582;H499L,S505N,W515K).IL2Rb(333-551;Y381S,Y384S,Y387S)	SDPTRVETATETAWISLVTAL ~~LL~~ VLGLN ~~N~~ AVLGLL LLR ~~K~~ QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLLEVTQLLLQQDKVPEPASLSSNHSLTSCFTNQ GYFFFHLPDALEIEACQV ~~S~~ FTSDPSSEEDPDEGVA GAPTGSSPQPLQPLSGEDDAYCTFSPSRDDLLLFSP SLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRD WDPQPLGPPTPGVPDLVDFQPPPELVLEAGEEV PDAGPREGVSFPWSRPPGQGEFRALNARLPLNTD AYLSLQELQGQDPTHLV	137
TPOR/MPLR(478-582;S505N,W515K).IL2Rb(333-551;Y364S,Y381S,Y384S,Y387S)	SDPTRVETATETAWISLVTALHLVLGLN ~~N~~ AVLGLL LLR ~~K~~ QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLLEVTQLLLQQDKVPEPASLSSNHSLTSCFTNQ G ~~S~~ FFFHLPDALEIEACQV ~~S~~ FTSDPSSEEDPDEGVA GAPTGSSPQPLQPLSGEDDAYCTFSPSRDDLLLFSP SLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRD WDPQPLGPPTPGVPDLVDFQPPPELVLEAGEEV PDAGPREGVSFPWSRPPGQGEFRALNARLPLNTD AYLSLQELQGQDPTHLV	138
TPOR/MPLR(478-582;H499L,S505N,W515K).IL2Rb(333-551;Y364S,Y381S,Y384S,Y387S)	SDPTRVETATETAWISLVTAL ~~LL~~ VLGLN ~~N~~ AVLGLL LLR ~~K~~ QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLLEVTQLLLQQDKVPEPASLSSNHSLTSCFTNQ G ~~S~~ FFFHLPDALEIEACQV ~~S~~ FTSDPSSEEDPDEGVA GAPTGSSPQPLQPLSGEDDAYCTFSPSRDDLLLFSP SLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRD WDPQPLGPPTPGVPDLVDFQPPPELVLEAGEEV PDAGPREGVSFPWSRPPGQGEFRALNARLPLNTD AYLSLQELQGQDPTHLV	139
TpoR(478-582;H499L,S505N,W515K).IL2Rb(393-433,518-551)	SDPTRVETATETAWISLVTAL ~~LL~~ VLGLN ~~N~~ AVLGLL LLR ~~K~~ QFPAHYRRLRHALWPSLPDLHRVLGQYLR DTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLEDEGVAGAPTGSSPQPLQPLSGEDDAYCTFP	143

Receptor	Amino acid sequence	SEQ ID NO:
	SRDDLLLFSPSGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV	
TpoR(478-582;S505N,W515K).IL2Rb(393-433,518-551)	SDPTRVETATETAWISLVTALHLVLGLN <u>NA</u> VLGGLLLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV	180
TpoR(478-582;H499L,S505N,W515K).IL2Rb(339-379,393-433,518-551)	SDPTRVETATETAWISLVTAL <u>LL</u> VLGLN <u>NA</u> VLGGLLLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLQQDKVPEPASLSSNHSLTSCFTNQGYFFHLPDALEIEACQDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV	181
TpoR(478-582;H499L,S505N,W515K).IL2Rb(393-433,518-551)	SDPTRVETATETAWISLVTAL <u>LL</u> VLGLN <u>NA</u> VLGGLLLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV	182
TpoR(478-582;S505N,W515K).IL2Rb(393-433,518-551). <u>P2A</u>	SDPTRVETATETAWISLVTALHLVLGLN <u>NA</u> VLGGLLLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLP <u>LE</u> DEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV <u>GSGATNFSLLKQAGDVEENPG</u>	184
TpoR(478-582;H499L,S505N,W515K).IL2Rb(339-379,393-433,518-551). <u>P2A</u>	SDPTRVETATETAWISLVTAL <u>LL</u> VLGLN <u>NA</u> VLGGLLLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLP <u>LE</u> QQDKVPEPASLSSNHSLTSCFTNQGYFFHLPDALEIEACQDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV <u>GSGATNFSLLKQAGDVEENPG</u>	185
TpoR(478-582;H499L,S505N,W515K).IL2Rb(393-433,518-551). <u>P2A</u>	SDPTRVETATETAWISLVTAL <u>LL</u> VLGLN <u>NA</u> VLGGLLLR <u>K</u> QFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLP <u>LE</u> DEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV <u>GSGATNFSLLKQAGDVEENPG</u>	186

*The underlined LE and SR are exemplary optional linker that may be inserted between two domains.

E. Expression of CACCRs

[0115] Provided herein are polynucleotides encoding any one of the CACCRs provided herein. Likewise, provided herein are expression vectors comprising such polynucleotides. In some embodiments, the vector is a viral vector. In some embodiments, the vector is not a viral vector. In some embodiments, the vector is a lentiviral vector. In some embodiments, the expression vector comprises a promoter, which may be selected from those known in the art. An exemplary promoter that can be used in the invention is the human elongation factor-1 alpha (EF-1 alpha or EF-1 α) promoter, which may be used in its full-length form, or truncated forms, or other variant forms (as described in the literature and disclosed in, *e.g.*, Wakabayashi-Ito, N. et al., J. Biol. Chem. 1994:269(47): 29831-29837; Montiel-Equihua, C. A. et al., Mol. Therapy, 2012, 20(7): 1400-1409). Any version of the EF1 alpha promoter is suitable and may be used as the sole promoter or together with other promoter elements. In some embodiments, the expression vector is a lentiviral vector and the promoter is a full-length EF-1 alpha promoter. Exemplary full-length EF1alpha promoter is shown below:

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GCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCC
CGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGC
GCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTCCCGAGG
GTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTCGCA
ACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTG
GCCTCTTTACGGGTTATGGCCCTTGCCTGCTTGAATTACTTCCACGCCCCCTGGC
TGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTT
CGAGGCCTTGCGCTTAAGGAGCCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGC
CTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCG
CTGCTTTTCGATAAGTCTCTAGCCATTTAAAATTTTGTATGACCTGCTGCGACGCT
TTTTTCTGGCAAGATAGTCTTGTAATGCGGGCCAAGATCTGCACACTGGTAT
TTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATG
TTCGGCGAGGCGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGT
CTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCC
CGCCCTGGGCGGCAAGGCTGGCCCGGTGGGCACCGAGTTGCGTGAGCGGAAAGA
TGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTC
GGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCTC
CAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCG

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ATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTT
 ATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTT
 GGCACCTGATGTAATTCTCCTTGGAATTTGCCCTTTTGTAGTTTGGATCTTGTT
 CATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTCTTCCATTTCAGGTGTCTG
 5 TGA (SEQ ID NO: 187)

[0116] Exemplary EF1alpha short promoter is shown below:

GCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCC
 CGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGC
 GCGGGGTAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTCCCGAGG
 10 GTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCA
 ACGGGTTTGCCGCCAGAACACAG (SEQ ID NO: 188)

[0117] In some embodiments, the expression vector comprises a polynucleotide expressing a CACCR and a polynucleotide expressing a chimeric antigen receptor (CAR).

[0118] In some embodiments, the CACCR and the CAR are expressed as a single
 15 polypeptide chain, separated by a linker. FIGs. 2B-2D show schematics of vectors that can be used to co-express the CACCR and a BCMA CAR of the disclosure. One or more recruiting domains may be joined in tandem in the CACCR to mimic signaling from one or more cytokines.

II. CAR-bearing Immune Cells

[0119] Provided herein are engineered immune cells comprising a polynucleotide
 20 encoding a B-cell maturation antigen (BCMA) chimeric antigen receptor (CAR) and a CACCR of the disclosure; and provided herein are engineered immune cells expressing a BCMA chimeric antigen receptor (BCMA CAR-T cell) and a CACCR of the disclosure. Examples of immune cells include T cells, e.g., alpha/beta T cells and gamma/delta T cells,
 25 B cells, natural killer (NK) cells, natural killer T (NKT) cells, invariant NKT cells, mast cells, myeloid-derived phagocytes, dendritic cells, killer dendritic cells, macrophages, and monocytes. In some embodiments, the engineered immune cells are CD4+ and/or CD8+ T cells. In some embodiments, the engineered immune cells are T cells exerting one or more T cell effector functions (or effector T cells). Immune cells also refer to cells derived from,
 30 for example without limitation, a stem cell. The stem cells can be adult stem cells, non-human embryonic stem cells, more particularly non-human stem cells, cord blood stem

cells, progenitor cells, bone marrow stem cells, induced pluripotent stem cells, totipotent stem cells or hematopoietic stem cells. The immune cells can be obtained from a natural source such as from a human patient or can be prepared from, for example, donor cells, stem cells or non-stem cells, according to methods known in the art.

5 [0120] Accordingly, in some embodiments, provided herein are BCMA CAR-T cells comprising a CACCR of the disclosure.

[0121] In some embodiments, a BCMA CAR can comprise an extracellular ligand-binding domain (e.g., a single chain variable fragment (scFv)), a transmembrane domain, and an intracellular signaling domain. In some embodiments, the extracellular ligand-binding domain, transmembrane domain, and intracellular signaling domain are in one
10 polypeptide, i.e., in a single chain. Multichain BCMA CARs and polypeptides are also provided herein. In some embodiments, the multichain BCMA CARs comprise: a first polypeptide comprising a transmembrane domain and at least one extracellular ligand-binding domain, and a second polypeptide comprising a transmembrane domain and at least
15 one intracellular signaling domain, wherein the polypeptides assemble together to form a multichain CAR.

[0122] The extracellular ligand-binding domain of a BCMA CAR specifically binds to BCMA.

[0123] In some embodiments, the extracellular ligand-binding domain of a BCMA CAR
20 comprises an scFv comprising the light chain variable (VL) region and the heavy chain variable (VH) region of a BCMA specific monoclonal antibody joined by a flexible linker. Single chain variable region fragments are made by linking light and/or heavy chain variable regions by using a short linking peptide (Bird et al., Science 242:423-426, 1988) (e.g. glycine-serine containing linkers). In general, linkers can be short, flexible
25 polypeptides and are generally comprised of about 20 or fewer amino acid residues. Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing
30 polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as E. coli. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as

ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[0124] The intracellular signaling domain of a BCMA CAR according to the invention is responsible for intracellular signaling following the binding of extracellular ligand-binding domain to the target resulting in the activation of the immune cell and immune response (Signals 1 and/or 2). The intracellular signaling domain has the ability to activate at least one of the normal effector functions of the immune cell in which the CAR is expressed. For example, the effector function of a T cell can be a cytolytic activity or helper activity including the secretion of cytokines.

[0125] In some embodiments, an intracellular signaling domain for use in a BCMA CAR can be the cytoplasmic sequences of, for example without limitation, the T cell receptor and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability. Intracellular signaling domains comprise two distinct classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation, and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal. Primary cytoplasmic signaling sequences can comprise signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or "ITAMs". ITAMs are well defined signaling motifs found in the intracytoplasmic tail of a variety of receptors that serve as binding sites for syk/zap70 class tyrosine kinases. Examples of ITAMs used in the invention can include as non-limiting examples those derived from TCR ζ , FcR γ , FcR β , FcR ϵ , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b and CD66d. In some embodiments, the intracellular signaling domain of the BCMA CAR can comprise the CD3 ζ signaling domain. In some embodiments the intracellular signaling domain of the BCMA CAR of the invention comprises a domain of a co-stimulatory molecule.

[0126] In some embodiments, the intracellular signaling domain of a BCMA CAR of the invention comprises a part of co-stimulatory molecule selected from the group consisting of fragment of 4-1BB (GenBank: AAA53133.) and CD28 (NP_006130.1).

[0127] CARs are expressed on the surface membrane of the cell. Thus, the BCMA CAR comprises a transmembrane domain. Suitable transmembrane domains for a BCMA CAR disclosed herein have the ability to (a) be expressed at the surface of a cell, preferably an

immune cell such as, for example without limitation, lymphocyte cells or Natural killer (NK) cells, and (b) interact with the ligand-binding domain and intracellular signaling domain for directing cellular response of immune cell against a predefined target cell. The transmembrane domain can be derived either from a natural or from a synthetic source. The

5 transmembrane domain can be derived from any membrane-bound or transmembrane protein. As non-limiting examples, the transmembrane polypeptide can be a subunit of the T cell receptor such as α , β , γ or δ , polypeptide constituting CD3 complex, IL-2 receptor p55 (a chain), p75 (β chain) or γ chain, subunit chain of Fc receptors, in particular Fc γ receptor III or CD proteins. Alternatively, the transmembrane domain can be synthetic and can

10 comprise predominantly hydrophobic residues such as leucine and valine. In some embodiments said transmembrane domain is derived from the human CD8 α chain (e.g., NP_001139345.1).

[0128] The transmembrane domain of the BCMA CAR can further comprise a stalk domain between the extracellular ligand-binding domain and said transmembrane domain.

15 A stalk domain may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. Stalk region may be derived from all or part of naturally occurring molecules, such as from all or part of the extracellular region of CD8, CD4, or CD28, or from all or part of an antibody constant region. Alternatively, the stalk domain may be a synthetic sequence that corresponds to a naturally occurring stalk

20 sequence, or may be an entirely synthetic stalk sequence. In some embodiments said stalk domain is a part of human CD8 α chain (e.g., NP_001139345.1).

[0129] In another particular embodiment, said transmembrane and hinge domains of the BCMA CAR comprise a part of human CD8 α chain.

[0130] Table 4 provides exemplary sequences of CAR components that can be used in the

25 BCMA CARs disclosed herein and the antibody and/or CAR sequences exemplified herein.

Table 4: Amino Acid Sequences relating to BCMA CARs

Domain	Amino acid sequence	SEQ ID NO:
CD8 hinge and transmembrane	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV HTRGLDFACDIYWAPLAGTCGVLLLSLVITLYC	101
4-1BB intracellular signaling	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE EEEGGCEL	102
CD3z intracellular signaling	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPPEMGGKPRRKNPQEGLYNELQKD	103

Domain	Amino acid sequence	SEQ ID NO:
	KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	
BFP	MSELIKENMHMKLYMEGTVDNHHFKCTSEGEKPYEGTQTMRIKVVEGGPLPFAFDILATSFLYGSKTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDTSLQDGCLIYNVKIRGVNFTSNGPVMQKKTLGWEAFTETLYPADGGLEGRNDMALKLVGGSHLIANIKTTYRSKKPAKNLKM PGVYYVDYRLERIKEANNETYVEQHEVAVARYCDLPSKLGHLN	104
P2A	GSGATNFSLLKQAGDVEENPGP	105
P5A2 anti-BCMA scFv	EVQLLES GGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISDSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQGTLVTVSSGGGGGSGGGGGGSEIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSWPLTFGQGGTKVEIK	140
P5A2 anti-BCMA CAR [including safety switch, the parentheses indicate signal sequence]	(MALPVTALLLPLALLLHAARP)EVQLLES GGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISDSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQGTLVTVSSGGGGGSGGGGGGSEIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSWPLTFGQGGTKVEIKGSGGGGSCPYSNPSLCSGGGGSCPYSNPSLCSGGGGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCKRG RKKLLYIFKQPFMRPVQTTQEEDGCS CRFP EEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	141
P5A2 VH	EVQLLES GGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISDSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQGTLVTVSS	144

Domain	Amino acid sequence	SEQ ID NO:
P5A2 VL	EIVLTQSPGTLSPGERATLSCRASQSVSSSYLA WYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQQYGSWPLTFGQGT KVEIK	145
VH CDR1 (Kabat)	SYAMN	146
VH CDR1 (Chothia);	GFTFSSY	147
VH CDR1 (extended)	GFTFSSYAMN	148
VH CDR2 (Chothia)	AISDSGGSTYYADSVKG	149
VH CDR2 (Kabat)	SDSGGS	150
VH CDR3	YWPMDI	151
VL CDR1	RASQSVSSSYLA	152
VL CDR2	DASIRAT	153
VL CDR3	QQYGSWPLT	154
C29 VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPM SWVRQAPGKGLEWVSAIGSGGSLPYADSVKGR FTISRDNSKNTLYLQMNSLRAEDTAVYYCARYW PMDIWGQGTLVTSS	155
C29 VL	EIVLTQSPGTLSPGERATLSCRASQSVSSSYLA WYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQQYQSWPLTFGQGT KVEIK	156
VH CDR1 (Kabat)	SYPMS	157
VH CDR1 (Chothia);	GFTFSSY	158
VH CDR1 (extended)	GFTFSSYPMS	159
VH CDR2 (Chothia)	AIGSGGSLPYADSVKG	160
VH CDR2 (Kabat)	GGSGGS	161
VH CDR3	YWPMDI	162
VL CDR1	RASQSVSSSYLA	163
VL CDR2	DASIRAT	164
VL CDR3	QQYQSWPLT	165
P5A2 BCMA CAR without a signal sequence	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAM NWVRQAPGKGLEWVSAISDSGGSTYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCARY WPMDIWGQGTLVTSSGGGGSGGGGSGGGGSEI VLTQSPGTLSPGERATLSCRASQSVSSSYLA WYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCQQYGSWPLTFGQGTK VEIKSGGGGSCPYSNPSLCSGGGGSCPYSNPSLC SGGGGSTTTTPAPRPPTPAPTIASQPLSLRPEACRPA AGGAVHTRGLDFACDIYWAPLAGTCGVLLLSL VITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQL YNELNLGRREEYDVLKRRGRDPGEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR	166
Linker	GGGGSGGGSGGGGS	167

Domain	Amino acid sequence	SEQ ID NO:
Off-switch ("R2")	GSGGGGSCPYSNP SLCSGGGGSCPYSNP SLCSGGGS	168
CD8 Hinge	TTTPAPRPPTPAPT IASQPLSLRPEACRPAAGGAVHTRGLDFACD	169
CD8 TM	IYIWAPLAGTCGVLLSLVIT	170
rituximab mimotope	CPYSNP SLC	183

[0131] In some embodiments of the BCMA CAR, the intracellular signaling domain comprises a CD3 ζ signaling domain. In some embodiments of the BCMA CAR, the intracellular signaling domain comprises a CD3 ζ signaling domain and additionally a second signaling domain. In some embodiments of the BCMA CAR, the intracellular signaling domain comprises a CD3 ζ signaling domain and a 4-1BB signaling domain. In some embodiments, the BCMA CARs disclosed herein comprise an extracellular ligand-binding domain that specifically binds BCMA, human CD8 α hinge and transmembrane domains, the CD3 ζ signaling domain, and 4-1BB signaling domain. In some embodiments, the BCMA specific CAR comprises the amino acid sequence of SEQ ID NO: 140 or 141. In some embodiments, the BCMA specific CAR comprises or consists of the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 140. In some embodiments, the BCMA specific CAR comprises or consists of the amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 141. In some embodiments, the BCMA CAR comprises the amino acid sequence of SEQ ID NO: 141, without the CD8 alpha leader sequence or signal sequence (MALPVTALLLPLALLLHAARP, SEQ ID NO: 89). In some embodiments, the BCMA CAR comprises the amino acid sequence of SEQ ID NO: 166.

