COMPOSITIONS AND METHODS REGARDING ENGINEERED AND NON-ENGINEERED γδ-T CELLS FOR TREATMENT OF HEMATOLOGICAL TUMORS

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Appl. No. 62/739,822, filed October 1, 2018, the contents of which are incorporated in the entirety for all purposes.

SEQUENCE LISTING

[0001.1] 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 December 17, 2019, is named ADC-0005-PCT_SL.txt and is 147,616 bytes in size.

BACKGROUND OF THE INVENTION

[0002] Adoptive cellular therapy has undergone near constant iteration for more than thirty (30) years, from early days focusing on basic lymphokine activation and/or tumor infiltration to more recent strategies engineering these immune cells to express genetically engineered antigen receptors, such as chimeric antigen receptors (CARs)s. While there have been some hints and indications of the curative potential of these approaches along the way, much still remains to be done. In particular, successful tumor eradication by CAR-T lymphocytes depends on CAR-T cell persistence and effector function, but an excess of either can trigger graft-versus-host effects in the patient. As such the art is testing myriad co-stimulation strategies in both T and NK cells, and in αβ T cells in particular, with a view to balancing efficacy with safety. Notably, the practical translation of any of these various approaches to alloeneic γδ T cells is at best uncertain, given the current lack of understanding around the co-stimulation requirements of γδ T cells as compared to αβ T cells. *See, e.g.*, Ribot *et al.*, "Searching for "signal 2": costimulation requirements of γδ T cells", *Cell. Mol. Life Sci.* (2011) 68:2345-2355.

[0003] Accordingly, improved strategies are still needed to improve the specificity or selectivity of the cells, to improve safety of the cells, for example by reducing or avoiding graft

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versus host (GVH) effects, to improve efficacy of the cells, for example, by avoiding suppression of effector functions, and to improve the activity and/or survival of the cells upon administration to subjects. Provided are methods, cells, compositions, kits, and systems that meet such needs.

SUMMARY OF INVENTION

[0004] Aspects of the invention include an isolated nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a binding domain that specifically binds to a tumor associated antigen (TAA) expressed on a surface of a hematological tumor cell; an, e.g., CD8α, hinge domain; an, e.g., CD8α, transmembrane domain; a costimulatory signaling region, optionally wherein the costimulatory signaling region is selected from a 4-1BB (CD137) costimulatory signaling region and a CD27 costimulatory signaling region; and a CD3ζ signaling domain.

[0005] Aspects of the invention further include a non-engineered γδ T cell as described herein as well as a γδ T cell comprising a nucleic acid encoding a CAR construct as described herein, wherein the γδ T cell functionally expresses the nucleic acid encoded CAR on the surface of the γδ T cell. Aspects of the invention further include a plurality of engineered or non-engineered γδ T cells as described herein. Aspects of the invention further include a method of making a γδ T cell or plurality of γδ T cells described herein wherein the method comprises transfecting γδ T cell(s) with a construct described herein. Aspects of the invention further include a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a γδ T cell or plurality of γδ T cells as described herein. Aspects of the invention further include contacting the hematological tumor cell with a tumor cell killing effective amount of a γδ T cell as described herein.

[0006] In one aspect, the present invention provides an isolated nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a binding domain that specifically binds to a tumor associated antigen (TAA) expressed on a surface of a hematological tumor cell; (b) a hinge domain, such as a CD8α hinge domain; (c) a transmembrane domain, such as a CD8α transmembrane domain; (d) a costimulatory signaling region or a combination of costimulatory signaling regions, optionally wherein the costimulatory signaling region is selected from a 4-1BB (CD137) costimulatory signaling region and a CD27 costimulatory signaling region; and (e) a signaling domain, such as a CD3ζ signaling domain. In some

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embodiments, the foregoing elements (a)-(e) are encoded on the sense strand of the isolated nucleic acid in 5' to 3' order.

[0007] In some embodiments, the binding domain specifically binds to CD20. In some embodiments, the binding domain selectively binds to an epitope within CD20 bound by, or competes for binding with, an anti-CD20 antibody selected from the group consisting of 3B9, 3H7, 2B7, and 9C11, preferably 3H7. In some embodiments, the binding domain comprises the complementary determining regions of an anti-CD20 antibody selected from the group consisting of 3B9, 3H7, 2B7, and 9C11, preferably 3H7.

[0008] In some embodiments, the isolated nucleic acid encodes a heavy chain variable region (HCVR) sequence and a light chain variable region (LCVR) sequence, e.g.,: wherein the HCVR and LCVR sequences are SEQ ID NO:99 and 107 respectively; a heavy chain complementarity determining region 1, 2, and 3 sequence of SEQ ID NOs: 101, 103, and 105 respectively, and a light chain complementarity determining region 1, 2, and 3 sequence of SEQ ID NOs: 109, 111, and 113 respectively; a heavy chain complementary determining region 3 (HCDR3) and a light chain CDR3 (LCDR3), wherein the HCDR3 and LCDR3 are selected from the group consisting of SEQ ID NO:345 and 353; 201 and 209; and 249 and 257; a heavy chain variable region (HCVR) sequence and a light chain variable region (LCVR) sequence, wherein the HCVR and LCVR sequences are selected from the group consisting of SEQ ID NO: 339 and 347; 195 and 203; and 243 and 251; and/or a heavy chain complementary determining region 3 (HCDR3) domain and a light chain CDR3 (LCDR3) domain, wherein the HCDR3 domain comprises an amino acid sequence of the formula X1—X2—X3—X4—X5—X6—X7—X8—X9—X10—X11—X12—X13—X14—X15—X16—X17—X18—X19, wherein X1=A, V or T; X2=K; X3=D; X4=P, F or G; X5=S or H; X6=Y; X7=G; X8=S or H; X9=G or F; X10=S or Y; X11=Y, N or S; X12=Y, G or H; X13=G, L or S; X14=Y, M or D; X15=Y, D or V; X16 =G, V or absent; X17=M or absent; X18=D or absent; X19=V or absent (SEQ ID NO: 369); and the LCDR3 domain comprises an amino acid sequence of the formula X1—X2—X3—X4—X5—X6—X7—X8—X9, wherein X1=Q; X2=Q; X3=R or S; X4=N, Y or F; X5=N, D, or Y; X6=W; X7=P; X8=L; X9=T (SEQ ID NO: 370).

[0009] In some embodiments, the isolated nucleic acid encodes a binding domain that specifically binds to CD19 or BCMA. In some embodiments, the binding domain specifically

binds to BCMA. In some embodiments, the binding domain selectively binds to an epitope within BCMA bound by, or competes for binding with, an anti-BCMA binding region having a sequence selected from the group consisting of SEQ ID NO: 27 and 28; SEQ ID NO: 29 and 30; and SEQ ID NO: and 31 and 32. In some embodiments, b. the binding domain comprises the complementarity determining regions of an anti-BCMA binding region having a sequence selected from the group consisting of SEQ ID NO: 27 and 28; SEQ ID NO: 29 and 30; and SEQ ID NO: and 31 and 32.

[0010] In some embodiments of any one of the foregoing, or as described herein, the CAR comprises: a CD8α hinge domain comprising SEQ ID NO:1 (PTPAPTIASQPLSLRPE ACRPAAGGAVHTRGLDFACDIY); or SEQ ID NO:2 (TTTPAPRPPTPAPTIASQPLSLR PEACRPAAGGAVHTRGLDFACDIY). In some embodiments of any one of the foregoing, or as described herein, the CAR comprises a CD8α transmembrane domain comprising SEQ ID NO:3 (IWAPLAGTCGVLLLSLVITLYC).

[0011] In some embodiments of any one of the foregoing, or as described herein, the CAR comprises a CD3ζ signaling domain comprising: SEQ ID NO:4 (RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR) or SEQ ID NO:5 (RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR).

[0012] In some embodiments of any one of the foregoing, or as described herein, the CAR comprises a 4-1BB costimulatory signaling region comprising SEQ ID NO:6 (KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL); or a CD27 costimulatory signaling region comprising SEQ ID NO:7. (QRRKYRSNKGESPVEPAEPCH YSCPREEEGSTIPIQEDYRKPEPACSP). In some embodiments of any one of the foregoing, or as described herein, the isolated nucleic acid encodes the 4-1BB costimulatory signaling region comprising SEQ ID NO:6 and the CD27 costimulatory signaling region comprising SEQ ID NO:7.