[0132] In some embodiments, a BCMA CAR of SEQ ID NO: 141 is encoded by the DNA sequence of SEQ ID NO: 171.

Table 5. BCMA CAR nucleotide sequences

Name	Nucleotide sequence	SEQ ID NO:
BCMA CAR nt	ATGGCACTGCCCCGTGACCGCCCTGCTGCTGCCTCTGGCCC TGCTGCTGCACGCCCGCCCGGCCTGAGGTGCAGCTGCTGG AGAGCGGAGGAGGCCTGGTGCAGCCAGGAGGCAGCCTG AGACTGTCCTGCGCAGCCTCTGGCTTCACCTTCAGCAGCT ACGCCATGAACTGGGTGAGGCAGGCACCTGGCAAGGGCC TGGAGTGGGTGAGCGCCATCTCCGACTCTGGCGGCAGCA CCTACTATGCCGATTCCGTGAAGGGCCGCTTCACAATCAG CCGGGATAACTCCAAGAATACCCTGTACCTGCAGATGAA CAGCCTGAGAGCCGAGGATACAGCCGTGTACTATTGCGC CAGGTATTGGCCAATGGACATCTGGGGCCAGGGCACACT GGTGACCGTGTCTAGCGGCGGAGGAGGCTCCGGAGGAGG AGGCTCTGGCGGCGGCGGCAGCGAGATCGTGCTGACACA GTCTCCAGGCACCCTGAGCCTGTCCCCAGGAGAGAGAGC CACCTGAGCTGTAGGGCCTCTCAGAGCGTGTCTCTAGC TACCTGGCCTGGTATCAGCAGAAGCCAGGCCAGGCCCCC AGACTGCTGATGTACGACGCCAGCATCAGGGCAACAGGC ATCCCCGATCGGTTCTCCGGCTCTGGCAGCGGCACCGACT TTACACTGACCATCAGCAGGCTGGAGCCCGAGGACTTCG CCGTGTACTATTGCCAGCAGTATGGCTCCTGGCCTCTGAC ATTTGGCCAGGGCACCAAGGTGGAGATCAAGGGCTCCGG CGGCGGAGGCTCTTGCCCTTACAGCAACCCATCCCTGTGC TCTGGAGGAGGAGGCTCCTGTCCCTATAGCAATCCCAGC CTGTGTCTCCGGCGGAGGAGGCTCTACCACAACCCCTGCA CCACGCCCCCTACACCAGCACCTACCATCGCCTCTCAGC CTCTGAGCCTGCGGCCCGAGGCCTGTAGGCCCGCCGCCG GCGGCGCCGTGCACACACGGGGCCTGGACTTTGCCTGCG ACATCTACATCTGGGCACCCCTGGCCGGCACATGTGGCG TGCTGCTGCTGAGCCTGGTCATCACCTGTACTGCAAGAG AGGCAGGAAGAAGCTGCTGTATATCTTCAAGCAGCCCTT CATGCGGCCCGTGCAGACAACCCAGGAGGAGGATGGCTG CTCCTGTCGTTCCAGAGGAGGAGGAGGGAGGATGTGA GCTGCGCGTGAAGTTTTCCCGGTCTGCCGACGCACCAGC ATACCAGCAGGGCCAGAACCAGCTGTATAACGAGCTGAA TCTGGGCCGGAGAGAGGAGTACGACGTGCTGGATAAGCG GCGGGGCCGGGACCCCGAGATGGGAGGCAAGCCTCGGA GAAAGAACCCACAGGAGGGCCTGTACAATGAGCTGCAG AAGGATAAGATGGCCGAGGCCTATTCTGAGATCGGCATG AAGGGAGAGAGGCGCCGGGGCAAGGGACACGACGGCCT GTACCAGGGCCTGTCCACAGCCACCAAGGACACCTATGA TGCCCTGCACATGCAGGCCCTGCCACCCAGATGA	171

[0133] In an embodiment, a BCMA CAR polypeptide of the invention comprises the following domains: optionally a leader sequence, a heavy chain variable (VH) region, linker, light chain variable (VL) region, off-switch, CD8 hinge, CD8 transmembrane

domain, 4-1BB signaling domain, and a CD3-zeta (CD3z or CD3ζ) domain. In some aspects of this embodiment, the BCMA CAR polypeptide comprises optionally a leader sequence that comprises the amino acid sequence shown in SEQ ID NO: 89, a VH region that comprises the amino acid sequence shown in SEQ ID NO: 144, a linker sequence that

5 comprises the amino acid sequence shown in SEQ ID NO: 167, a VL region that comprises the amino acid sequence shown in SEQ ID NO: 145, an off-switch region that comprises the amino acid sequence shown in SEQ ID NO: 168, a CD8 hinge region that comprises the amino acid sequence shown in SEQ ID NO: 169, a CD8 TM region that comprises the amino acid sequence shown in SEQ ID NO: 170, a 4-1BB signaling domain that comprises

10 the amino acid sequence shown in SEQ ID NO: 102, and a CD3z signaling domain that comprises the amino acid sequence shown in SEQ ID NO: 103. In some aspects of this embodiment, the BCMA CAR polypeptide comprises optionally a leader sequence that comprises the amino acid sequence shown in SEQ ID NO: 89, a VH region that comprises the amino acid sequence shown in SEQ ID NO: 144, a linker sequence that comprises the

15 amino acid sequence shown in SEQ ID NO: 167, a VL region that comprises the amino acid sequence shown in SEQ ID NO: 145, an off-switch region that comprises the amino acid sequence shown in SEQ ID NO: 168, a CD8 hinge region and TM region that comprises the amino acid sequence shown in SEQ ID NO: 101, a 4-1BB signaling domain that comprises the amino acid sequence shown in SEQ ID NO: 102, and a CD3z signaling domain that

20 comprises the amino acid sequence shown in SEQ ID NO: 103. In some embodiments, a different leader sequence is used.

[0134] In other aspects of this embodiment, the BCMA CAR polypeptide comprises optionally a leader sequence that comprises the amino acid sequence shown in SEQ ID NO: 89, a VH region that comprises the amino acid sequence shown in SEQ ID NO: 155, a

25 linker sequence that comprises the amino acid sequence shown in SEQ ID NO: 167, a VL region that comprises the amino acid sequence shown in SEQ ID NO: 156, an off-switch region that comprises the amino acid sequence shown in SEQ ID NO: 168, a CD8 hinge region that comprises the amino acid sequence shown in SEQ ID NO: 169, a CD8 TM region that comprises the amino acid sequence shown in SEQ ID NO: 170, a 4-1BB

30 signaling domain that comprises the amino acid sequence shown in SEQ ID NO: 102, and a CD3z signaling domain that comprises the amino acid sequence shown in SEQ ID NO: 103.

[0135] In another embodiment, a BCMA CAR polypeptide of the invention comprises the following domains: optionally a leader sequence, a heavy chain variable (VH) region,

linker, light chain variable (VL) region, an off-switch, CD8 hinge, CD8 transmembrane domain, 4-1BB signaling domain, and a CD3-zeta (CD3z or CD3 ζ) domain, wherein the off-switch optionally is omitted and/or only one or the other of the 4-1BB signaling domain and the CD3z signaling domain is present, but not both.

5 [0136] In aspects of either of these embodiments, the extracellular binding region of the BCMA CAR comprises a VH region that comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 146, 147, or 148; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 149 or 150; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 151; and comprises a VL region comprising a VL CDR1
10 comprising the amino acid sequence shown in SEQ ID NO: 152; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 153; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 154.

[0137] In alternative aspects of either of these embodiments, the extracellular binding region of the BCMA CAR comprises a VH region that comprises a VH CDR1 comprising
15 the amino acid sequence shown in SEQ ID NO: 157, 158 or 159; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 160 or 161; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 162; and comprises a VL region comprising a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 163; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 164; and a VL CDR3
20 comprising the amino acid sequence shown in SEQ ID NO: 165.

[0138] In some embodiments, a BCMA CAR can be introduced into an immune cell as a transgene via a vector. In some embodiments, the vector can also contain, for example, a selection marker which provides for identification and/or selection of cells which received the vector.

25 [0139] In some embodiments of the invention, a bicistronic vector comprising the nucleotide sequence of SEQ ID NO: 172 encodes a predicted CACCR BCMA CAR polypeptide having the predicted amino acid sequence of SEQ ID NO: 173, which comprises the following domains: a CD8 alpha signal sequence (e.g. SEQ ID NO: 89), transmembrane/JAK2 binding domain - TpoR(478-582;S505N,W515K) (e.g. SEQ ID NO:
30 13), IL2Rb(393-433,518-551) (e.g. SEQ ID NO: 77), P2A (e.g. SEQ ID NO: 105), CD8 alpha signal sequence (e.g., SEQ ID NO:89), and P5A2 anti-BCMA CAR with a safety switch (e.g. SEQ ID NO: 166). In aspects of this embodiment, the bicistronic vector further

comprises, following the polypeptide coding sequence, a wild-type woodchuck hepatitis virus posttranscriptional regulatory element WPRE of e.g. nucleotide sequence SEQ ID NO: 174 or a mutant WPRE ("mWPRE") of e.g. nucleotide sequence of SEQ ID NO: 175. In other aspects of this embodiment, the bicistronic vector does not include a wild-type WPRE or a mutant WPRE. In some aspects, the disclosure provides an engineered immune cell that comprises the polynucleotide sequence of SEQ ID NO: 172 and expresses a CACCR comprising the amino acid sequence of SEQ ID NO: 184 or 99, and a BCMA CAR comprising the amino acid sequence of SEQ ID NO: 166. In some embodiments, the disclosure provides an engineered immune cell that expresses a CACCR comprising the amino acid sequence of SEQ ID NO: 180, 184 or 99, and a BCMA CAR comprising the amino acid sequence of SEQ ID NO: 166. In some embodiments, the engineered immune cell is an engineered T cell. In some embodiments, the engineered T cell is an allogeneic T cell. In some embodiments, the engineered T cell is an autologous T cell.

[0140] SEQ ID NO: 172:

[0141] atggccctgccagtaccgccctgctgctgccactggccctgctgctgcacgcagcaaggccatcagaccctactag
 agtcgagaccgctaccgagaccgcttgatctctctggtgaccgccctgcacctggtgctgggcctgaacgccgtgctgggcctgc
 tgcgtctgaggaagcagttccagcacactaccggagactgaggcacgcactgtggccaagcctgcccacctgcacaggggtgc
 tgggacagtatctgaggatacagccgccctgagccacctaaggcaaccgtgtccgacacatgcgaggaggtggaaccaagtc
 tgcgtggaatcctgcaaaatcctctgagcggacacccctgcccctgctcgcaggacgaggagtgaggcaggagcaccacccggca
 gctccccccagcctctgcagccactgtccggagaggacgatgcatactgcacattcccttctcgggacgatctgctgctgttctctcc
 aagcggacaggagagtttcgggccctgaacgccagactgccctgaataccgacgcctatctgagcctgcaggagctgcaggg
 acaggacccacacacctggtgggatccggagccaccaacttctccctgctgaagcaggccggcgatgtggaggagaatccagg
 ccccatggtctgcccgtcaccgcactgctgctgccctggctctgctgctgcacgccgaagacccgaggtccagctgctggaat
 ctgggggaggactggtgcagcctggaggcagcctgagactgtcctgcgcagcatctggcttcaccttcagctcctacgccatgaac
 tgggtgaggcaggcaccaggcaaggactggagtgggtgtctgccatctccgactctggcggcagcacctactatgccgattccg
 tgaaggccgcttcacaatcagccgggataactccaagaataccctgtacctgcagatgaattccctgagagccgaggatacagcc
 gtgtactattgcgccaggtattggccatggacatctggggccagggcacactggtgacctgtcttccggaggaggaggtccgg
 aggaggaggtctgcgccggcgccgagagatcgtgctgacacagtctcctggcaccctgagcctgtccccaggagagagag
 ccacctgagctgtaggcctctcagagcgtgtcctctagctacctggcctggtatcagcagaagcccgccaggcccttagactg
 ctgatgtacgacgccagcatcagggaacaggcatccctgatcggttctccggtctggcagcggaaaccgactttacactgacat
 cagcaggctggagcccaggacttcgctgtactattgccagcagtatggctcctggcctctgacatttggccagggcaccaagg
 tggagatcaagggtccggcgccggaggctcttcccatacagcaacccatccctgtgctctggaggaggaggtcctgtccttat
 agcaatcctagcctgtgctccggcgaggaggctctaccacaacccagcaccgaaggccacctacacctgcaccaacctcgcct

ctcagccactgagcctgagacccgaggcctgtaggcctgcagcaggaggagcagtgacacccggggactggactttgcctgcg
 atatctacatctgggcaccactggcaggaacatgtggcgtgctgctgctgagcctgggtcatcacctgtactgcaagagaggcagg
 aagaagctgctgtatatcttaagcagcccttatgcccctgtgcagacaacccaggaggaggatggctgctcctgtcggttccca
 gaggaggaggaggaggatgtgagctgcgcgtgaagtttcccggtctgccgacgcaccagcataccagcagggccagaacca
 5 gctgtataacgagctgaatctgggccggagagaggagtacgacgtgctggataagaggagggaagagatcccagatggggag
 gcaagccacggagaaaagaacccccaggagggcctgtacaatgagctgcagaaggataagatggccgaggcctatagcgagatc
 ggcataagggagagaggcgccggggcaaggacacgacggcctgtatcagggcctgtccaccgctaccaaagacacctatga
 tgctctgcacatgcaggctctgccaccaagatga

[0142] SEQ ID NO: 173:

10 [0143] MALPVTALLLPLALLLHAARSPDTRVETATETAWISLVTALHLVLGLNA
 VLGLLLLRKQFPAHYRRLRHALLWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEE
 VEPSLLEILPKSSERTPLPLEDEGVAGAPTGSSPQLQPLSGEDDAYCTFPSRDDLLL
 FSPSQGGEFRALNARLPLNTDAYLSLQELQGQDPTHLVGSGATNFSLLKQAGDVEE
 NPGPMALPVTALLLPLALLLHAARPEVQLLES GGGLVQPGGSLRLSCAASGFTFSSY
 15 AMNWVRQAPGKGLEWVSAISDSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLR
 AEDTAVYYCARYWPMDIWGQGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTL S
 LSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLMYDASIRATGIPDRFSGSGSG
 TDFTLTISRLEPEDFAVYYCQQYGSWPLTFGQGTKVEIKSGGGGSCPYSNPSLCSG
 GGGSCPYSNPSLCSGGGGSTTPAPRPPTAPTIASQPLSLRPEACRPAAGGAVHTRG
 20 LDFACDIYWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDG
 CSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRG
 RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLS
 TATKDTYDALHMQALPPR

[0144] SEQ ID NO: 174: wt WPRE

25 [0145] aatcaacctctggattacaaaatttgtaaagattgactggattcttaactatgttgctcctttacgctatgtggatacgt
 gctttaatgcctttgtatcatgctattgcttcccgatggccttcattttctcctcctgtataaatcctggtgctgtctctttataggagttgt
 ggcccggtgtcaggcaacgtggcgtggtgtgactgtgtttgctgacgcaacccccactggtggggcattgccaccacctgtcagc
 tcctttccgggactttgcctttccccctccctattgccacggcggaactcatcgccgctgcttgcctgctgacaggggctcg
 gctgttgggcactgacaattccgtggtgtgtcggggaagctgacgtcctttccatggctgctgcctgtgttgcacctggattctgc
 30 gcgggacgtccttctgtacgtcccttcggccctcaatccagcggaccttccctcccgcgctgctgccggctctgcggcctcttcc
 gcgtcttcgcttcgcccctcagacgagtcggatctcccttggggcgccctccccgcctg

[0146] SEQ ID NO: 175: mWPRE

[0147] aatcaacctctggattacaaaatttgtgaaagattgactgggtattcttaactatgttgctcctttacgctatgtggatacgcct
 gctttaatgcctttgtatcatgctattgcttcccgtatggccttcattttctcctccttgataaatcctgggtgctgtctctttataggagtgtg
 ggcccgttgcaggcaacgtggcgtgggtgacactgtgtttgctgacgcaacccccactgggtggggcattgccaccacctgtcagc
 tcctttccgggactttcgctttccccctccctattgccacggcggaactcatcgccgcctgccttgcccgcctgctggacaggggctcg
 5 gctgttgggcactgacaattccgtggtgtgctggggaatcatcgctcctttcctggctgctgcctgtgttccacctggattctgcg
 cgggacgtccttctgctacgtcccttcggccctcaatccagcggaccttctcccgcgccctgctgccggctctgcggcctcttcg
 cgtcttcgccttcgcctcagacgagtcggatctccctttggcgccctccccgcctg

[0148] In embodiments of the invention, a bicistronic vector comprising the nucleotide
 sequence of SEQ ID NO: 176 encodes a predicted CACCR BCMA CAR polypeptide
 10 having the predicted amino acid sequence of SEQ ID NO: 177, which comprises the
 following domains: CD8 signal peptide (e.g. SEQ ID NO: 89), transmembrane/JAK2
 binding domain - TpoR(478-582;H499L,S505N,W515K) (e.g. SEQ ID NO: 12),
 IL2Rb(339-379,393-433,518-551) (e.g. SEQ ID NO: 78), P2A (e.g. SEQ ID NO: 105), CD8
 alpha signal sequence (e.g., SEQ ID NO:89), and P5A2 BCMA CAR with a safety switch
 15 (e.g. SEQ ID NO: 166). In aspects of this embodiment, the bicistronic vector further
 comprises, following the polypeptide coding sequence, a wild-type WPRE of e.g. SEQ ID
 NO: 174 or a mutant WPRE ("mWPRE") of e.g. nucleotide sequence of SEQ ID NO: 175.
 In other aspects of this embodiment, the bicistronic vector does not include a wild-type
 WPRE or a mutant WPRE. In some aspects, the disclosure provides an engineered immune
 20 cell that comprises the polynucleotide sequence of SEQ ID NO: 176 and expresses a
 CACCR comprising the amino acid sequence of SEQ ID NO: 185 or 100, and a BCMA
 CAR comprising the amino acid sequence of SEQ ID NO: 166. In some embodiments, the
 disclosure provides an engineered immune cell that expresses a CACCR comprising the
 amino acid sequence of SEQ ID NO: 181, 185 or 100, and a BCMA CAR comprising the
 25 amino acid sequence of SEQ ID NO: 166. In some embodiments, the engineered immune
 cell is an engineered T cell. In some embodiments, the engineered T cell is an allogeneic T
 cell. In some embodiments, the engineered T cell is an autologous T cell.