[0013] In some embodiments of any one of the foregoing, or as described herein, the isolated nucleic acid further encodes a secreted cytokine; or a secreted common gamma chain interleukin; or a secreted common gamma chain interleukin such as IL-15, preferably wherein the secreted

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common gamma chain interleukin such as IL-15 comprises an interleukin polypeptide sequence operably linked to a secretion signal sequence (e.g., a secretion signal of SEQ ID NO: 33 or 49). In some embodiments, the isolated nucleic acid encodes a secreted IL-15, preferably wherein the IL-15 comprises the sequence of SEQ ID NO:34, more preferably wherein the IL-15 comprises the sequence of 34 operably linked to a secretion signal sequence of SEQ ID NO:33, or wherein the IL-15 comprises the sequence of SEQ ID NO: 34 operably linked to a secretion signal sequence of SEQ ID NO: 49. In some cases, the secreted cytokine, common gamma chain interleukin, and/or IL-15 are encoded carboxy terminal to the binding region, hinge and transmembrane domains, signaling domain, and/or costimulation endodomain. In some cases, the secreted cytokine, common gamma chain interleukin, and/or IL-15 are encoded on the sense strand 3' of the region encoding the binding region, hinge and transmembrane domains, signaling domain, and/or costimulation endodomain.

[0014] In some embodiments, the nucleic acid encodes a multi-cistronic linker region configured to facilitate translation of the CAR and the secreted cytokine, common gamma chain cytokine, or IL-15 as separate polypeptides. In some embodiments, the multi-cistronic linker region encodes a self cleavage and/or a cleavage polypeptide sequence. In some cases, the self-cleavage sequence is a P2A, F2A, T2A, or E2A self cleavage sequence. In some cases, the cleavage sequence is a furin cleavage sequence. In some cases, the cleavage sequence (e.g., furin cleavage sequence) is amino terminal to a self cleavage sequence. In some embodiments, the multi-cistronic linker region encodes an internal ribosome entry site. In some embodiments, the nucleic acid encodes a multi-cistronic linker region amino terminal to the interleukin or cytokine or interleukin or cytokine secretion signal, preferably wherein the multicistronic linker region comprises a sequence of any one of SEQ ID NOs: 43-45, 47, or 52-55 or a combination thereof, or encodes an internal ribosome entry site, e.g., SEQ ID NO: 56 or 60.

[0015] In some embodiments, the binding domain specifically binds to CD20 and the nucleic acid encodes SEQ ID NO:8, 9, 10, 11, 12, 46, 48, or 57 and 58. In some embodiments, the nucleic acid comprises the sequence of SEQ ID NO: 13, 14, 15, 16, 17, 50, 51, or 59. In some embodiments, the binding domain specifically binds to BCMA and the nucleic acid encodes SEQ ID NO: 35, 36, 37, or 38. In some embodiments, the nucleic acid comprises the sequence of SEQ ID NO: 39, 40, 41, or 42.

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[0016] In some aspects, the present invention provides a polypeptide or plurality of polypeptides encoded by any one of the foregoing isolated nucleic acids, or as described herein. In some embodiments, the present invention provides a T cell, such as a γδ T cell that comprises any one of the foregoing polypeptides or plurality of polypeptides. In some embodiments, the T cell expresses a functional binding domain as described herein on the surface of the T cell. In some embodiments, the T cell secretes a cytokine such as a common gamma chain interleukin, e.g., IL-15.

[0017] In some embodiments, the T cell exhibits *in vitro* and/or *in vivo* cell killing activity against a hematological tumor cell that exhibits cell surface expression of a tumor associated antigen (TAA). In some embodiments, the hematological tumor cell killing activity of said, e.g., γδ, T cell is greater than an innate level of *in vitro* and/or *in vivo* hematological tumor cell killing activity in a control, e.g., γδ, T cell that does not comprise a CAR construct. In some embodiments, the, e.g., γδ, T cell exhibits the increased hematological tumor cell killing activity against HLA class I^+ hematological tumor cells. In some embodiments, the hematological tumor cell killing activity or increased hematological tumor cell killing activity persists for, for about, for at least, or for at least about, 6 days to 180 days after first contact with the hematological tumor cell.

[0018] In some embodiments, the, e.g., γδ, T cell proliferates in response to contact with a hematological tumor cell that exhibits cell surface expression of the tumor associated antigen (TAA). In some embodiments, the, e.g., γδ, T cell exhibits increased proliferation in response to contact with a hematological tumor cell that exhibits cell surface expression of the tumor associated antigen (TAA) as compared to a control, e.g., γδ, T cell that does not functionally express the nucleic acid encoded CAR on the surface of the, e.g., γδ, T cell. In some embodiments, the, e.g., γδ, T cell proliferates in a host organism that comprises the hematological tumor cell that exhibits cell surface expression of the tumor associated antigen (TAA).

[0019] In some embodiments, the, e.g., γδ, T cell proliferation or increased, e.g., γδ, T cell proliferation persists for, for about, for at least, or for at least about, 6 days to 180 days after first contact with the hematological tumor cell. In some embodiments, the, e.g., γδ, T cell expresses pro-inflammatory cytokine(s), such as tumor necrosis factor alpha and/or interferon gamma after contact with the hematological tumor cell. In some embodiments, the, e.g., γδ, T cell expresses pro-inflammatory cytokine(s), such as tumor necrosis factor alpha and/or interferon gamma after

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contact with the hematological tumor cell, in an amount greater than a control T cell that does not functionally express the nucleic acid encoded CAR on the surface of the cell.

[0020] In some embodiments, the, e.g., γδ, T cell exhibits reduced, substantially reduced, essentially no, or no graft versus host response when introduced into an allogeneic host in comparison to a graft versus host response exhibited by an αβ T cell administered to an allogeneic host. In some embodiments, the T cell is a γ T cell. In some embodiments, the T cell is a δ T cell. In some embodiments, the T cell is a γδ T cell. In some embodiments, the T cell is a δ1, a δ2, a δ3, or a δ4 T cell, preferably a $\delta 2^-$ δ T cell, more preferably a δ1 δ T cell. In some embodiments, the T cell is a δ1, a δ2, a δ3, or a δ4 γδ T cell, preferably a $\delta 2^-$ γδ T cell, more preferably a δ1 γδ T cell.

[0021] In another aspect, the present invention provides a plurality of any one of the foregoing, e.g., γδ, T cells, or a plurality of , e.g., γδ, T cells as described herein. In some embodiments, the plurality comprises at least about 10^8, e.g., γδ, T cells, preferably from about 10^8, e.g., γδ, T cells to about 10^{11}, e.g., γδ, T cells. In some embodiments, the the plurality comprises a composition that is at least 60%, 80%, or from about 60% or 80% to about 90% or 95% δ1, δ2, δ3, or δ4 γδ T cells, preferably δ1 or δ2 γδ T cells, more preferably $\delta 2^-$ γδ T cells, most preferably δ1 γδ T cells.

[0022] In some embodiments, the present invention provides a method of making an, e.g., γδ, T cell as described herein, or a plurality of, e.g., γδ, T cells as described herein, wherein the method comprises transfecting the T cell(s) with a construct comprising an isolated nucleic acid sequence as described herein. In some cases, the method comprises, e.g., gamma, retroviral transduction. In some cases, the method comprises *ex vivo* expansion of the T cell(s), wherein the *ex vivo* expansion is performed before transfection and/or after transfection of the isolated nucleic acid sequence. In some cases, the method comprises *ex vivo* expansion of the T cell(s), wherein the *ex vivo* expansion is performed before transfection and after transfection of the isolated nucleic acid sequence. In some cases, the method comprises *ex vivo* expansion of the T cell(s), wherein the *ex vivo* expansion is performed after transfection of the isolated nucleic acid sequence. In some embodiments, the method comprises producing the from about 10^8, e.g., γδ, T cells to about 10^{11}, e.g., γδ, T cells that functionally express a CAR described herein within about 30 days of transfection.

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[0023] In another aspect, the present invention provides a pharmaceutical composition comprising a pharmaceutically acceptable excipient and an, e.g., γδ, T cell described herein.

[0024] In another aspect, the present invention provides a method of killing a hematological tumor cell, the method comprising contacting the hematological tumor cell with a tumor cell killing effective amount of an, e.g., γδ, T cell, plurality of such cells, and/or pharmaceutical composition comprising such cells as described herein. In some embodiments, the method comprises introducing a therapeutically effective amount of the, e.g., γδ, T cell(s) or the pharmaceutical composition into a host organism comprising the hematological tumor cell. In some embodiments, the method comprises introducing into a host organism comprising the hematological tumor cell a therapeutically effective amount of the, e.g., γδ, T cell(s) or the pharmaceutical composition and simultaneously or sequentially administering one or more methods to elevate common gamma chain cytokine(s).