[0149] SEQ ID NO: 176:

[0150] atggccctgccagtaccgccctgctgctgccactggccctgctgctgcacgcagcaaggccatcagaccctactag
 30 agtcgagaccgctaccgagaccgttgatctctctggtgaccgccctgctgctggtgctgggcctgaacgccgtgctgggcctgc
 tgctgctgaggaagcagttcccagcacactaccggagactgaggcacgcactgtggccaagcctccccacctgcacaggggtgc
 tgggacagtatctgaggatacagccgccctgagccacctaaggcaaccgtgtccgacacatgcgaggaggtggaaccaagtc

tgctggaatcctgcaaaatcctctgagcggacacccctgcccctgctcgagcagcaggacaaggtgcccagcctgcctccct
 gagctccaaccacagcctgacctcctgctttacaatcagggtactcttttccacctgcctgacgccctggagatcgaggcctgtc
 aggatgaggagtgaggcaggagcacctaccggctctagcccacagccactgcagccactgtctggagaggacgatgcctactgca
 cattccccagccgggacgatctgctgctgtttcccttctggacaggagaggtccgggcccctgaacgcaagactgccactgaata
 5 ccgacgcctatctgtctctgcaggagctgcagggccaggaccccacacacctggtgggatccggagccaccaacttctccctgctg
 aagcaggccggcgatgtggaggagaatccaggccccatggctctgcccgtcaccgcactgctgctgcccctggctctgctgctgc
 acgccgcaagaccgaggtccagctgctggaatctgggggaggactggtgcagcctggaggcagcctgagactgtcctgcgca
 gcatctggcttcacctcagctcctacgcatgaactgggtgaggcaggcaccaggcaagggactggagtgggtgtctgccatctc
 cgactctggcggcagcacctactatgccgattccgtgaaggggccgcttcacaatcagccgggataactccaagaataacctgtacct
 10 gcagatgaattccctgagagccgaggatacagccgtgtactattgcccaggtattggcccatggacatctggggccagggcaca
 ctggtgacctgtcttccggaggaggaggtccggaggaggaggtctgcccggcggcgagcgagatcgtgctgacacagtct
 cctggcacccctgagcctgtccccaggagagagagccacctgagctgtaggccctctcagagcgtgtccttagctacctggcctg
 gtatcagcagaagcccggccaggcccctagactgtctgtatgacgcccagcatcagggcaacaggcatccctgatcggttctcc
 ggctctggcagcgggaaccgactttactgaccatcagcaggtggagcccaggacttcgctgtactattgccagcagtatgg
 15 ctctggcctctgacatttggccaggccaccaaggtggagatcaagggtccggcggcgaggctcttgcatacagcaacca
 tccctgtgctctggaggaggaggtcctgtccttatagcaatcctagcctgtgctccggcggaggaggtctaccacaaccccagca
 ccaaggccacctacacctgcaccaaccatgcctctcagccactgagcctgagacccgaggcctgtaggcctgcagcaggagga
 gcagtgcacacccgggactggactttgctgcatatctacatctgggcaccactggcaggaacatgtggcgtgctgctgctgag
 cctggtcatcacctgtactgcaagagaggcaggaagaagctgctgtatatcttcaagcagcccttatgcccctgtgcagacaac
 20 ccaggaggaggatggctgctcctgtcggttcccaggaggaggaggaggatgtgagctgcccgtgaagtttccggctgccc
 gacgcaccagcataccagcagggccagaaccagctgtataacgagctgaatctggccggagagaggagtacgactgctgga
 taagaggagggggaagatcccagatgggaggcaagccacggagaaagaacccccaggagggcctgtacaatgagctgcag
 aaggataagatggccgagccctatagcgagatcgcatgaaggagagaggcgggggcaagggacacgacggcctgtatc
 agggcctgtccaccgctaccaagacacctatgatgctctgcacatgcaggctctgccaccaagatga

25 [0151] SEQ ID NO: 177

[0152] MALPVTALLLPLALLLHAARPSDPTRVETATETAWISLVTALLLVLGLNA
 VLGLLLLRKQFPAHYRRLRHAWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEE
 VEPSLLEILPKSSERTPLPLLEQQDKVPEPASLSSNHSLTSCFTNQGYFFHLPDALEI
 EACQDEGVAGAPTGSSPQPLQPLSGEDDAYCTFSPSRDDLLLFSPSGQGEFRALNARL
 30 PLNTDAYLSLQELQGQDPHTLVGSGATNFSLLKQAGDVEENPGPMALPVTALLLPL
 ALLLHAARPEVQLLES GGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLE
 WWSAISDSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARYWP
 MDIWGQGTLVTVSSGGGGSGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQ

SVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFA
 VYYCQQYGSWPLTFGQGTKVEIKSGGGGSCPYSNP SLCSGGGGSCPYSNP SLCSG
 GGGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLA
 GTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCE
 5 LRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
 PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ
 ALPPR

[0153] In embodiments of the invention, a bicistronic vector comprising the nucleotide sequence of SEQ ID NO: 178 encodes a predicted CACCR BCMA CAR polypeptide
 10 having the predicted amino acid sequence of SEQ ID NO: 179, which comprises the following domains: CD8 signal peptide (e.g. SEQ ID NO: 89), transmembrane/JAK2 binding domain - TpoR(478-582; H499L,S505N,W515K) (e.g. SEQ ID NO: 12), IL2Rb(393-433,518-551) (e.g. SEQ ID NO: 77), P2A (e.g. SEQ ID NO: 105), CD8 alpha signal sequence (e.g., SEQ ID NO:89), and P5A2 BCMA CAR with a safety switch (e.g.
 15 SEQ ID NO: 141). In aspects of this embodiment, the bicistronic vector further comprises, following the polypeptide coding sequence, a wild-type WPRE of e.g. SEQ ID NO: 174 or a mutant WPRE ("mWPRE") of e.g. nucleotide sequence of SEQ ID NO: 175. In other aspects of this embodiment, the bicistronic vector does not include a wild-type WPRE or a mutant WPRE.

20 [0154] In some embodiments, the disclosure provides an engineered immune cell that comprises the polynucleotide sequence of SEQ ID NO: 178 and expresses a CACCR comprising the amino acid sequence of SEQ ID NO: 186 or 143 and a BCMA CAR comprising the amino acid sequence of SEQ ID NO: 166. In some embodiments, the disclosure provides an engineered immune cell that expresses a CACCR comprising the
 25 amino acid sequence of SEQ ID NO: 182, 186 or 143, and a BCMA CAR comprising the amino acid sequence of SEQ ID NO: 166. In some embodiments, the engineered immune cell is an engineered T cell. In some embodiments, the engineered T cell is an allogeneic T cell. In some embodiments, the engineered T cell is an autologous T cell.

[0155] SEQ ID NO: 178:

30 [0156] atggccctgccagtgaccgccctgctgctgccactggccctgctgctgcacgcagcaaggccatcagaccctactag
 agtcgagaccgctaccgagaccgcttgatctctctggtgaccgccctgctgctggtgctgggcctgaacgccgtgctgggcctgc
 tgctgctgaggaagcagttcccagcacactaccggagactgaggcacgcactgtggccaagcctgcccacactgcacaggggtgc

tgggacagtatctgagggatacagccgacctgagcccacctaaggcaaccgtgtccgacacatgcgaggaggtggaaccaagtc
 tgctggaatcctgcaaaatcctctgagcggacacccctgcccctgctcgaggacgaggagtgaggagcagcacaacccggca
 gctccccccagcctctgcagccactgtccggagaggacgatgcatactgcacattcccttctcgggacgatctgctgtgttctctcc
 aagcggacagggagagtttcgggacctgaacgccagactgcccctgaataccgacgcctatctgagcctgcaggagctgcaggg
 5 acaggacccccacacacctggtgggatccggagccaccaacttctccctgctgaagcaggccggcgatgtggaggagaatccagg
 ccccatggctctgcccgtcaccgactgctgtgcccctggctctgctgctgcacgccgcaagacccgaggtccagctgctggaat
 ctgggggaggactggtgcagcctggaggcagcctgagactgtcctgcgagcatctggcttcaccttcagctcctacgccatgaac
 tgggtgaggcaggcaccaggcaaggagctggagtgggtgtctgccatctccgactctggcggcagcacctactatgccgattccg
 tgaaggccgcttcacaatcagccgggataactccaagaataccctgtacctgcagatgaattccctgagagccgaggatacagcc
 10 gtgtactattgcccaggtattggccatggacatctggggccagggcacactggtgacctgtcttccggaggaggaggctccgg
 aggaggaggctctggcggcgccggcagcgagatcgtgctgacacagtctcctggcaccctgagcctgtccccaggagagagag
 ccacctgagctgtaggcctctcagagcgtgtcctctagctacctggcctggtatcagcagaagcccggccaggcccttagactg
 ctgatgtacgagccagcatcagggaacaggcatccctgatcggttctccggctctggcagcggaaaccgactttacactgacat
 cagcaggctggagcccaggacttcgctgtactattgccagcagtatggctcctggcctctgacatttggccagggcaccaagg
 15 tggagatcaagggtccggcgaggaggtcttggccatacagcaaccatccctgtgctctggaggaggaggctcctgtccttat
 agcaatcctagcctgtgctccggcgaggaggtctaccacaacccagcaccagggccacctacacctgcaccaacctgcct
 ctgagccactgagcctgagacccgaggcctgtaggcctgcagcaggaggagcagtgacacccggggactggactttgctgctg
 atatctacatctgggcaccactggcaggaacatgtggcgtgctgctgctgagcctggtcatccctgtactgcaagagaggcagg
 aagaagctgctgtatatcttcaagcagcccttatgcccctgtgcagacaacccaggaggaggatggctgctcctgtcgttccca
 20 gaggaggaggaggaggatgtgagctgcgctgaagtttcccggtctgccgacgcaccagcataccagcagggccagaacca
 gctgtataacgagctgaatctggccggagagaggagtacgacgtgctggataagaggagggaagagatcccagatgggag
 gcaagccacggagaaagaacccccaggagggcctgtacaatgagctgcagaaggataagatggccgaggcctatagcgagatc
 ggcatgaaggagagaggcgccggggcaaggacacgacggcctgtatcagggcctgtccaccgctaccaaaagacacctatga
 tgctctgcacatgcaggctctgccaccaagatga

25 [0157] SEQ ID NO: 179:

[0158] MALPVTALLLPLALLLHAARPSDPTRVETATETAWISLVTALLLVLGLNA
 VLGLLLLRKQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEE
 VEPSLLEILPKSSERTPLPLEDEGVAGAPTGSSPQLQPLSGEDDAYCTFPSRDDLLL
 FSPSQGEFRALNARLPLNTDAYLSLQELQGQDPTHLVGSGATNFSLLKQAGDVEE
 30 NPGPMALPVTALLLPLALLLHAARPEVQLLES GGGLVQPGGSLRLSCAASGFTFSSY
 AMNWVRQAPGKGLEWVSAISDSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLR
 AEDTAVYYCARYWPMDIWGQGLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTL
 LSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSG

TDFTLTISRLEPEDFAVYYCQQYGSWPLTFGQGTKVEIKSGGGGSCPYSNPSLCSG
 GGGSCPYSNPSLCSGGGGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG
 LDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDG
 CSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG
 5 RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS
 TATKDTYDALHMQALPPR

[0159] In some embodiments of the invention, a bicistronic vector encodes a polypeptide that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 173, 177 or 179. In some embodiments, the invention further
 10 provides a cell, such as an immune cell, e.g. a T cell, that contains a bicistronic vector that encodes a polypeptide that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 173, 177 or 179. In some
 embodiments, the invention further provides a method of treating a disease characterized by the expression of BCMA, such as multiple myeloma, comprising administering such a cell
 15 to a patient suffering from the disease.

[0160] In some embodiments, the CAR-immune cell (e.g., CAR-T cell) of the disclosure comprises a polynucleotide encoding a suicide polypeptide, such as for example RQR8 or R2. See, *e.g.*, WO2013153391A, which is hereby incorporated by reference in its entirety. In some
 20 embodiments, a suicide polypeptide is expressed on the surface of the cell. In some
 embodiments, a suicide polypeptide is included in the CAR construct. In some
 embodiments, a suicide polypeptide is not part of the CAR construct.

[0161] In some embodiments, the extracellular domain of any one of the CARs disclosed herein may comprise one or more epitopes specific for (specifically recognized by) a monoclonal antibody. These epitopes are also referred to herein as mAb-specific epitopes.
 25 Exemplary mAb-specific epitopes are disclosed in International Patent Publication No. WO 2016/120216, which is incorporated herein in its entirety. In these embodiments, the extracellular domain of the CARs comprises antigen binding domains that specifically bind to a target of interest and one or more epitopes that bind to one or more monoclonal antibodies (mAbs). CARs comprising the mAb-specific epitopes can be single-chain or
 30 multi-chain.

[0162] The inclusion of epitopes specific for monoclonal antibodies in the extracellular domain of the CARs described herein allows sorting and depletion of engineered immune

cells expressing the CARs. In some embodiments, allowing for depletion provides a safety switch in case of deleterious effects, e.g., upon administration to a subject.

[0163] Methods of preparing engineered immune cells for use in immunotherapy are also provided herein. In some embodiments, the methods comprise introducing a CACCR and a
5 CAR into immune cells, and expanding the cells. In some embodiments, the invention relates to a method of engineering an immune cell comprising: providing a cell and expressing a CACCR, and expressing at the surface of the cell at least one CAR. In some embodiments, the method comprises: transfecting the cell with at least one polynucleotide encoding a CACCR, and at least one polynucleotide encoding a CAR, and expressing the
10 polynucleotides in the cell. In some embodiments, the method comprises: transfecting the cell with at least one polynucleotide encoding a CACCR, at least one polynucleotide encoding a CAR, and expressing the polynucleotides in the cell.

[0164] In some embodiments, the polynucleotides encoding the CACCR and CAR are present in one or more expression vectors for stable expression in the cells. In some
15 embodiments, the polynucleotides are present in viral vectors for stable expression in the cells. In some embodiments, the viral vectors may be for example, lentiviral vectors or adenoviral vectors.

[0165] In some embodiments, polynucleotides encoding polypeptides according to the present disclosure can be mRNA which is introduced directly into the cells, for example by
20 electroporation. In some embodiments, CytoPulse electroporation technology, such as PulseAgile, can be used to transiently permeabilize living cells for delivery of material into the cells (e.g. US 6,078,490; PCT/US2011/000827; and PCT/US2004/005237). Parameters can be modified in order to determine conditions for high transfection efficiency with minimal mortality.

[0166] Also provided herein are methods of transfecting an immune cell, e.g a T cell. In some embodiments, the method comprises: contacting a T cell with RNA and applying to
25 the T cell an agile pulse sequence. In some embodiments, a method of transfecting an immune cell (e.g. T cell) comprising contacting the immune cell with RNA and applying to the cell an agile pulse sequence.

[0167] In some embodiments, the method can further comprise a step of genetically
30 modifying a cell by inactivating at least one gene expressing, for example without limitation, a component of the TCR, a target for an immunosuppressive agent, an HLA

gene, and/or an immune checkpoint protein such as, for example, PDCD1 or CTLA-4. By inactivating a gene it is intended that the gene of interest is not expressed in a functional protein form. In some embodiments, the gene to be inactivated is selected from the group consisting of, for example without limitation, TCR α , TCR β , CD52, GR, deoxycytidine kinase (DCK), PD-1, and CTLA-4. In some embodiments the method comprises inactivating one or more genes by introducing into the cells a rare-cutting endonuclease able to selectively inactivate a gene by selective DNA cleavage. In some embodiments the rare-cutting endonuclease can be, for example, a transcription activator-like effector nuclease (TALE-nuclease) or CRISPR-based endonuclease (e.g Cas-9 or Cas12a).