[0025] In some embodiments, the administering one or more methods to elevate common gamma chain cytokine(s) comprises administering simultaneously with introducing the, e.g., γδ, T cell(s) or sequentially an amount of common gamma chain cytokine(s) effective to increase proliferation, cytotoxic activity, persistence, or the combination thereof of the introduced, e.g, γδ, T cell(s), preferably wherein the method comprises administering IL-2, more preferably wherein the method comprises administering IL-15. In some embodiments, the one or more methods to elevate common gamma chain cytokine(s) comprise administering an amount of common gamma chain cytokine(s) effective to increase proliferation, cytotoxic activity, persistence, or the combination thereof of the introduced T cell(s) before and/or after introducing the T cell(s). In some embodiments, the one or more methods to elevate common gamma chain cytokine(s) comprises lymphodepletion before introducing the T cell(s).

[0026] In some embodiments, the one or more methods to elevate common gamma chain cytokine(s) comprises secretion of one or more common gamma chain cytokine(s) from the introduced T cell(s). In some embodiments, the method reduces the *in vivo* tumor burden in the host organism, and/or increases the mean survival time of the host organism as compared to a control organism, wherein the control organism is not treated with the T cell(s) or the pharmaceutical composition. In some embodiments, the method is a method of treating cancer in a subject in need thereof. In some embodiments, the present invention provides an, e.g., γδ, T cell,

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plurality of, e.g., γδ, T cell(s), or a pharmaceutical composition described herein for use in treating a hematological tumor cell in a subject in need thereof.

[0027] In one aspect, the present invention provides a method of treating cancer by administering a therapeutically effective amount of γδ T cells, wherein the cancer comprises hematological tumor cells that exhibit cell surface expression of CD20; or administering a therapeutically effective amount of γδ T cells, wherein the cancer comprises hematological tumor cells that exhibit cell surface expression of BCMA. In some embodiments, the method comprises simultaneously with the administering of γδ T cells or sequentially, administering one or more methods to elevate common gamma chain cytokine(s). In some embodiments, the method comprises performing a plurality of administrations of the γδ T cells, wherein the interval between the plurality of administrations is at least about a week, preferably at least about 2, 3, 4, 5, 6, 7, 8, or 12 weeks, and/or no more than once every 6 or 12 months. In some embodiments, the present invention provides a pharmaceutical composition for use in any one of the foregoing methods of treatment.

INCORPORATION BY REFERENCE

[0028] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] **FIG. 1** is a schematic illustration of an embodiment of a chimeric antigen receptor (CAR) containing one costimulatory signaling endodomain (left) or two costimulatory signaling endodomains (right). As used herein costimulatory signaling endodomains are also referred to as costimulation endodomains or costimulatory endodomains. Exemplary costimulatory signaling endodomains useful in exemplary CARs include, without limitation, CD28; CD137 (41BB); CD278 (ICOS); CD27; CD134 (OX40); TLR2, and combinations thereof.

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[0030] **FIG. 2** illustrate sequences of binding domains that specifically bind to an epitope within CD20. Figure 2 discloses SEQ ID NOS 335-363, 99, 364, 107, and 365-368, respectively, in order of appearance.

[0031] **FIG. 3** illustrates induction of apoptosis CD20-expressing normal B cells by untransduced Vδ1 cells and Vδ1 cells transduced with various CD20-specific CAR constructs.

[0032] **FIG. 4** illustrates potent cytotoxic activity of CD20-specific CAR γδ T cells against lymphoma cell lines

[0033] **FIG. 5** illustrates cytotoxicity of engineered CAR γδ T cells described herein against Raji cells. Top: Binding domains containing CDRs of 3B9, 2B7, 3H7, and 9C11 are tested in a CAR construct encoding a 4-1BB costimulation endodomain and CD3ζ signaling domain.

[0034] **FIG. 6** illustrates cytotoxicity of engineered CAR γδ T cells described herein against Raji cells. Top: Binding domains containing CDRs of 3H7 are tested. Bottom: cytotoxicity against Raji cells of γδ T cells expressing a CAR containing a 3H7 binding domain, a CD3ζ signaling domain, and various costimulation endodomains as indicated a is demonstrated. The 3H7-CD27z CAR has a 3H7 binding domain, CD8α hinge and transmembrane domain, CD27 costimulation endodomain, and CD3ζ signaling domain. 3H7-5.1 has a 3H7 binding domain, a CD8α hinge and transmembrane domain, a 4-1BB costimulation endodomain, and a CD3ζ signaling domain.

[0035] **FIG. 7** illustrates results of a cytotoxicity assay with re-challenge of the indicated γδ T cells. Arrows indicate time of re-administration.

[0036] **FIG. 8** illustrates *in vivo* efficacy of γδ T cells described herein in a subcutaneous Raji cell NOD scid gamma (NSG) mouse model.

[0037] **FIG. 9** illustrates enumeration of intratumoral CD20-specific γδ CAR-T cells *in vivo* indicating *in vivo* expansion of γδ CAR-T cells and tumor clearance.

[0038] **FIG. 10** illustrates *in vivo* proliferation of CD20-specific γδ CAR-T cells in $CD20^+$ lymphoma tumor and other organs.

[0039] **FIG. 11** illustrates *in vivo* efficacy of γδ T cells described herein in a disseminated Raji cell NOD scid gamma (NSG) mouse model.

[0040] **FIG. 12** illustrates effective treatment of a disseminated Raji tumor with CD20-specific γδ CAR-T cells in the SRG-15 mouse model that expresses human IL-15, without induction of a Graft versus Host (GVH) response. In contrast, CD20-specific αβ CAR-T cells elicit a lethal GVH response.

[0041] **FIG. 13** illustrates a manufacturing process for production of engineered γδ CAR-T cells and non-engineered γδ CAR-T cells.

[0042] **FIG. 14** illustrates therapeutic efficacy and persistence of CD20 CAR Vδ1 T cells expressing sIL15 in NSG mice subcutaneously implanted with Raji cells and subsequently re-challenged (Day 62) with Raji cells at a different implantation site.

[0043] **FIG. 15** illustrates transduction efficiency of Vδ1 cells with indicated anti-B cell maturation antigen (BCMA) scFv CAR constructs. BCMA is also known as tumor necrosis factor receptor superfamily member 17 (TNFRSF17).

[0044] **FIG. 16** illustrates cytotoxic activity of Vδ1 T cells transduced with various anti-BCMA CAR constructs against a panel of multiple myeloma BCMA+ cell lines. The SCABER-Luc cell line is a control cell line that is BCMA-negative.

[0045] **FIG. 17** illustrates cytotoxic activity of Vδ1 T cells transduced with various anti-BCMA CAR constructs against a panel of multiple myeloma and Burkitt lymphoma BCMA+ cell lines.

[0046] **FIG. 18** illustrates in vivo therapeutic efficacy of anti-BCMA CAR Vδ1 T cells against subcutaneously implanted NCI-H929 cells.

DETAILED DESCRIPTION

Definitions:

[0047] For purposes of interpreting this specification, the following definitions will apply, and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth conflicts with any document incorporated herein by reference, the definition set forth below shall control. Unless defined otherwise, all technical and scientific

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terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

[0048] "About" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more preferably ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

[0049] The term "γδ T-cells (gamma delta T-cells)" as used herein refers to a subset of T-cells that express a distinct T-cell receptor (TCR), namely γδTCR, on their surface, composed of one γ-chain and one δ-chain. The term "γδ T-cells" specifically includes all subsets of γδ T-cells, including, without limitation, Vδ1 and Vδ2, Vδ3 γδ T cells, as well as naïve, effector memory, central memory, and terminally differentiated γδ T-cells. As a further example, the term "γδ T-cells" includes Vδ4, Vδ5, Vδ7, and Vδ8 γδ T cells, as well as Vγ2, Vγ3, Vγ5, Vγ8, Vγ9, Vγ10, and Vγ11 γδ T cells. In some embodiments, the γδ T-cells are $V\delta1^-$, $V\delta2^-$, or $V\delta1^-$ and $V\delta2^-$. Compositions and methods for making and using engineered and non-engineered γδ T cells and/or sub-types thereof include, without limitation, those described in US 2016/0175358; WO 2017/197347; US 9499788; US 2018/0169147; US 9907820; US 2018/0125889 and US 2017/0196910, the contents of each of which are incorporated by reference for all purposes, including the said compositions and methods for making and using engineered and non-engineered γδ T cells and/or sub-types thereof. The present application further contemplates T cells, or other engineered leukocytes or lymphocytes, that express one γ-chain or one δ-chain, optionally in combination with a second polypeptide to form a functional TCR. Such engineered leukocytes or lymphocytes, that express one γ-chain or one δ-chain may be used in the methods or present in the compositions described herein.