10 [0168] In another aspect, a step of genetically modifying cells can comprise: modifying immune cells (e.g. T cells) by inactivating at least one gene expressing a target for an immunosuppressive agent, and; expanding the cells, optionally in the presence of the immunosuppressive agent.

[0169] In some embodiments, the engineered immune cells (e.g. T cells) provided herein exhibit improved cytotoxicity, increased expansion, and/or increased levels of memory phenotype markers relative to engineered immune cells that do not express the CACCR.

[0170] In some embodiments, the engineered immune cells (e.g. T cells) provided herein exhibit (i) increased *in vivo* persistence, (ii) increased STAT activation, (iii) increased cytotoxicity, (iv) increased levels of memory phenotype markers, (v) increased expansion (proliferation), or combinations of these functional features constitutively, relative to engineered immune cells that do not express the CACCR. In some embodiments, the improvement in the one or more functional features described herein is tunable, dependent upon the mutations/modifications introduced to the CACCR. In some embodiments, STATs activated by the engineered immune cell comprising one or more CACCRs disclosed are STAT1, STAT2, STAT3, STAT4, STAT5, STAT6, or combinations thereof. In one embodiment, memory phenotype markers that are increased or maintained by the immune cell comprising the CACCR include stem cell memory (Tscm) marker and central memory (Tcm) marker.

[0171] In some embodiments, the improvement in one or more functional features exhibited by an engineered immune cell comprising a CACCR provided herein is at least about 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 25 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold,

100 fold, 125 fold, 150 fold, 200 fold, 250 fold, 300 fold, 350 fold, 400 fold, 450 fold, or even about 500 fold, including values and ranges therebetween, compared to an immune cell that does not express the CACCR.

[0172] In some embodiments, the improvement in one or more functional features exhibited by an engineered immune cell comprising a CACCR provided herein is at least about 10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, 75%, 80%, 90%, 100%, 125%, 150%, 200%, 250%, 300%, 350%, 400%, or even about 500%, including values and ranges therebetween, compared to an engineered immune cell that does not express the CACCR.

10 III. Therapeutic Methods

[0173] Provided herein are pharmaceutical compositions comprising cells bearing the CACCRs and CARs of the disclosure.

[0174] Engineered CACCR-bearing and BCMA CAR-bearing immune cells (e.g. BCMA CAR-T cells) obtained by the methods described above, or cell lines derived from such engineered immune cells, can be used as a medicament. In some embodiments, such a medicament can be used for treating a disorder such as for example a viral disease, a bacterial disease, a cancer, an inflammatory disease, an immune disease, or an aging-associated disease. In some embodiments, the cancer is a solid cancer. In some embodiments the cancer is a liquid cancer. The cancer can be selected from the group consisting of gastric cancer, sarcoma, lymphoma, leukemia, head and neck cancer, thymic cancer, epithelial cancer, salivary cancer, liver cancer, stomach cancer, thyroid cancer, lung cancer, ovarian cancer, breast cancer, prostate cancer, esophageal cancer, pancreatic cancer, glioma, leukemia, multiple myeloma, B cell malignancy, diffused large B-cell lymphoma, renal cell carcinoma, bladder cancer, cervical cancer, choriocarcinoma, colon cancer, oral cancer, skin cancer, and melanoma. In some embodiments, the subject is a previously treated adult subject with locally advanced or metastatic melanoma, squamous cell head and neck cancer (SCHNC), ovarian carcinoma, sarcoma, or relapsed or refractory classic Hodgkin's Lymphoma (cHL).

[0175] In some embodiments, engineered immune cells, or a cell line derived from the engineered immune cells, can be used in the manufacture of a medicament for treatment of a disorder in a subject in need thereof. In some embodiments, the disorder can be, for example, a cancer, an autoimmune disorder, or an infection.

[0176] Also provided herein are methods for treating subjects in need of such treatment.

[0177] As used herein, the term “subject” refers to any vertebrate including, without limitation, humans and other primates (*e.g.*, chimpanzees, cynomolgous monkeys, and other apes and monkey species), farm animals (*e.g.*, cattle, sheep, pigs, goats and horses),
5 domestic mammals (*e.g.*, dogs and cats), laboratory animals (*e.g.*, rabbits, rodents such as mice, rats, and guinea pigs), and birds (*e.g.*, domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like). In some embodiments, the subject is a mammal. In exemplary embodiments, the subject is a human.

[0178] In some embodiments the method comprises providing immune cells of the disclosure, bearing the CACCRs and CARs (*e.g.*, a BCMA CAR) described herein to a
10 subject in need thereof.

[0179] In some embodiments, CACCR and CAR-bearing T cells of the invention can undergo robust *in vivo* T cell expansion and can persist for an extended amount of time.

[0180] Methods of treatment of the invention can be ameliorating, curative or
15 prophylactic. The method of the invention may be either part of an autologous immunotherapy or part of an allogenic immunotherapy treatment.

[0181] In another aspect, the invention provides a method of inhibiting tumor growth or progression in a subject who has a tumor, comprising administering to the subject an effective amount of CACCR-expressing and CAR-expressing (*e.g.*, BCMA CAR-
20 expressing) immune cells as described herein. In another aspect, the invention provides a method of inhibiting or preventing metastasis of cancer cells in a subject, comprising administering to the subject in need thereof an effective amount of engineered immune cells as described herein. In another aspect, the invention provides a method of inducing tumor regression in a subject who has a tumor, comprising administering to the subject an
25 effective amount of engineered immune cells as described herein.

[0182] In some embodiments, the engineered T cells herein can be administered parenterally in a subject. In some embodiments, the engineered T cells herein can be administered intravenously in a subject.

[0183] Also provided is the use of any of the engineered T cells provided herein in the
30 manufacture of a medicament for the treatment of cancer or for inhibiting tumor growth or progression in a subject in need thereof.

[0184] In some embodiments, treatment can be administered into subjects undergoing an immunosuppressive treatment. Indeed, the invention preferably relies on cells or population of cells, which have been made resistant to at least one immunosuppressive agent due to the inactivation of a gene encoding a receptor for such immunosuppressive agent. In this aspect, the immunosuppressive treatment should help the selection and expansion of the T cells according to the invention within the subject. The administration of the cells or population of cells according to the invention may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a subject subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous or intralymphatic injection, or intraperitoneally. Cells bearing the CACCRs and CARs (e.g. a BCMA CAR) of the disclosure or the pharmaceutical compositions thereof may be administered via one or more of the following routes of administration: intravenous, intraocular, intravitreal, intramuscular, subcutaneous, topical, oral, transdermal, intraperitoneal, intraorbital, by implantation, by inhalation, intrathecal, intraventricular, via the ear, or intranasal.

[0185] In some embodiments the administration of the cells or population of cells (bearing the CACCRs and CARs, e.g., a BCMA CAR, of the disclosure) can comprise administration of, for example, about 10^4 to about 10^9 cells per kg body weight including all integer values of cell numbers within those ranges. In some embodiments the administration of the cells or population of cells can comprise administration of about 10^4 to 10^5 cells per kg body weight, 10^5 to 10^6 cells per kg body weight, 10^6 to 10^7 cells per kg body weight, 10^7 to 10^8 cells per kg body weight, or 10^8 to 10^9 cells per kg body weight, . The cells or population of cells can be administered in one or more doses. In some embodiments, said effective amount of cells can be administered as a single dose. In some embodiments, said effective amount of cells can be administered as more than one dose over a period of time. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the subject. The cells or population of cells may be obtained from any source, such as a blood bank or a donor. While individual needs vary, determination of optimal ranges of effective amounts of a given cell type for a particular disease or conditions within the skill of the art. An effective amount means an amount which provides a therapeutic or prophylactic benefit. The dosage administered will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment,

if any, frequency of treatment and the nature of the effect desired. In some embodiments, an effective amount of cells or composition comprising those cells are administered parenterally. In some embodiments, administration can be an intravenous administration. In some embodiments, administration can be directly done by injection within a tumor.

5 [0186] In line with the above, an appropriate dosage of a cell of the invention (e.g., a CACCR-expressing and a BCMA CAR-expressing allogeneic or autologous T cells) may be between about 7×10^6 cells and about 480×10^6 cells. In certain embodiments, an appropriate dose may range from about 20×10^6 cells/dose to about 480×10^6 cells/dose, more particularly about 20×10^6 cells/dose, about 40×10^6 cells/dose, about 60×10^6
10 cells/dose, about 80×10^6 cells/dose, about 100×10^6 cells/dose, about 120×10^6 cells/dose, about 160×10^6 cells/dose, about 240×10^6 cells/dose, about 300×10^6 cells/dose, about 320×10^6 cells/dose, about 360×10^6 cells/dose, about 400×10^6 cells/dose, about 440×10^6 cells/dose, or about 480×10^6 cells/dose.

[0187] In certain embodiments, when the weight of the subject is 50 kg or more, and at
15 least one dose of cells is to be administered to the patient, an appropriate dose may range from about 20×10^6 cells/dose to about 480×10^6 cells/dose, more particularly about 20×10^6 cells/dose, about 40×10^6 cells/dose, about 80×10^6 cells/dose, about 120×10^6 cells/dose, about 240×10^6 cells/dose, about 320×10^6 cells/dose, about 360×10^6 cells/dose, or about 480×10^6 cells/dose. In certain embodiments, when the weight of the
20 subject is less than 50 kg, and at least one dose of cells is to be administered to the patient, an appropriate dose may range from about 7×10^6 cells/dose to about 360×10^6 cells/dose, more particularly about 7×10^6 cells/dose, about 14×10^6 cells/dose, about 20×10^6 cells/dose, about 80×10^6 cells/dose, about 240×10^6 cells/dose, or about 360×10^6 cells/dose.

25 [0188] The methods can further comprise administering one or more agents to a subject prior to administering the engineered immune cells bearing a CAR (e.g., a BCMA CAR) and a CACCR provided herein. In certain embodiments, the agent is a lymphodepleting (preconditioning) regimen. For example, methods of lymphodepleting a subject in need of such therapy comprise administering to the subject specified beneficial doses of
30 cyclophosphamide (between $200 \text{ mg/m}^2/\text{day}$ and $2000 \text{ mg/m}^2/\text{day}$, about $100 \text{ mg/m}^2/\text{day}$ and about $2000 \text{ mg/m}^2/\text{day}$; e.g., about $100 \text{ mg/m}^2/\text{day}$, about $200 \text{ mg/m}^2/\text{day}$, about $300 \text{ mg/m}^2/\text{day}$, about $400 \text{ mg/m}^2/\text{day}$, about $500 \text{ mg/m}^2/\text{day}$, about $600 \text{ mg/m}^2/\text{day}$, about 700

mg/m²/day, about 800 mg/m²/day, about 900 mg/m²/day, about 1000 mg/m²/day, about 1500 mg/m²/day or about 2000 mg/m²/day) and specified doses of fludarabine (between 20 mg/m²/day and 900 mg/m²/day, between about 10 mg/m²/day and about 900 mg/m²/day; e.g., about 10 mg/m²/day, about 20 mg/m²/day, about 30 mg/m²/day, about 40 mg/m²/day, 5 about 40 mg/m²/day, about 50 mg/m²/day, about 60 mg/m²/day, about 70 mg/m²/day, about 80 mg/m²/day, about 90 mg/m²/day, about 100 mg/m²/day, about 500 mg/m²/day or about 900 mg/m²/day). An exemplary dosing regimen involves treating a subject comprising administering daily to the patient about 300 mg/m²/day of cyclophosphamide in combination or before or after administering about 30 mg/m²/day of fludarabine for three 10 days prior to administration of a therapeutically effective amount of engineered immune cells to the patient.

[0189] In some embodiments, notably in the case when the engineered cells provided herein have been gene edited to eliminate or minimize surface expression of CD52, lymphodepletion further comprises administration of an anti-CD52 antibody, such as 15 alemtuzumab (CAS (Chemical Abstract Service) Registry # 216503-57-0). In some embodiments, the CD52 antibody is administered at a dose of about 1-20 mg/day IV, e.g., about 13 mg/day IV for 1, 2, 3 or more days, or about 20 mg/day IV for 1, 2, 3 or more days. In some embodiments, the CD52 antibody is administered at a dose of about 20-30 mg/day IV, e.g., about 30 mg/day IV for 1, 2, 3 or more days. The antibody can be 20 administered in combination with, before, or after administration of other elements of a lymphodepletion regime (e.g., cyclophosphamide and/or fludarabine).

[0190] In some embodiments, an effective amount of engineered immune cells can be administered to the patient after the initial or first dosing (i.e., redosing). The second or subsequent dosing can be the same as, or higher or lower than, the amount of engineered 25 immune cells of previous dosing. In some embodiments, a lymphodepleting regimen is administered to the patient before a second or subsequent dosing of the engineered immune cells. In some embodiments, the second or subsequent dosing is not preceded with a lymphodepletion regimen.

[0191] In certain embodiments, compositions comprising CACCR and CAR-expressing 30 (e.g., a BCMA CAR-expressing) immune effector cells disclosed herein may be administered in conjunction with any number of chemotherapeutic agents.

[0192] In certain embodiments, patients who have received CACCR BCMA CAR T cells of the disclosure are subsequently administered an effective amount of ribuximab and/or dasatinib to reduce or mitigate any potential safety concerns during the course of CAR T treatment. In certain embodiments, the patients receive BCMA CAR T therapy for the treatment of multiple myeloma or other BMCA positive cancers.

[0193] IV. Kits and Articles of Manufacture

[0194] The present disclosure provides kits comprising any one or more of the CACCR- and CAR-bearing cells described herein (e.g. a cell, for example an immune cell such as a T cell, bearing a CACCR and a BCMA CAR), and pharmaceutical compositions thereof. The present disclosure also provides articles of manufacture comprising any one or more of the CACCR- and CAR-bearing (e.g., BCMA CAR-bearing) CAR-I cells described herein (e.g. a cell, for example an immune cell such as a T cell, bearing a CACCR and a BCMA CAR), pharmaceutical compositions thereof, and kits described herein.

[0195] The following examples are included for illustrative purposes and are not intended to limit the scope of the disclosure.

[0196] All patent and non-patent documents referenced throughout this disclosure are incorporated by reference herein in their entirety for all purposes.

[0197] FIG. 1 shows a schematic representation of an engineered constitutively active chimeric cytokine receptor as disclosed herein and used in the following examples. FIG. 2 shows schematic representations of truncated cytotoil polypeptides of the disclosure that are components of the CACCR in the following examples. FIGs. 2B-2D show exemplary bicistronic vectors for the expression of both a CACCR and a CAR, which can be used in experiments and procedures such as those described below for the generation of cells that co-express a CAR and a CACCR.

EXAMPLES

Example 1: Optimized IL2Rb-derived cytotoils more closely mimic signaling of IL-15, rather than IL-2

[0198] IL-2 and IL-15 are two cytokines that naturally signal through a heterodimeric cytokine receptor comprised of the common-gamma chain and IL2Rb. In spite of sharing the same native receptors, IL-2 and IL-15 exert different effects on T cell differentiation and persistence. Whereas IL-2 induces short-lived effector differentiation, IL-15 promotes the

generation of long-lived memory T cells. Furthermore, increased serum concentrations of IL-15 has been shown to correlate positively with patient response to CAR-T cell therapy. CACCRs that mimic the signaling and effects of IL-15, rather than IL-2, are therefore preferred. We sought to determine if the truncated IL2Rb cytotails more closely mimicked
5 IL-2 or IL-15 signaling.

[0199] To this end, we utilized CAR-T cells comprising an exemplary BCMA CAR bearing the P5A2 scFv directed towards BCMA, coupled to rituximab mimotopes, 4-1BB and CD3z signaling domains (see US Pat. 10,294,304, incorporated herein by reference). BCMA specific CAR-T cells co-expressing the truncated IL2Rb tails were generated, and
10 their gene expression profiles compared to control CAR-T cells that had been exposed to exogenous recombinant human IL-2 or IL-15. To make lentivirus encoding a CACCR and BCMA CAR, HEK293T cells were plated at 0.45 million cells per mL in 2mL of DMEM (Gibco) supplemented with 10% FBS (Hyclone) per well of a 6-well plate the day before transfection. On the day of transfection, the lentivirus was prepared by mixing together
15 lentiviral packaging vectors 1.5 ug psPAX2, 0.5 ug pMD2G, and 0.5 ug of the appropriate transfer CAR vector in 250 uL Opti-MEM (Gibco) per well of the 6-well plate ("DNA mix"). 10 uL Lipofectamine 2000 (Invitrogen) in 250 uL Opti-MEM was incubated at room temperature for 5 minutes and then added to the DNA mix. The mixture was incubated at room temperature for 20 minutes and the total volume of 500 uL was slowly added to the
20 sides of the wells containing HEK293T. One day post-transfection, the media from each well of HEK293T cells in the 6-well plate was replaced with 2mL per well of T cell transduction media, i.e., X-Vivo-15 supplemented with 10% FBS. Two days post-transfection, the lentiviral supernatants from HEK293T cells were harvested and passed through a 0.45 micron filter (EMD Millipore) to remove cell debris, and crude lentiviral
25 supernatants were used directly for T cell transduction. On Day 0, purified T cells were activated in X-Vivo-15 medium (Lonza) supplemented with 100 IU/mL human IL-2 (Miltenyi Biotec), 10% FBS (Hyclone), and human T TransAct (Miltenyi Biotec, Cat# 130-111-160, 1:100 dilution) in a Grex-24 plate (Wilson Wolf, cat# 80192M). On Day2, T cells were resuspended at 0.5 million cells per mL in T cell transduction media, transduced with
30 an equal volume of crude lentiviral supernatant along with 100 IU/mL human IL-2 in a Grex-24 plate. On Day 5, CACCR-expressing CAR-T cells were fed by replacing the spent media with T cell expansion media, i.e., X-Vivo-15 supplemented with 5% human AB serum (Gemini Bio), along with 100 IU/mL human IL-2. At this time, control CAR-T cells

lacking CACCR were expanded in either 100 U/mL human IL-2 only, or 100 U/mL human IL-2 and 10 ng/mL human IL-15 (Miltenyi Biotec). Cells were expanded into larger G-Rex vessels (Wilson Wolf) as needed using T cell expansion media and the respective concentrations of recombinant cytokines. On Day 13, cells were stained with the Zombie

5 NIR Fixable Viability Kit (Biolegend), labelled with a BUV395-conjugated CD3 antibody (Biolegend) and an anti-idiotypic antibody specific for the P5A2 scFv, then FACS-sorted to enrich for CAR⁺ T cells. Sorted CAR⁺ T cells were then cultured in Grex-24 plates for a further 2 days in T cell expansion media, with CACCR BCMA CAR⁺ T cells left in the absence of exogenous cytokines, and with sorted control CAR⁺ T cells either left in the

10 absence of exogenous cytokines, treated with 100 U/mL human IL-2, or treated with 10 ng/mL human IL-15. On Day 15, live CAR⁺ T cells were enriched using the Easy Sep Dead Cell Removal Kit (StemCell Technologies), and cell pellets were snap-frozen for subsequent RNA extraction and NanoString gene expression analysis (Human CAR-T Panel; NanoString Technologies). See FIG. 3A.