[0050] As used herein, the term "T lymphocyte" or "T cell" refers to an immune cell that expresses or has expressed CD3 (CD3+) and a T Cell Receptor (TCR+). T cells play a central role in cell-mediated immunity. A T cell that "has expressed" CD3 and a TCR has been engineered to eliminate CD3 and/or TCR cell surface expression.

[0051] As used herein, the term "TCR" or "T cell receptor" refers to a dimeric heterologous cell surface signaling protein forming an alpha-beta or gamma-delta receptor or combinations

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thereof. αβTCRs recognize an antigen presented by an MHC molecule, whereas γδTCR can recognize an antigen independently of MHC presentation.

[0052] The term "MHC" (major histocompatibility complex) refers to a subset of genes that encodes cell-surface antigen-presenting proteins. In humans, these genes are referred to as human leukocyte antigen (HLA) genes. Herein, the abbreviations MHC or HLA are used interchangeably.

[0053] "Activation", as used herein, refers to the state of a T cell that has been sufficiently stimulated to induce detectable cellular proliferation. Activation can also be associated with induced cytokine production, and detectable effector functions. The term "activated T cells" refers to, among other things, T cells that are undergoing cell division.

[0054] The term "antibody," as used herein, refers to an immunoglobulin molecule which specifically binds with an antigen. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. Antibodies are typically tetramers of immunoglobulin molecules. The antibodies in the present invention may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, Fv, Fab and $F(ab)_2$, as well as single chain antibodies and humanized antibodies (Harlow et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, N.Y.; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426).

[0055] The term "antibody fragment" refers to a portion of an intact antibody and refers to the antigenic determining variable regions of an intact antibody. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')2, and Fv fragments, linear antibodies, scFv antibodies, and multispecific antibodies formed from antibody fragments.

[0056] An "antibody heavy chain," as used herein, refers to the larger of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations.

[0057] An "antibody light chain," as used herein, refers to the smaller of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations. κ and λ light chains refer to the two major antibody light chain isotypes.

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[0058] By the term "synthetic antibody" as used herein, is meant an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage as described herein. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using synthetic DNA or amino acid sequence technology which is available and well known in the art.

[0059] The term "antigen" or "Ag" as used herein is defined as a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA that comprises a nucleotide sequence or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an "antigen" as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full-length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a "gene" at all. It is readily apparent that an antigen can be generated, synthesized, or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid.

[0060] The term "epitope" includes any protein determinant, lipid or carbohydrate determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of active surface groupings of molecules such as amino acids, lipids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics. An antibody is said to specifically bind an antigen when the equilibrium dissociation constant (K_D) is in the range of $10^{-6} – 10^{-12}$M.

[0061] Antibodies 3B9, 9C11, 3H7, 2B7, and 10F2 represent exemplary embodiments of antibodies that specifically recognize CD20. These antibodies, fragments thereof, and their

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complementary determining regions, are also described in U.S. 2009/0035322, where they are referred to as 3B9-10, 9C11-14, 3H7-6, 2B7-7, and 10F2-13 respectively. As described herein, these antibodies, fragments thereof, and their complementary determining regions, are useful in generating anti-CD20 chimeric antigen receptor (CAR) constructs and engineering and using CAR-T cells for treating hematological tumors that express CD20.

[0062] Binding domains 21587N, 16747P, 16711P, and 16716P represent exemplary embodiments of binding domains that specifically recognize BCMA. These antibodies, fragments thereof, and their complementary determining regions, are also described in U.S. 16/516,028, filed July 18, 2019, the contents of which are incorporated by reference in the entirety and for all purposes and in particular for the binding domains, antibodies, antibody fragments, complementarity determining regions, polypeptides containing said complementarity determining regions, nucleic acids encoding for said complementarity determining regions, and epitope specificities and assays for determining epitope specificity described therein. In some cases, 21587N, 16747P, 16711P, and 16716P are referred to as H2aM21587N, H1H16747P, H1H16711P, H1H16716P respectively. As described herein, these antibodies, fragments thereof, and their complementary determining regions, are useful in generating anti-BCMA chimeric antigen receptor (CAR) constructs and engineering and using CAR-T cells for treating hematological tumors that express BCMA.