15 [0200] The data in FIGs. 3B-3C show that CACCR BCMA CAR-T cells bearing truncated IL2Rb tails more closely mimic IL-15, rather than IL-2, signaling. As an example, the CACCR TpoR(478-582;S505N,W515K).IL2Rb(393-433,518-551) and TpoR(478-582;H499L,S505N,W515K).IL2Rb(339-379,393-433,518-551) were tested. FIG. 3A is a schematic diagram of the experimental design and workflow for sample preparation. FIG.

20 3B shows the gene expression profile of CACCR BCMA CAR-T cells compared to that of control CAR-T cells treated with IL-2 from Days 13-15. FIG. 3C shows the gene expression profile of CACCR CAR-T cells compared to that of control CAR-T cells treated with IL-15 from Days 13-15. Log2 fold change (FC) of each sample was calculated by normalization to control CAR-T cells that were left untreated from Days 13-15. The R2 values and best-fit

25 line (solid line) as determined by linear regression analysis are shown on each graph. Data shown is one representative of two donors. While the gene expression profiles of CACCR CAR-T cells showed no correlation with IL-2-treated samples (FIG. 3B), they correlated positively with IL-15-treated samples (FIG. 3C). These suggest that CACCR bearing the truncated IL2Rb tails more closely mimic the downstream signaling and transcriptional

30 responses of IL-15, instead of IL-2.

Example 2: Constitutive cytokine receptors enhance the in vitro cytotoxicity of CARs directed towards a liquid tumor target

[0201] The CACCRs were cloned into CAR construct directed towards a marker for a hematological malignancy (i.e. BCMA) and the long-term cytotoxicity against the BCMA positive target cell line was evaluated.

[0202] Target cells stably expressing the firefly luciferase and GFP reporters were generated by lentiviral transduction. 10,000 Luc-GFP-labelled target cells were plated in 100 uL per well in a white flat-bottomed 96-well tissue culture plate. Cryopreserved CAR-T cells (with TRAC/CD52 double knockouts) were thawed, counted, and the percentage of CAR-T cells across all samples were normalized to the sample with the lowest transduction efficiency by the addition of non-transduced (NTD) T cells. CAR-T cells in a volume of 100 uL were then added to each well of target cells at the indicated Effector:Target (E:T) ratios in triplicates. As a “Targets only” negative control, 100 uL of media, instead of T cells, was added to target cells. After two or three days, wells were mixed by gentle pipetting, and 100 uL of each T cell-containing well was transferred to a new white flat-bottomed 96-well tissue culture plate containing 10,000 freshly-plated Luc-GFP-labelled target cells in 100 uL. “Targets only” wells received fresh media in place of T cells. The new plate was incubated at 37°C, while the number of live target cells remaining in the old 96-well plate was determined using the ONE-Glo Luciferase Assay System (Promega) according to manufacturer’s instructions. The percentage of live target cells was calculated by normalizing the luciferase signal of to that of “Targets only” wells, and percentage cytotoxicity was calculated as 100% - % live target cells. Serial transfers to fresh target cells and luciferase readouts were performed every two or three days until all cytotoxic activity has ceased.

[0203] FIG. 4 shows that CACCRs improved the cytotoxic activity of CAR-T cells directed towards BCMA, a liquid tumor target. FIG. 4 shows the cytotoxicity of a BCMA CAR (P5A2 scFv) against the MM.1S multiple myeloma cell line at an E:T=10:1, indicating co-expression of a CACCR in the BCMA CAR T cells with TRAC/CD52 double knockouts (dKO) increased the long-term cytotoxicity of CAR-T cells. The CAR T cells used in the following examples all contained the TRAC/CD52 dKO.

Example 3: CACCRs enhance the in vivo activity of CAR-T cells

[0204] CAR-T cell therapies, such as those targeting CD19 and BCMA, have attained unprecedented clinical success in the treatment of hematological malignancies. While a high rate of complete responses has been achieved, this is transient as most patients eventually relapse. Furthermore, CAR-T cells have attained more limited success for the treatment of solid tumors. Among the reasons for relapse and the lack of response include insufficient CAR-T cell expansion and persistence, as well as CAR-T cell functional inhibition by immune-suppressive microenvironments. Since our in vitro characterization of CACCR CAR-T cells revealed improvements in target-driven proliferation, persistence, potency and exhaustion profiles, we next investigated whether these functional enhancements translated into improved anti-tumor activity in vivo.

[0205] To interrogate the in vivo activity of CACCR CAR-T cells in the context of hematological malignancies, we utilized CAR-T cells with TRAC/CD52 dKO bearing the BCMA specific P5A2 scFv coupled to 4-1BB and CD3 ζ signaling domains in an orthotopic xenograft model of multiple myeloma. T cell receptor (TCR)-deficient BCMA CAR-T cells were generated by Transcription Activator-Like Effector Nucleases (TALEN)-mediated knockout to avoid potential confoundance from TCR-driven xenoreactivity. 8-10 weeks old female NSG mice were irradiated with 1 Gy one day prior to intravenous inoculation of 5×10^6 MM.1S-Luc-GFP. 14 days after tumor implantation, mice were randomized based on tumor burden, and dosed intravenously with either 1×10^6 or 3×10^6 of the indicated CAR-T cells (n=10 per group). Tumor progression was monitored by bioluminescent imaging. On Day 30 post T cell dose, mice that had received 3×10^6 CAR-T cells were bled for the enumeration of BCMA CAR-T cells in the periphery. Specifically, 50 uL of whole blood from each mouse was subjected to red blood cell lysis using ACK Lysing Buffer (Gibco), Fc-blocked and stained with the following antibody cocktail diluted in PBS+1%BSA: FITC-conjugated anti-mouse CD45 (Biolegend), BV421-conjugated anti-human CD45 (Biolegend) and an anti-idiotypic antibody specific for the P5A2 scFv. Finally, samples were washed in PBS and cell pellets were resuspended in 130 uL PBS+1%BSA containing 123count eBeads counting beads (Thermo Fisher) (10 uL counting beads in 120 uL PBS+1%BSA) prior to FACS analysis.

[0206] FIGs. 5A-5C show that CACCRs improved the in vivo anti-tumor activity and persistence of BCMA CAR-T cells against orthotopic multiple myeloma. FIGs. 5A-5B

show tumor progression in response to treatment with either 1×10^6 or 3×10^6 of the indicated CAR-T cells, respectively. Although control BCMA CAR-T cells were able to mediate initial tumor regression, this response was short-lived as tumors relapsed 5 days after cell infusion. However, CACCR coexpressing CAR-T cells significantly delayed tumor relapse and improved the durability of response. Statistics in FIGs. 5A-5B represent ** $p < 0.01$ and *** $p < 0.001$ based on repeated measures one-way ANOVA with Tukey's multiple comparisons from Days 6-34 for FIG. 5A and Days 6-44 for FIG. 5B. FIG. 5C shows the number of BCMA CAR-T cells present in the peripheral blood of mice treated with 3×10^6 CAR-T cells 30 days after T cell infusion. Coincident with tumor relapse observed in mice treated with control BCMA CAR-T cells, control BCMA CAR-T cells could no longer be detected in the periphery. In contrast, CACCR BCMA CAR-T cells that were superior at preventing tumor relapse were also more significantly abundant in vivo. Statistics in FIG. 5C represent * $p < 0.05$ and **** $p < 0.0001$ based on ordinary one-way ANOVA with Tukey's multiple comparisons. These suggest that improved CACCR CAR-T cell persistence in part mediated enhanced long-term tumor control and prolonged the durability of response.

[0207] FIGs. 5 D-5E show the long-term survival of mice that had received 1×10^6 or 3×10^6 CAR T cells, respectively. At both CAR T cell doses, BCMA CAR T cells bearing the TpoR(478-582;H499L,S505N,W515K).IL2Rb(339-379,393-433,528-551) CACCR dramatically prolonged overall survival.

20 **Example 4: Evaluate CACCRs for BCMA CAR T cells**

[0208] The modular nature of CACCRs provide the flexibility of fusing signaling domains derived from different cytokine receptor of interest. Among these, IL-7, IL-2 and IL-15 have well-established roles in promoting T cell survival, expansion and effector function. We therefore hypothesized that BCMA CAR T cells may functionally benefit from CACCRs bearing the IL7Ra or IL2/15Rb signaling domains, and sought to identify which, if any, of these CACCRs could provide the most enhancement to BCMA CAR T cells.

[0209] BCMA-expressing MM.1S or Molp8 target cells stably expressing the firefly luciferase and GFP reporters were generated by lentiviral transduction. 10,000 Luc-GFP-labelled target cells were plated in 100 uL per well in a white flat-bottomed 96-well tissue culture plate. Cryopreserved TRAC/CD52 dKO CAR T cells were thawed, counted, and the percentage of CAR T cells across all samples were normalized to the sample with the lowest

transduction efficiency by the addition of non-transduced (NTD) T cells. CAR T cells in a volume of 100 uL were then added to each well of target cells at an E:T=10:1 or 3:1 in triplicates. As a “Targets only” negative control, 100 uL of media, instead of T cells, was added to target cells. After two or three days, wells were mixed by gentle pipetting, and 100 uL of each T cell-containing well was transferred to a new white flat-bottomed 96-well tissue culture plate containing 10,000 freshly-plated Luc-GFP-labelled target cells in 100 uL. “Targets only” wells received fresh media in place of T cells. The new plate was incubated at 37°C, while the number of live target cells remaining in the old 96-well plate was determined using the ONE-Glo Luciferase Assay System (Promega) according to manufacturer’s instructions. The percentage of live target cells was calculated by normalizing the luciferase signal of to that of “Targets only” wells, and percentage cytotoxicity was calculated as 100% - % live target cells. Serial transfers to fresh target cells and luciferase readouts were performed every two or three days until all cytotoxic activity has ceased.

[0210] FIG. 6 shows the functional screening of TRAC/CD52 dKO CACCR-BCMA CAR T cells bearing the indicated CACCRs using an in vitro serial killing assay. Two target cell lines expressing varying levels of endogenous BCMA were used – moderate-expressing MM.1S-Luc-GFP (high BCMA expressing cell line) and low-expressing Molp8-Luc-GFP (low BCMA expressing cell line). FIG. 6A shows a schematic of the bicistronic CACCR-BCMA CAR lentiviral construct. The BCMA CAR construct containing the clone P5A2 scFv and R2 was used throughout this example. The CACCR was cloned downstream of the EF1a promoter and upstream of a 2nd generation BCMA CAR. A wildtype WPRE sequence was included to stabilize transcript expression. FIGs. 6B and 6C show the serial killing activity of CACCR-BCMA CARs bearing IL2Rb-derived CACCRs against MM.1S-Luc-GFP and Molp8-Luc-GFP, respectively. All IL2Rb-derived CACCRs tested improved the activity of BCMA CAR T cells, and the TpoR(478-582;H499L,S505N,W515K).IL2Rb(339-379,393-433,528-551) CACCR conferred the greatest enhancement. FIGs. 6D and 6E show the serial killing activity of CACCR-BCMA CAR bearing the most active IL2Rb-derived CACCR and an IL7Ra-bearing CACCR. Unlike the IL2Rb-derived CACCR, the IL7Ra-bearing CACCR did not enhance the activity of BCMA CAR T cells in either target cell line.

[0211] To evaluate the in vivo anti-tumor activity of CACCR-BCMA CARs, we utilized an orthotopic xenograft model of multiple myeloma in which disease initially establishes in

the bone marrow and relapse subsequently occurs in extramedullary sites. Briefly, 8-10 weeks old female NSG mice were irradiated with 1 Gy one day prior to intravenous inoculation of 5×10^6 MM.1S-Luc-GFP. 15 days post tumor implantation, mice were randomized based on tumor burden and the indicated numbers of TRAC/CD52 dKO
 5 CACCR-BCMA CAR T cells were intravenously infused per mouse (n=10 mice per group). Thereafter, tumor burden was monitored twice weekly by bioluminescent imaging.

[0212] FIG. 7 shows the anti-tumor activity of 5×10^6 CACCR-BCMA CAR T cells bearing either an IL7Ra- or IL2Rb-derived CACCR relative to the unmodified BCMA CAR. FIG. 7A shows the tumor progression of each group. Under the experimental
 10 condition, although unmodified BCMA CAR T cells were able to mediate initial tumor regression, disease eventually relapsed. The IL7Ra-derived CACCR did not enhance the activity of BCMA CAR T cells. While the TpoR(478-582;H499L,S505N,W515K).IL2Rb(393-379,393-433,528-551) CACCR showed some delay in tumor relapse, the TpoR(478-582;H499L,S505N,W515K).IL2Rb(339-379,393-433,528-551) CACCR provided the most
 15 durable response. The in vivo performance of these CACCRs corroborated the rank-ordering of in vitro activity in FIG. 6. Graph shows mean $\pm$ sem; *p<0.05 and *p<0.001 based on RM one-way ANOVA with Tukey's multiple comparisons from Days 6-48. FIG. 7B shows the response of individual mice in each group. Mice that had received unmodified BCMA CAR T cells responded non-uniformly initially, and eventually all relapsed with
 20 extramedullary tumors. In contrast, mice that had received CACCR-BCMA CARs bearing the TpoR(478-582;H499L,S505N,W515K).IL2Rb(339-379,393-433,528-551) CACCR responded uniformly initially, and showed durable long-term responses with 9/10 mice remaining tumor-free at the end of the study.

[0213] FIG. 8 shows anti-tumor activities of two IL2Rb-derived CACCRs relative to the
 25 unmodified BCMA CAR at a dose of either 1×10^6 or 3×10^6 , all TRAC/CD52 dKO. Ten female NSG mice were tested per group. Each mice received 5×10^6 MM.1S luc2 GFP cells. FIG. 8A shows the overall survival of mice receiving a low dose of 1×10^6 CAR T cells. While the TpoR(478-582;S505N,W515K).IL2Rb(393-433,528-551) CACCR had no effect on overall survival relative to unmodified BCMA CAR T cells, the TpoR(478-
 30 582;H499L,S505N,W515K).IL2Rb(339-379,393-433,528-551) CACCR dramatically prolonged overall survival. FIG. 8B shows tumor progression of mice that had received 3×10^6 CAR T cells. Although all groups underwent initial tumor regression, mice that had received the unmodified BCMA CAR T cells quickly relapsed. Both CACCRs were able to

delay relapse, with the TpoR(478-582;H499L,S505N,W515K).IL2Rb(339-379,393-433,528-551) CACCR resulted in the most durable response. FIG. 8C shows the response of individual mice that had received 3×10^6 CAR T cells. Compared to 1/10 tumor-free mice in the unmodified BCMA CAR T-treated group, BCMA CAR T cells coexpressing the TpoR(478-582;H499L,S505N,W515K).IL2Rb(339-379,393-433,528-551) CACCR mediated tumor clearance in 5/10 mice by the end of the study. Taken together, these data demonstrate that among the IL7Ra- and IL2Rb-derived CACCRs tested, the TpoR(478-582;H499L,S505N,W515K). IL2Rb(339-379,393-433,528-551) CACCR conferred the greatest enhancement to BCMA CAR T cells.

10 **Example 5: Manufacturability and Functional activity of CACCR-BCMA CAR**

[0214] CAR T cells are commonly generated by viral-based gene delivery, which imposes an upper limit on vector cargo size. While coexpressing additional modifications with a CAR in the same vector may enhance CAR T cell activity, increasing the cargo size may compromise transduction efficiency and product manufacturability. We next examined functional activity and CAR⁺ T cell yield of a number of CACCR-BCMA CARs and compare to those of their unmodified counterpart.

[0215] BCMA CAR T cells were generated as follows. To make lentivirus encoding the respective BCMA CARs, HEK293T cells were plated at 0.45 million cells per mL in 2mL of DMEM (Gibco) supplemented with 10% FBS (Hyclone) per well of a 6-well plate the day before transfection. On the day of transfection, the lentivirus was prepared by mixing together lentiviral packaging vectors 1.5 ug psPAX2, 0.5 ug pMD2G, and 0.5 ug of the appropriate transfer CAR vector in 250 uL Opti-MEM (Gibco) per well of the 6-well plate ("DNA mix"). 10 uL Lipofectamine 2000 (Invitrogen) in 250 uL Opti-MEM was incubated at room temperature for 5 minutes and then added to the DNA mix. The mixture was incubated at room temperature for 20 minutes and the total volume of 500 uL was slowly added to the sides of the wells containing HEK293T. 1 day post-transfection, the media from each well of HEK293T cells in the 6-well plate was replaced with 2mL per well of T cell transduction media, i.e., X-Vivo-15 supplemented with 10% FBS. 2 days post-transfection, the lentiviral supernatants from HEK293T cells were harvested and passed through a 0.45 micron filter (EMD Millipore) to remove cell debris, and crude lentiviral supernatants were used directly for T cell transduction. On Day 0, purified T cells were activated in X-Vivo-15 medium (Lonza) supplemented with 100 IU/mL human IL-2

(Miltenyi Biotec), 10% FBS (Hyclone), and human T TransAct (Miltenyi Biotec, Cat# 130-111-160, 1:100 dilution) in a Grex-24 plate (Wilson Wolf, cat# 80192M). On Day 2, T cells were resuspended at 0.5 million cells per mL in T cell transduction media, transduced with an equal volume of crude lentiviral supernatant along with 100 IU/mL human IL-2 in a Grex-24 plate. On Day 5, cells were fed by replacing the spent media with T cell expansion media, i.e., X-Vivo-15 supplemented with 5% human AB serum (Gemini Bio), along with 100 IU/mL human IL-2. On Day 6, the TCR α constant (TRAC) and CD52 genes were knocked out by Transcription Activator-Like Effector Nucleases (TALEN)-mediated gene editing. See e.g., US2016/0145337. Cells were expanded into larger G-Rex vessels (Wilson Wolf) as needed using T cell expansion media and 100 IU/mL human IL-2. On Day 14, TCR α /b $^{-}$ cells were purified using the EasySep Human TCR α /b depletion kit (Stem Cell Technologies) and rested overnight in T cell expansion media and 100 IU/mL human IL-2 before cryopreservation on Day 15. On Days 5 and 15, CAR positivity was determined by flow cytometry using a PE-conjugated anti-idiotypic antibody for the detection of the P5A2 scFv.

[0216] FIG. 9 shows the manufacturability and yield of T cells bearing CACCR-BCMA CAR transduced in a lentiviral construct. FIG. 9A shows a schematic of a CACCR-BCMA CAR lentiviral construct, in which the strong EF1 α promoter drives expression of both the CACCR and the BCMA CAR from a bicistronic open reading frame and transcript expression is stabilized by the wildtype (wt) WPRE sequence. FIG. 9B shows the initial transduction efficiency on Day 5 (3 days post-transduction) and Day 15 (final day) of the CAR T cell production process. As expected, CACCR-BCMA CAR transduce less efficiently on Day 5 due to their larger cargo size. However, CACCRs confer CACCR-BCMA CARs with a proliferative advantage, allowing CACCR-BCMA CAR $^{+}$ T cells to enrich faster and reach comparable levels of CAR positivity at the end of production on Day 15. FIG. 9C shows that the yield of CACCR-BCMA CAR $^{+}$ T cells is equal to or greater than that of unmodified BCMA CAR $^{+}$ T cells. Yield is defined by the final number of CAR $^{+}$ T cells obtained at the end of production per million pan-T cells starting material on Day 0 of production. Data in FIGs. 9B and 9C show mean $\pm$ sem from 3 donors. Although CACCR-BCMA CARs have a larger cargo size, their lower initial transduction efficiency of CACCR-BCMA CARs was offset by their faster enrichment over the course of production, allowing them to retain comparable manufacturability to their unmodified counterparts.

[0217] It has been reported that the wildtype WPRE may encode a truncated X-protein that may pose an oncogenic risk. To mitigate this safety risk, wildtype WPRE may be mutated (e.g. by inactivating the start codon) to abrogate X-protein expression. As the CACCR-BCMA CAR lentiviral vector initially tested contained the wildtype WPRE
5 sequence, we sought to determine if substitution to the mutant sequence would impact the expression and functionality of CACCR-BCMA CAR.

[0218] BCMA CAR T cells were generated as follows. To make lentivirus encoding the respective BCMA CARs, HEK293T cells were plated at 0.45 million cells per mL in 2mL of DMEM (Gibco) supplemented with 10% FBS (Hyclone) per well of a 6-well plate the
10 day before transfection. On the day of transfection, the lentivirus was prepared by mixing together lentiviral packaging vectors 1.5 ug psPAX2, 0.5 ug pMD2G, and 0.5 ug of the appropriate transfer CAR vector in 250 uL Opti-MEM (Gibco) per well of the 6-well plate ("DNA mix"). 10 uL Lipofectamine 2000 (Invitrogen) in 250 uL Opti-MEM was incubated at room temperature for 5 minutes and then added to the DNA mix. The mixture was
15 incubated at room temperature for 20 minutes and the total volume of 500 uL was slowly added to the sides of the wells containing HEK293T. One day post-transfection, the media from each well of HEK293T cells in the 6-well plate was replaced with 2mL per well of T cell transduction media, i.e., X-Vivo-15 supplemented with 10% FBS. 2 days post-transfection, the lentiviral supernatants from HEK293T cells were harvested and passed
20 through a 0.45 micron filter (EMD Millipore) to remove cell debris, and crude lentiviral supernatants were used directly for T cell transduction. On Day 0, purified T cells were activated in X-Vivo-15 medium (Lonza) supplemented with 100 IU/mL human IL-2 (Miltenyi Biotec), 10% FBS (Hyclone), and human T TransAct (Miltenyi Biotec, Cat# 130-111-160, 1:100 dilution) in a Grex-24 plate (Wilson Wolf, cat# 80192M). On Day 2, T cells
25 were resuspended at 0.5 million cells per mL in T cell transduction media, transduced with an equal volume of crude lentiviral supernatant along with 100 IU/mL human IL-2 in a Grex-24 plate. On Day 5, cells were fed by replacing the spent media with T cell expansion media, i.e., X-Vivo-15 supplemented with 5% human AB serum (Gemini Bio), along with 100 IU/mL human IL-2. On Day 6, the TCRa constant (TRAC) and CD52 genes were
30 knocked out by Transcription Activator-Like Effector Nucleases (TALEN)-mediated gene editing. Cells were expanded into larger G-Rex vessels (Wilson Wolf) as needed using T cell expansion media and 100 IU/mL human IL-2. On Day 14, TCRa/b⁺ cells were purified using the EasySep Human TCRa/b depletion kit (Stem Cell Technologies) and rested

overnight in T cell expansion media and 100 IU/mL human IL-2 before cryopreservation on Day 15. On Days 5 and 15, CAR positivity was determined by flow cytometry using a PE-conjugated anti-idiotypic antibody for the detection of the P5A2 scFv.

[0219] The in vitro cytotoxicity of BCMA CAR T cells were evaluated in a serial killing assay as follows. BCMA-expressing MM.1S or Molp8 target cells stably expressing the firefly luciferase and GFP reporters were generated by lentiviral transduction. 10,000 Luc-GFP-labelled target cells were plated in 100 uL per well in a white flat-bottomed 96-well tissue culture plate. Cryopreserved TRAC/CD52 dKO CAR T cells were thawed, counted, and the percentage of CAR T cells across all samples were normalized to the sample with the lowest transduction efficiency by the addition of non-transduced (NTD) T cells. CAR T cells in a volume of 100 uL were then added to each well of target cells at an E:T=3:1 in triplicates. As a “Targets only” negative control, 100 uL of media, instead of T cells, was added to target cells. After two or three days, wells were mixed by gentle pipetting, and 100 uL of each T cell-containing well was transferred to a new white flat-bottomed 96-well tissue culture plate containing 10,000 freshly plated Luc-GFP-labelled target cells in 100 uL. “Targets only” wells received fresh media in place of T cells. The new plate was incubated at 37°C, while the number of live target cells remaining in the old 96-well plate was determined using the ONE-Glo Luciferase Assay System (Promega) according to manufacturer’s instructions. The percentage of live target cells was calculated by normalizing the luciferase signal of to that of “Targets only” wells, and percentage cytotoxicity was calculated as 100% - % live target cells. Serial transfers to fresh target cells and luciferase readouts were performed every two or three days until all cytotoxic activity has ceased.

[0220] FIG. 10 shows the effects of substitution to the mutant (mut) WPRE sequence on CACCR-BCMA CART cell manufacturability and function. FIG. 10A shows a schematic for vectors used with either the wildtype or mutant WPRE sequences. FIG. 10B shows the initial and final CAR positivity of CACCR-BCMA CARs. CACCR-BCMA CAR transduction efficiency (Day 5) and enrichment (Day 15) were not impacted by the switch to mutWPRE. FIG. 10C shows that the final CAR⁺ yield of CACCR-BCMA CARs were not impacted by the switch to mutWPRE. FIG. 10D shows that although CACCR-BCMA CAR T cells had a lower CAR expression than their unmodified counterpart, CAR expression levels on CACCR-BCMA CAR T cells were not impacted by the switch to mutWPRE. Data in FIGs. 10 B-10D show mean±sem from 3 donors. FIG. 10E shows the in

vitro serial killing activity of CACCR-BCMA CARs against the MM.1S-Luc-GFP and Molp8-Luc-GFP cell lines. Compared to unmodified BCMA CAR T cells, CACCR-BCMA CAR T cells bearing either the wildtype or mutant WPRE both showed enhanced serial killing activity, demonstrating that the switch to mutWPRE does not impair CACCR-
5 BCMA CAR function. Data shown is mean±sem from one out of two donors that yielded similar results.

[0221] Serial killing was performed using PBMC-based TRAC/CD52 dKO CAR T cells at an E:T=10:1. Enhanced serial killing activity was maintained in PBMC-derived CACCR BCMA CAR T cells. See FIG. 10F. Data shown represents mean±sem of 3 donors.

10 [0222] As shown in FIG. 9, the lower initial transduction efficiencies of CACCR-BCMA CARs were offset by the faster enrichment and growth advantage of the transduced CAR T cells, resulting in comparable yields of CAR T cells to that of unmodified CAR T cells. Nevertheless, we sought to further improve CACCR-BCMA CAR T cell transduction efficiencies and yields by exploring alternative vector designs that reduced the size of the
15 cargo, at the same time, maintaining CACCR-BCMA CAR functional enhancements. By benchmarking yields and functional activity to the original vectors shown in FIG. 10A, three vector designs were interrogated: (i) an EF1a-driven vector without the 3' WPRE sequence, (ii) a vector driven by the minimal EF1a(short) promoter, and (iii) a vector driven by the MND promoter (myeloproliferative sarcoma virus enhancer negative control region
20 deleted, dl587rev primer-binding site substituted).

[0223] TRAC/CD52 dKO CACCR-BCMA CAR T cells were generated and characterized as previously described.

[0224] To evaluate CACCR signaling, CACCR-BCMA CAR T cells at the end of the production process were serum starved in 100 uL serum-free RPMI (Corning) for 4 hours in
25 humidified incubator at 37°C with 5% CO₂. As a positive control, exogenous recombinant human IL-2 (10 ng/mL; Miltenyi) was added to unmodified CAR T cells during the last 30 minutes of the 4-hour serum starvation. After 4 hours, an antibody cocktail comprising BUV395-conjugated anti-human CD3 (Biolegend) and FITC-conjugated v5 tag monoclonal antibody (Thermo Fisher) were added to the cells and allowed to incubate for the final 20
30 minutes. Cells were then fixed by adding 35 uL of 16% paraformaldehyde to each 100 uL sample and allowed to incubate for 15 minutes at 37°C. Cells were then washed three times with PBS, and permeabilized in 100% cold methanol for 1 or 2 nights at -20°C. On the day

of FACS analysis, cells were washed three times with PBS, Fc-blocked, and stained with AlexaFluor647-conjugated anti-mouse/human Stat5 (pY694) (BD Biosciences) diluted in PBS+1%BSA. After a one hour incubation at room temperature in the dark, cells were washed three times before FACS analysis.

5 [0225] Serial killing assays were performed at the indicated E:T ratios as previously described.

[0226] In some cases, in vivo anti-tumor activity was assessed using an orthotopic model of multiple myeloma. 8-10 weeks old female NSG mice were irradiated with 1 Gy one day prior to intravenous inoculation of 5×10^6 MM.1S-Luc-GFP. 14 days after tumor
10 implantation, mice were randomized based on tumor burden, and dosed intravenously with 3×10^6 of the indicated CAR T cells (n=10 per group). Tumor progression was monitored by bioluminescent imaging.

[0227] FIG. 11 shows the effects of WPRE removal from the original vector. FIG. 11A is a schematic of CACCR-BCMA CAR vectors used. FIGs. 11B and 11C show the final CAR
15 positivity and CAR⁺ yields, respectively, of the indicated constructs. Despite a vector cargo reduction, removing WPRE did not significantly improve the final CACCR-BCMA CAR percentage or yield. FIGs. 11C & 11D show CAR expression level and CACCR signaling strength as determined by pSTAT5 staining intensity, respectively. WPRE removal did not reduce CAR expression or CACCR signaling strength in the quiescent CACCR-BCMA
20 CAR T cell product. Data in FIGs. 11B-11D show mean $\pm$ sem of 3 donors. FIGs. 11F-11H show serial killing activity of three different IL2Rb-derived CACCR-BCMA CARs with or without WPRE. While cytotoxicity of the TpoR(478-582;S505N,W515K). IL2Rb(393-433,528-551) (FIG. 11F) and TpoR(478-582;H499L,S505N,W515K). IL2Rb(393-433,528-551) CACCRs were slightly reduced upon WPRE removal, the most active TpoR(478-
25 582;H499L,S505N,W515K).IL2Rb(339-379,393-433,528-551) CACCR was not impacted by WPRE removal. Data in FIGs. 11F-11H show mean $\pm$ sem from one out of two donors that yielded similar results.

[0228] FIG. 12 shows the effects of substituting the full-length (FL) EF1a promoter for the weaker minimal EF1a(short) (S) promoter. FIG. 12A is a schematic of CACCR-BCMA
30 CAR vectors used. FIG. 12B shows the final CAR positivity of the indicated constructs driven by the full-length (FL) or short (S) EF1a promoters. Utilizing the smaller EF1a(short) promoter improved overall transduction efficiency and final CAR percentage

for both the unmodified and CACCR-BCMA CAR T cells. FIGs. 12C and 12D show serial killing activity of CAR T cells driven by the EF1a or EF1a(short) promoter against MM.1S-Luc-GFP and Molp8-Luc-GFP target cells, respectively. While substitution to the EF1a(short) promoter did not affect the activity of unmodified BCMA CAR T cells,
 5 EF1a(short)-driven CACCR-BCMA CAR T cells were less efficacious than their EF1a (full-length)-driven counterparts. Although constructs with the EF1a(short) promoter improved CACCR-BCMA CAR manufacturability, the accompanying loss in CACCR-BCMA CAR activity undermined further use of EF1a(short) CACCR CAR constructs. Data shown is from one representative donor.

10 [0229] FIG. 13 shows the effects of substituting the full-length (FL) EF1a promoter for the MND promoter. FIG. 13A is a schematic of CACCR-BCMA CAR vectors used. FIGs. 13B & 13C show the final CAR positivity and yield of the indicated constructs driven by the EF1a or MND promoters. Utilizing the smaller MND promoter improved overall transduction efficiency, final CAR percentage and yield of CACCR-BCMA CART cells.
 15 FIG. 13D shows CAR expression level. CACCR-BCMA CAR T cells driven by the MND promoter have higher CAR expression, suggesting that the MND promoter is stronger than the EF1a promoter. Data in FIGs. 13 B-13D show mean $\pm$ sd of 2 donors. FIGs. 13 E & 13F show serial killing activity of CAR T cells driven by the EF1a or MND promoter against MM.1S-Luc-GFP and Molp8-Luc-GFP. FIG. 13E shows that activity of the TpoR(478-
 20 582;H499L,S505N,W515K).IL2Rb(339-379,393-433,528-551) CACCR was reduced when driven by the MND promoter. This was corroborated with an alternate CACCR in FIG. 13F, where substitution to the MND promoter abrogated enhancement by the TpoR(478-582;S505N,W515K).IL2Rb(393-433,528-551) CACCR. Data in FIGs. 13 E & 13F show mean $\pm$ sem from one out of two donors that yielded similar results. FIG. 13G shows in vivo
 25 anti-tumor activity of CACCR-BCMA CARs driven by either the EF1a or MND promoter. Compared to the EF1a-driven CACCR-BCMA CAR T cells that enhanced anti-tumor efficacy, substitution to the MND promoter abrogated all functional enhancements. Data in FIG. 13G shows mean $\pm$ sem; ***p<0.01 and ****p<0.0001 based on RM one-way ANOVA with Tukey's multiple comparisons from Days 5-48.

30 [0230] Taken together, the original vector format of EF1a-TpoR(478-582;H499L,S505N,W515K).IL2Rb(339-379,393-433,528-551)-BCMA CAR.WPRE remained the most functionally active while retaining comparable manufacturability to its unmodified BCMA CAR counterpart. Since the use of wildtype or mutant WPRE did not

impact CACCR-BCMA CARactivity (FIG. 10) and mutWPRE mitigates potential safety risks associated with the X-protein expression, EF1a-TpoR(478-582;H499L,S505N,W515K).IL2Rb(339-379,393-433,528-551)-BCMA CAR.mutWPRE was selected for further studies.

5 Example 6: Functional characterization of potency and persistence of CACCR-BCMA CARs

[0231] To elucidate the mechanisms by which CACCR-BCMA CARs improve CAR T cell activity, we characterized the function and phenotype of CACCR-BCMA CART cells following repeated target exposure in vitro.

10 [0232] TRAC/CD52 dKO CAR T cells were generated as previously described. On Day 0 of the assay, cryopreserved TRAC/CD52 dKO CAR T cells were thawed, and the percentage of CAR T cells across all samples were normalized to the sample with the lowest transduction efficiency by the addition of non-transduced (NTD) T cells. 3×10^5 CAR⁺ T cells and 1×10^5 MM.1S-Luc-GFP- target cells were plated at an E:T of 3:1 in a total volume
15 of 750 uL per well in a 48-well tissue culture plate. Duplicate wells were set up for each condition. As a comparator, 10 ng/mL recombinant human IL-15 (Miltenyi) was added to cocultures of unmodified CAR T cells and target cells. Every 2 or 3 days thereafter, wells were mixed thoroughly by gentle pipetting, and 200 uL from each well was removed be to either discarded or used for flow cytometric analysis as outlined below. Wells were then
20 replenished with 2×10^5 fresh MM.1S-Luc-GFP- target cells in a volume of 200 uL. In IL-15-treated wells, 10 ng/mL recombinant human IL-15 was replenished twice weekly.

[0233] To assess CAR T cell expansion and memory T cell differentiation, 200 uL was removed from each well of the 48-well plate and re-plated into a well of a 96-well U-bottom plate. Cells were washed with PBS and stained with the Zombie-NIR Fixable Viability kit
25 (Biolegend). Cells were then washed with PBS, Fc-blocked, and stained with a PE-conjugated anti-idiotypic antibody for the detection of the P5A2 scFv, BV605-conjugated anti-human CD4 (Biolegend), BV510-conjugated anti-human CD8 (Biolegend), PE/Cy7-conjugated anti-human CD62L (Biolegend) and BV785-conjugated anti-human CD45RO diluted in PBS+1%BSA. After washing, cells were resuspended 100 uL PBS+1%BSA
30 containing 123count eBeads counting beads (Thermo Fisher) (10 uL counting beads in 90 uL PBS+1%BSA) prior to flow cytometric analysis.

[0234] To evaluate activation-induced cell death (AICD), 200 uL was removed from each well of the 48-well plate and re-plated into two wells of a 96-well U-bottom plate at 100 uL per well. 1×10^5 MM.1S-Luc-GFP- target cells in a volume of 100 uL was added to one of these wells in the 96-well U-bottom plate, while the other well was left unstimulated as a baseline comparison. After 4 hours, the percentages of dead CAR T cells were determined by flow cytometry. Briefly, cells were washed with PBS and stained with the Zombie-NIR Fixable Viability kit (Biolegend). Cells were then washed with PBS, Fc-blocked, and stained with a PE-conjugated anti-idiotypic antibody for the detection of the P5A2 scFv, BV605-conjugated anti-human CD4 (Biolegend) and BV510-conjugated anti-human CD8 (Biolegend) diluted in PBS+1%BSA.

[0235] For intracellular cytokine staining, 200 uL was removed from each well of the 48-well plate and 100 uL was re-plated into a well of a 96-well U-bottom plate. 1×10^5 MM.1S-Luc-GFP- target cells in a volume of 100 uL was added to one of these wells in the 96-well U-bottom plate, along with a Protein Transport Inhibitor Cocktail (Invitrogen). After 4 hours, cells were stained for surface markers and intracellular cytokines. Briefly, cells were washed with PBS and stained with the Zombie-NIR Fixable Viability kit (Biolegend). Cells were then washed with PBS, Fc-blocked, and stained with a PE-conjugated anti-idiotypic antibody for the detection of the P5A2 scFv, BV605-conjugated anti-human CD4 (Biolegend) and BV510-conjugated anti-human CD8 (Biolegend) diluted in PBS+1%BSA. After washing, cells were fixed and permeabilized using the BD Cytofix/Cytoperm kit (BD Biosciences) according to manufacturer's instructions and stained with a PE/Cy7-conjugated anti-human IFN γ (Biolegend), BV785-conjugated anti-human TNF α (Biolegend) and PE/Dazzle594-conjugated anti-human IL-2 (Biolegend) prior to flow cytometric analysis.

[0236] FIG. 14 shows CAR T cell expansion following repeated target exposures in two donors. Shown insets are CAR T cell expansion profiles during the first 15 days of the assay. In both donors, the CACCR-BCMA CAR (EF1a-TpoR(478-582;H499L,S505N,W515K).IL2Rb(339-379,393-433,528-551)-BCMA CAR_mutWPRE) demonstrated the greatest target-driven expansion capacity. Data are presented as mean $\pm$ sem.

[0237] FIG. 15 shows the sensitivity of CAR T cells to AICD with increasing exposure to target cells. FIG. 15A shows that CAR T cells that had undergone one prior round of target exposure (on Day 0) were comparably sensitive to AICD following a second target

exposure, regardless of CACCR expression or IL-15 treatment. FIG. 15B shows that after multiple rounds of target exposure on Day 7, unmodified BCMA CAR T cells were highly sensitive to AICD, whereas CACCR-BCMA CART cells and IL-15-treated BCMA CAR T cells were protected from AICD. Without intending to be limited to a specific mechanism, the data suggest that cytokine signaling – through CACCRs or supplemented IL-15 – can help CAR T cells resist AICD and improve survival, particularly following chronic target exposure. Data are presented as mean±sd from one out of two donors that yielded similar results.

[0238] FIG. 16 shows cytokine expression profiles of CAR T cells based on intracellular cytokine staining on Day 7 of the assay. FIG. 16A shows the percentage of CAR+ T cells that expressed the indicated cytokines. Compared to the unmodified BCMA CAR, CACCR-BCMA CART cells and IL-15-treated CAR T cells expressed higher levels of effector cytokines. FIG. 16B shows CAR T cell polyfunctionality, as determined by their ability to coexpress 2 or 3 effector cytokines. Compared to unmodified BCMA CAR T cells, CART cells with the EF1a-TpoR(478-582;H499L,S505N,W515K).IL2Rb(339-379,393-433,528-551)-BCMA CAR mutWPRE construct demonstrated the greatest extent of polyfunctionality. Data shows mean±sd from one out of two donors that yielded similar results.

[0239] FIG. 17 shows the memory phenotype of CAR T cells on Day 15 of the assay. Compared to unmodified BCMA CAR T cells, CACCR-BCMA CART cells showed a slight enrichment of the stem cell memory (T_{SCM}) and central memory (T_{CM}) populations, which are known to mediate long-term CAR T cell persistence.

[0240] To monitor the in vivo pharmacokinetics of CACCR-BCMA CART cells, we tracked CAR T cell expansion and persistence in an orthotopic model of multiple myeloma using the Molp8-Luc-GFP cell line. The Molp8-Luc-GFP cell line expresses lower levels of BCMA than MM.1S-Luc-GFP and represents a model of multiple myeloma that is highly treatment-resistant. Briefly, 8-10 weeks old female NSG mice were irradiated with 1 Gy one day prior to intravenous inoculation of 2×10^6 Molp8-Luc-GFP. 8 days post tumor implantation, mice were randomized based on tumor burden and either 1×10^6 or 5×10^6 TRAC/CD52 dKO BCMA CAR T cells were intravenously infused per mouse (n=10 mice per group). Thereafter, tumor burden was monitored twice weekly by bioluminescent imaging, and mice that had received 5×10^6 CAR T cells were bled at the indicated

timepoints for the enumeration of BCMA CAR T cells in the periphery. Specifically, 50 uL of whole blood from each mouse was subjected to red blood cell lysis using ACK Lysing Buffer (Gibco), Fc-blocked and stained with the following antibody cocktail diluted in PBS+1%BSA: FITC-conjugated anti-mouse CD45 (Biolegend), BV421-conjugated anti-human CD45 (Biolegend) and PE-conjugated anti-idiotypic antibody for the detection of the P5A2 scFv. Finally, samples were washed in PBS and cell pellets were resuspended in 250 uL PBS+1%BSA containing 123count eBeads counting beads (Thermo Fisher) (10 uL counting beads in 240 uL PBS+1%BSA) prior to flow cytometric analysis.

[0241] FIG. 18 shows the expansion and persistence of BCMA CAR T cells in Molp8-Luc-GFP-bearing mice that had received 5×10^6 CAR T cells. FIG. 18A shows that while unmodified BCMA CAR T cells underwent an initial expansion, its numbers quickly declined within 2 weeks of CAR T cell infusion. In contrast, CACCR-BCMA CAR T cells expanded comparably or better than their unmodified counterparts and showed prolonged persistence in vivo. FIG. 18B shows CAR expression levels (gMFI) in BCMA CAR T cells from peripheral blood. Although no differences in CAR expression was detected in the CAR T cell product in vitro (FIG. 11D), the EF1a-TpoR(478-582;H499L,S505N,W515K). IL2Rb(339-379,393-433,528-551)-BCMA CAR_noWPRE construct showed lower CAR expression relative to its WPRE-bearing counterparts in vivo. Data shows mean $\pm$ sem.

[0242] FIGs. 19A-19B show the overall survival of mice that had received either 1×10^6 or 5×10^6 CAR T cells. FIG. 19B shows the extended results. At both doses, CACCR-BCMA CAR T cells prolonged overall survival. Notably, median survival of 1×10^6 CACCR-BCMA CAR -treated mice was 61 days, which was comparable to the median survival of 57 days in mice treated with 5×10^6 unmodified BCMA CAR T cells.

[0243] FIG. 20 shows the tumor burden of individual mice that had received 5×10^6 CAR T cells. Mice treated with unmodified BCMA CAR T cells uniformly showed disease progression beyond Day 36. By contrast, infusions of CACCR-BCMA CAR T cells were able to substantially delay disease progression in a subset of mice. Therefore, the greater persistence of CACCR-BCMA CAR T cells correlates with improved tumor control.

Example 7: The activity of CACCR-BCMA CART cells is target-dependent

[0244] For CACCR-BCMA CAR T cells to be efficacious and safe, we next investigated whether their activity is governed by CAR-mediated target recognition, and that CACCR signaling in the absence of CAR engagement is insufficient to induce cytotoxic responses.

[0245] To evaluate whether the cytotoxicity and effector responses of CACCR-BCMA CAR T cells, an overnight cytotoxicity assay was performed using either REH-Luc-GFP stably overexpressing BCMA (REH-BCMA) or the parental BCMA-negative REH-Luc-GFP as target cells. TRAC/CD52 dKO CAR T cells were generated as previously described.

5 Cryopreserved TRAC/CD52 dKO CAR T cells were thawed, and the percentage of CAR T cells across all samples were normalized to the sample with the lowest transduction efficiency by the addition of non-transduced (NTD) T cells. 10,000 target cells in a volume of 100 uL were added to each well of a white flat-bottomed 96-well tissue culture plate. CAR T cells in a volume of 100 uL were then added to each well of target cells at the

10 indicated E:T ratios in triplicates. As a “Targets only” negative control, 100 uL of media, instead of T cells, was added to target cells. 24 hours later, the number of live target cells remaining was determined using the ONE-Glo Luciferase Assay System (Promega) according to manufacturer’s instructions. The percentage of live target cells was calculated by normalizing the luciferase signal of to that of “Targets only” wells, and percentage

15 cytotoxicity was calculated as 100% - % live target cells.

[0246] To evaluate cytokine secretion profiles of CACCR-BCMA CAR T cells, 2.5×10^5 REH-BCMA or parental REH cells in a volume of 250 uL were plated in each well of a 24-well plate. Cryopreserved TRAC/CD52 dKO CAR T cells were thawed, and the percentage of CAR T cells across all samples were normalized to the sample with the lowest

20 transduction efficiency by the addition of non-transduced (NTD) T cells. CAR T cells in a volume of 250 uL were then added to each well of target cells at and E:T=1:1 in duplicates. To assess whether CACCR-BCMA CAR T cells secreted elevated basal levels of cytokines, CAR T cells were cultured alone, and 250 uL media was added in place of target cells. 24 hours later, plates were spun down and 200 uL of supernatant from each well was harvested

25 and stored at -80°C for Luminex analysis. On the day of Luminex analysis, frozen culture supernatants were thawed and diluted either 4-fold or 16-fold prior to analysis using the MILLIPLEX MAP Human High Sensitivity T Cell Panel Premixed 21-plex - Immunology Multiplex Assay according to manufacturer’s instructions.

[0247] FIG. 21 shows that CACCR-BCMA CAR T cells do not elaborate cytotoxic

30 effector functions in the absence of BCMA. FIG. 21A shows the cytotoxicity of CACCR-BCMA CAR T cells after 24-hour coculture with either REH-BCMA or BCMA-negative REH. As expected, the unmodified BCMA CAR and CACCR-BCMA CART cells showed a dose-dependent killing of REH-BCMA target cells. By contrast, neither the unmodified

BCMA CAR nor CACCR-BCMA CAR T cells were able to kill BCMA-negative REH cells. FIG. 21B shows the cytokine secretion profiles of CACCR-BCMA CAR T cells following 24 hour exposure to REH-BCMA, BCMA-negative REH or in media alone (T cells only). As expected, all BCMA CAR T cells secreted high and comparable levels of IFN γ and GM-CSF, and to a lesser degree IL-2, in response to REH-BCMA. In response to BCMA-negative REH, or with CAR T cells alone, all BCMA CAR T cells secreted little to no cytokines, with CACCR-BCMA CAR T cells showing a very similar profile to their unmodified counterpart. These data demonstrate that CACCRs do not increase the BCMA-independent cytotoxicity or effector functions of CACCR-BCMA CAR T cells.

[0248] To assess whether CACCR signaling alone is sufficient to drive the overt expansion of CACCR-BCMA CAR T cells, we performed a growth factor independent assay, where CACCR-BCMA CAR T cells were cultured in the absence of targets or exogenous cytokines. TRAC/CD52 dKO CAR T cells were generated as previously described. Cryopreserved TRAC/CD52 dKO CAR T cells were thawed, and the percentage of CAR T cells across all samples were normalized to the sample with the lowest transduction efficiency by the addition of non-transduced (NTD) T cells. 2.5×10^5 CAR T cells/mL were plated at a volume of 1.5-2 mL in 24-well tissue culture plates in duplicates. As a positive control, unmodified BCMA CAR T cells were cultured in the presence of 10 ng/mL recombinant human IL-15 that was replenished weekly. At the indicated timepoints, wells were mixed thoroughly by pipetting, and 100 μ L was harvested from each well for CAR T cell enumeration by flow cytometric analysis. Briefly, cells were washed with PBS and stained with the Zombie-NIR Fixable Viability kit (Biolegend). Cells were then washed with PBS, Fc-blocked, and stained with a PE-conjugated anti-idiotypic antibody for the detection of the P5A2 scFv, BV605-conjugated anti-human CD4 (Biolegend) and BV510-conjugated anti-human CD8 (Biolegend), diluted in PBS+1%BSA. After washing, cells were resuspended 100 μ L PBS+1%BSA containing 123count eBeads counting beads (Thermo Fisher) (10 μ L counting beads in 90 μ L PBS+1%BSA) prior to flow cytometric analysis. On Day 70 of the assay, each well was diluted 2-fold by the addition of media, and CAR T cell counts for subsequent timepoints were normalized accordingly by multiplying 2-fold.

[0249] FIG. 22A shows CACCR-BCMA CAR T cells being more persistent than unmodified BCMA CAR T cells in the absence of targets and exogenous cytokines. Compared to unmodified CAR T cells that declined within 3 weeks, CACCR-BCMA CAR

T cells showed prolonged persistence in the growth factor-independent assay. Nevertheless, CACCR-BCMA CAR T cells did not show overt proliferation, but instead underwent a slow but steady decline. The data indicate that while CACCR signaling can prolong CACCR-BCMA CAR T cell survival, CACCR signaling alone is insufficient to drive CACCR-BCMA CAR T cell expansion, nor can it sustain indefinite survival. Growth factor-independent assay was repeated using IL2 as the growth factor, and the ability of CACCR BCMA CAR T cells to expand in the absence of target and exogenous cytokine stimulation was further assessed in vitro by cell counting and Ki-67 analysis.

[0250] Briefly, TRAC/CD52 dKO CAR T cells were suspended in assay medium (X-VIVO15 medium (Lonza) containing 10% FBS (Hyclone)) to a final concentration of 1×10^6 cells/ml on Day 0. 500 μ l (5×10^5 cells) of each sample was plated in quadruplicate wells in a 24-well tissue culture plate. 1 ml of assay medium was added to each well to bring the final volume to 1.5 ml. 1.5 ml of PBS were added to the outer wells of the 24-well plate to prevent media evaporation. Twice per week, cells in each well were mixed and 750 μ l from each well was transferred to a new 24-well plate containing 750 μ l of fresh assay medium. As a positive control, 1 ml of irradiated MM.1S cells suspended at a final concentration of 5×10^5 cells/ml was added in place of assay medium, along with the addition of IL-2 at a final concentration of 50 IU/ml. Twice per week, cells in each positive control well were mixed and 750 μ l from each well was transferred to a new 24-well plate containing 750 μ l of fresh assay medium, along with the addition IL-2 at a final concentration of 50 IU/ml. The volume remaining in each well was used to measure Total Viable Cells (TVC) using the NC-200 analyzer. The assay continued until all cell counts in each test sample read zero on the NC-200 instrument. The viable cell fold expansion was calculated by dividing the TVC of a specific time point by the TVC of Day 0. For each time point after day 0, the TVC value was normalized by a multiple of 2 to account for the sample dilution occurring twice per week.

[0251] For the Ki-67 analysis assessing cells in active cell cycle, on days 0, 3, 7 and 14, cells were harvested and washed with 2 mL of BSA-containing stain buffer (BD Biosciences). Cells were suspended in 100 μ L of stain buffer containing pre-titrated amounts of each of the primary antibody. For CAR detection, 5 μ L of FITC-conjugated soluble BCMA protein was added. Cells were stained for 30 min at 4°C and washed twice with 2 mL of PBS. After the last wash, the supernatant was aspirated and the

cell pellet was loosened up using a vortex. Two milliliters of cold 70% ethanol were added dropwise to the cell pellet while vortexing and then the mixture was vortexed for an additional 30 seconds. Cells in 70% ethanol were incubated at -20 °C for 1 hour and then washed three times with BioLegend Cell Staining buffer. Cells were suspended in 100 µL of BioLegend Cell Staining buffer and a pre-titrated amount of human anti-Ki-67 antibody was added. Cells were stained in the dark for 30 min at room temperature and then washed twice with BioLegend Cell Staining buffer. Finally, the cell pellet was suspended in BioLegend Cell Staining buffer for flow cytometry analysis.

[0252] The data show that CACCR BCMA CAR T cells do not display target- and IL-2-independent growth in vitro. FIG. 22B shows that BCMA and CACCR BCMA CAR T cells display minimal or no expansion during the first 3 days. After this, CACCR BCMA CAR T cells persisted for ~ 2 weeks, followed by a gradual decline over time. By Day 42, BCMA CAR T cells and CACCR BCMA CAR T cells were non-detectable. FIG. 22C shows that approximately 30-40% of CAR⁺ cells in all three donors were expressing Ki-67⁺ on Day 0. However, Ki-67 expression substantially decreased by day 3 of IL-2 independent culture and was undetectable by Day 14. FIG. 22D shows that both BCMA CAR T cells and CACCR BCMA CAR T cells were capable of robust and comparable expansion when cultured with irradiated target cells and IL-2.

[0253] The data confirmed that CACCR did not sustain indefinite survival of CACCR-BCMA CAR T cells growth in the absence of IL2 or target, either in T cell-based or PBMC-based CAR T cells derived from multiple donors.

[0254] The growth factor-independent assay presented is but one way for assessing aberrant growth, as the CAR T cells have never seen target and are therefore quiescent. We additionally sought to evaluate whether the expansion and persistence of CACCR-BCMA CART cells remained finite in recently activated CACCR-BCMA CAR T cells once targets had been cleared. To this end, CACCR-BCMA CAR T cells were first exposed repeatedly to BCMA-expressing target cells to drive their full activation and expansion, before target cells were withdrawn from these cultures. Briefly, TRAC/CD52 dKO CAR T cells were generated as previously described. On Day 0 of the assay, cryopreserved TRAC/CD52 dKO CAR T cells were thawed, and the percentage of CAR T cells across all samples were normalized to the sample with the lowest transduction efficiency by the addition of non-transduced (NTD) T cells. 3×10^5 CAR⁺ T cells and 1×10^5 MM.1S-Luc-GFP- target cells

were plated at an E:T of 3:1 in a total volume of 750 uL per well in a 48-well tissue culture plate. As a comparator, 10 ng/mL recombinant human IL-15 (Miltenyi) was added to cocultures of unmodified CAR T cells and target cells. Every 2 or 3 days for the first 7 days, wells were mixed thoroughly by gentle pipetting, and 200 uL from each well was discarded.

5 Wells were then replenished with 2×10^5 fresh MM.1S-Luc-GFP- target cells in a volume of 200 uL. On Day 9, target cells were either continued to be added every 2 or 3 days for the remainder of the assay, or withdrawn completely. In IL-15-treated wells, 10 ng/mL recombinant human IL-15 was replenished twice weekly. Duplicate wells were set up for each condition. At the indicated timepoints, wells were mixed thoroughly by pipetting, and
10 50-200 uL was harvested from each well for CAR T cell enumeration by flow cytometric analysis. Briefly, cells were washed with PBS and stained with the Zombie-NIR Fixable Viability kit (Biolegend). Cells were then washed with PBS, Fc-blocked, and stained with a PE-conjugated anti-idiotypic antibody for the detection of the P5A2 scFv, BV605-conjugated anti-human CD4 (Biolegend) and BV510-conjugated anti-human CD8
15 (Biolegend), diluted in PBS+1%BSA. After washing, cells were resuspended 100 uL PBS+1%BSA containing 123count eBeads counting beads (Thermo Fisher) (10 uL counting beads in 90 uL PBS+1%BSA) prior to flow cytometric analysis.

[0255] FIG. 23A shows the expansion profiles of recently activated BCMA CAR T cells that were either continually stimulated with target cells, or had targets withdrawn. BCMA
20 CAR T cells that were continually stimulated with targets demonstrated proliferative potential. However, when targets were withdrawn, CACCR-BCMA CAR T cells ceased proliferation and instead underwent a slow and steady decline. This demonstrates that even after robust activation, the continued expansion and persistence of CACCR-BCMA CAR T cells is still dependent on target-induced CAR activation.

25 [0256] Long-term persistence of CACCR BCMA CAR T cells in the absence of target was next evaluated in vivo in non-tumor-bearing mice. To facilitate long-term in vivo tracking via bioluminescent imaging, CAR T cells labelled with the Click Beetle Red (CBR) luciferase reporter were generated. Briefly, lentivirus encoding the BCMA CARs and BFP-CBR were generated as described above. TRAC/CD52 dKO human CAR T cells
30 were generated and cryopreserved as described above, with the exception that on Day 2, T cells resuspended at 0.5 million cells per mL were co-transduced with equal volumes of CAR and BFP-CBR crude lentiviral supernatants. The percentage of CAR- and CBR-transduced cells was determined by flow cytometry using a PE-conjugated anti-idiotypic

antibody specific for the BCMA scFv and the BFP fluorescent reporter, respectively. On the day of T cell dosing, cryopreserved CAR T cells were thawed and normalized to the sample with the lower transduction efficiency by the addition of non-transduced (NTD) T cells.

7×10⁶ TRAC/CD52 CAR⁺ T cells were intravenously infused into non-tumor-bearing 8-10 weeks old female NSG mice. Starting on the day of T cell dosing and 1-3 times per week thereafter, in vivo T cell pharmacokinetics was determined by bioluminescent imaging. At the indicated timepoints, mice were bled for the enumeration of BCMA CAR T cells in the periphery. Specifically, 50 uL of whole blood from each mouse was subjected to red blood cell lysis using ACK Lysing Buffer (Gibco), Fc-blocked and stained with the following antibody cocktail diluted in PBS+1%BSA: FITC-conjugated anti-mouse CD45 (Biolegend), AlexaFluor647-conjugated anti-human CD45 (Biolegend), PE-conjugated anti-idiotypic antibody specific for the BCMA scFv and BUV395-conjugated anti-human CD3 (used as a surrogate for human T cell receptor; BD Biosciences). Finally, samples were washed in PBS and cell pellets were resuspended in 250 uL PBS+1%BSA containing 123count eBeads counting beads (Thermo Fisher) (10 uL counting beads in 240 uL PBS+1%BSA) prior to flow cytometric analysis.

[0257] FIG. 23B shows that CACCR BCMA CAR T cells did not display aberrant target-independent growth in non-tumor bearing mice. Compared to NTD and BCMA CAR T cells, CACCR BCMA CAR T cells had increased persistence and survival. However, CACCR BCMA CAR T cells showed no evidence of overt target-independent expansion and instead underwent an eventual decline. T cell luminescence at each timepoint is expressed as a fold change relative to that of Day 0. FIG. 23C shows comparable weight gain between mice that had received CACCR BCMA CAR T cells and BCMA CAR T cells. FIG. 23D shows that no enrichment of T cell receptor (TCR)-expressing T cells was observed, suggesting that CACCR did not increase the xenoreactive potential of BCMA CAR T cells. The data suggest that BCMA CAR T expressing a CACCR will not likely increase the potential of GvHD when administered to patients.

[0258] CAR T cells that have recently undergone robust target-induced activation may have a higher threshold for their return to quiescence. To determine if the activity of CACCR BCMA CAR T cells could be reined in once target cells were cleared, the expansion and long-term persistence of CBR-labeled TRAC/CD52 dKO CAR T cells in mice bearing subcutaneous tumors was evaluated by bioluminescent imaging.

[0259] CBR-labeled TRAC/CD52 dKO CAR T cells were generated by two-vector co-transduction as described above, with the exception that CAR+CBR+ double-transduced T cells were FACS-sorted based on staining with a PE-conjugated anti-idiotypic antibody specific for the BCMA scFv, as well as the BFP reporter. In parallel, unlabeled

5 TRAC/CD52 dKO CAR T cells were generated. 8-10 weeks old female NSG mice were subcutaneously implanted with 3×10^6 unlabeled Molp8 cells. 17 days post tumor-implantation, mice were randomized based on tumor burden and a total of 10×10^6 (9.5×10^6 unlabeled spiked with 0.5×10^6 CBR-labeled) TRAC/CD52 dKO CAR T cells were intravenously infused per mouse (n=7 or 8 mice per group). Starting 1 day after T cell
10 dosing and 1 or 2 times per week thereafter, in vivo T cell pharmacokinetics was determined by bioluminescent imaging, and subcutaneous tumor burden was determined by caliper measurements. Tumor burden was calculated using the formula Tumor volume = (width² x length/2).

[0260] The data in FIGs. 23E-23G demonstrate that in vivo expansion of CACCR BCMA
15 CAR T cells was target-dependent, and that they returned to quiescence following target clearance. FIG. 23E shows initial tumor progression in CAR T cell-treated mice, which was followed by tumor regression starting on Day 11. CACCR BCMA CAR T cells effectively eliminated tumors in 8/8 mice. By contrast, BCMA CAR T cells mediated tumor clearance in 5/7 mice. FIG. 23F shows the long-term CAR T cell pharmacokinetics in tumor-bearing
20 mice. In response to tumor target, both BCMA and CACCR BCMA CAR T cells underwent robust expansion, with peak expansion on Day 11. This was followed by a comparable rate of decline in both BCMA and CACCR BCMA CAR T cells. FIG. 23G shows comparable weight gain between mice that had received CACCR BCMA CAR T cells and BCMA CAR T cells.

25 **Example 8 Inhibition of the CACCR-BCMA CAR T cells**

[0261] To minimize any potential safety concerns in clinical development, the BCMA CAR construct was designed to incorporate two rituximab mimotopes, which would sensitize the BCMA CAR T cells to rituximab-mediated depletion. We next tested the effects of rituximab on the BCMA CAR T cells with or without the CACCR. The results
30 demonstrate that exposure of both types of BCMA CAR T cells to rituximab significantly decreased viable CAR T cells in a dose-responsive manner, as shown either in a

complement-dependent cytotoxicity assay (FIG. 24A) or NK cell-mediated antibody-dependent cellular cytotoxicity assay (FIG. 24B).

[0262] Next, we tested the effect of rituximab on decreasing BCMA CAR T cells, with or without the CACCR, in vivo. Briefly, 8-10 weeks old female NSG mice were irradiated with 1 Gy one day prior to intravenous inoculation of 5×10^6 MM.1S-Luc-GFP. 14 days post tumor implantation, mice were randomized based on tumor burden and 3×10^6 TRAC/CD52 dKO CAR T cells were intravenously infused per mouse (n=10 mice per group). Rituximab (10 mg/kg) or PBS vehicle control was dosed intraperitoneally starting on the day of CAR T cell dosing and then daily for 4 additional days (n=9 or 10 per group). Tumor burden was monitored twice weekly by bioluminescent imaging. 7 days after CAR T cell dosing, mice were bled for the enumeration of BCMA CAR T cells in the periphery. Specifically, 50 uL of whole blood from each mouse was subjected to red blood cell lysis using ACK Lysing Buffer (Gibco), Fc-blocked and stained with the following antibody cocktail diluted in PBS+1%BSA: FITC-conjugated anti-mouse CD45 (Biolegend), BV421-conjugated anti-human CD45 (Biolegend) and PE-conjugated anti-idiotypic antibody specific for the P5A2 scFv. Finally, samples were washed in PBS and cell pellets were resuspended in 250 uL PBS+1%BSA containing 123count eBeads counting beads (Thermo Fisher) (10 uL counting beads in 240 uL PBS+1%BSA) prior to flow cytometric analysis.

[0263] FIGs. 24C-24D show the effects of rituximab treatment on CAR T cell numbers and tumor growth kinetics. FIG. 24C shows the CAR T cell counts in peripheral blood 7 days post-CAR T cell dosing. In PBS-treated tumor-bearing mice, CACCR BCMA CAR T cells showed increased expansion compared to BCMA CAR T cells. However, treatment with rituximab resulted in efficient and complete elimination of both CAR T cells, demonstrating that CACCR BCMA CAR T cells remain susceptible to rituximab-mediated depletion in vivo. FIG. 24D shows tumor burden following rituximab-mediated CAR T cell depletion. While CAR T cells effectively controlled tumor growth in PBS-treated mice, CAR T cell elimination by rituximab compromised anti-tumor efficacy, as shown by sub-optimal tumor clearance and subsequent tumor progression.

[0264] The data in FIGs. 25A-25B demonstrate that the activities of both the BCMA CAR T cells and CACCR BCMA CAR T cells can also be inhibited by the tyrosine kinase inhibitor dasatinib. It has been reported that dasatinib inhibits TCR signaling, T cell

proliferation and effector function. We tested in an in vitro cell killing assay the effect of 150 nM dasatinib on the activities of the BCMA CAR T cells with or without the CACCR. The results in FIGs. 25A-25B show that dasatinib abolished the cytotoxicity (FIG. 25A) and inhibited cytokine secretion by both types of BCMA CAR T cells (FIG. 25B), though we
5 observed that the effects were reversible when dasatinib was washed out of the cultures (data not shown).

Example 9 Long-term in vivo pharmacokinetics of CACCR BCMA CAR T cells

[0265] The long-term in vivo pharmacokinetics of CACCR BCMA CAR T cells was additionally evaluated in an orthotopic model of multiple myeloma. 8-10 weeks old female
10 NSG mice were irradiated with 1 Gy one day prior to intravenous inoculation of 5×10^6 MM.1S-Luc-GFP. 15 days post tumor implantation, mice were randomized based on tumor burden and a suboptimal dose of 3×10^6 TRAC/CD52 dKO CACCR-BCMA CAR T cells were intravenously infused per mouse (n=10 mice per group). Thereafter, tumor burden was monitored twice weekly by bioluminescent imaging. At the indicated timepoints starting
15 from Day 30, mice were bled for the enumeration of CACCR BCMA CAR T cells in the periphery. Specifically, 50 uL of whole blood from each mouse was subjected to red blood cell lysis using ACK Lysing Buffer (Gibco), Fc-blocked and stained with the following antibody cocktail diluted in PBS+1%BSA: FITC-conjugated anti-mouse CD45 (Biolegend), BV421-conjugated anti-human CD45 (Biolegend) and PE-conjugated anti-idiotypic antibody
20 specific for the BCMA scFv. Finally, samples were washed in PBS and cell pellets were resuspended in 250 uL PBS+1%BSA containing 123count eBeads counting beads (Thermo Fisher) (10 uL counting beads in 240 uL PBS+1%BSA) prior to flow cytometric analysis.

[0266] FIGs. 26A-26C again demonstrate that in vivo expansion of CACCR BCMA CAR T cells was target-dependent. FIG. 26A shows various anti-tumor response in individual
25 mouse following treatment with a suboptimal dose of 3×10^6 TRAC/CD52 dKO CACCR BCMA CAR T cells, at which dose disease relapsed significantly in some mice, and completely regressed in others. FIG. 26B shows that disease relapse was followed by a sharp increase in CACCR BCMA CAR T cell expansion. FIG. 26C shows that in mice where tumors were cleared, no expansion of CACCR BCMA CAR T cells was observed,
30 and the numbers of CACCR BCMA CAR T cells declined to the limit of detection by Day 80. Taken together, these demonstrate that active expansion of CACCR BCMA CAR T

cells was target-dependent, and that constitutive signaling through the CACCR alone was insufficient to drive target-independent CAR T cell expansion or indefinite survival.

Example 10 The effect of CACCR on BCMA CAR T cell in an orthotopic model of multiple myeloma

- 5 [0267] The effect of CACCR on BCMA CAR T cell in vivo activity and pharmacokinetics was next evaluated in an orthotopic model of multiple myeloma, in which disease initially establishes in the bone marrow and relapse subsequently occurs in extramedullary sites. 8-10 weeks old female NSG mice were irradiated with 1 Gy one day prior to intravenous inoculation of 5×10^6 MM.1S-Luc-GFP. 15 days post tumor
- 10 implantation, mice were randomized based on tumor burden and the indicated numbers of TRAC/CD52 dKO CACCR-BCMA CAR T cells were intravenously infused per mouse (n=10 mice per group). Thereafter, tumor burden was monitored twice weekly by bioluminescent imaging. At the indicated timepoints, mice were bled for the enumeration of CACCR BCMA CAR T cells in the periphery.
- 15 [0268] FIGs. 27A-27D show that CACCR improved the anti-tumor efficacy and expansion of BCMA CAR T cells in the orthotopic model of multiple myeloma. FIG. 27A shows the anti-tumor efficacy in response to the indicated numbers of CAR T cells. Compared to BCMA CAR T cell-treated groups that relapsed by Day 38, mice that had received CACCR BCMA CAR T cells showed improved durability of response. FIGs. 27B-
- 20 27D show CAR T cell pharmacokinetics in the peripheral blood of mice that had received 1×10^6 (FIG. 27B), 3×10^6 (FIG. 27C), or 5×10^6 (FIG. 27D) CAR T cells. Consistent with improved anti-tumor efficacy, the results show that CACCR increased the expansion of BCMA CAR T cells in vivo.