[0063] The term "chimeric antigen receptors (CARs)," as used herein, may refer to artificial T-cell receptors, T-bodies, single-chain immunoreceptors, chimeric T-cell receptors, or chimeric immunoreceptors, for example, and encompass engineered receptors that graft an artificial specificity onto a particular immune effector cell. CARs may be employed to impart the specificity of a monoclonal antibody onto a T cell, thereby allowing a large number of specific T cells to be generated, for example, for use in adoptive cell therapy. In specific embodiments, CARs direct specificity of the cell to a tumor associated antigen, for example. In some embodiments, CARs comprise an intracellular activation domain (allowing the T cell to activate upon engagement of targeting moiety with target cell, such as a target tumor cell), a transmembrane domain, and an extracellular domain that may vary in length and comprises a disease- or disorder-associated, *e.g.*, a tumor-antigen binding region. In particular aspects, CARs comprise fusions of single-chain variable fragments (scFv) derived from monoclonal antibodies, fused to CD3-zeta a transmembrane domain and endodomain. The specificity of other CAR designs may be derived

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from ligands of receptors (e.g., peptides) or from pattern-recognition receptors, such as Dectins. In certain cases, the spacing of the antigen-recognition domain can be modified to reduce activation-induced cell death. In certain cases, CARs comprise domains for additional co-stimulatory signaling, such as CD3ζ, FcR, CD27, CD28, CD137, DAP 10/12, and/or OX40, ICOS, TLRs (*e.g.*, TLR2), etc. In some cases, molecules can be co-expressed with the CAR, including co-stimulatory molecules, reporter genes for imaging (e.g., for positron emission tomography), gene products that conditionally ablate the T cells upon addition of a pro-drug, homing receptors, chemokines, chemokine receptors, cytokines, and cytokine receptors. Furthermore, one skilled in the art will understand that a costimulatory domain need not be encoded solely by a full-length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to elicit the desired immune response.

[0064] The term "anti-tumor effect" as used herein, refers to a biological effect which can be manifested by a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, or amelioration of various physiological symptoms associated with the cancerous condition. An "anti-tumor effect" can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies of the invention in prevention of the occurrence of tumor in the first place.

[0065] The term "auto-antigen" means, in accordance with the present invention, any self-antigen which is mistakenly recognized by the immune system as being foreign. Auto-antigens comprise, but are not limited to, cellular proteins, phosphoproteins, cellular surface proteins, cellular lipids, nucleic acids, glycoproteins, including cell surface receptors.

[0066] As used herein, the term "autologous" is meant to refer to any material derived from an individual which is later to be re-introduced into the same individual.

[0067] As used herein, the term "allogeneic" refers to material derived from an animal which is later introduced into a different animal of the same species.

[0068] The term "therapeutically effective amount" refers to the amount of a composition that will elicit a biological or medical response of a tissue, system, or subject that is being sought by the researcher, veterinarian, medical doctor or other clinician. The term "therapeutically effective amount" includes that amount of a composition that, when administered, is sufficient to prevent

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development of, or alleviate to some extent, one or more of the signs or symptoms of the disorder or disease (*e.g.*, hematological cancer) being treated. The therapeutically effective amount will vary depending on the composition, the disease and its severity and the age, weight, etc., of the subject to be treated.

[0069] To "treat" a disease as the term is used herein, means to reduce the frequency or severity of at least one sign or symptom of a disease or disorder experienced by a subject.

[0070] Administration *"in combination with"* one or more further therapeutic agents includes simultaneous (concurrent) and sequential administration in any order.

[0071] The term *"pharmaceutically acceptable"*, as used herein, refers to a material, including but not limited, to a salt, carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively nontoxic, i.e., the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

[0072] "Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

[0073] "Isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

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[0074] Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

[0075] The terms "patient," "subject," "individual," and the like are used interchangeably herein, and refer to any animal, amenable to the methods described herein. In certain non-limiting embodiments, the patient, subject or individual is a human.

[0076] By the term "specifically binds," as used herein with respect to an antibody, is meant an antibody which recognizes a specific antigen, but does not substantially recognize or bind other molecules in a sample. For example, an antibody that specifically binds to an antigen from one species may also bind to that antigen from one or more species. But, such cross-species reactivity does not itself alter the classification of an antibody as specific. In another example, an antibody that specifically binds to an antigen may also bind to different allelic forms of the antigen. However, such cross reactivity does not itself alter the classification of an antibody as specific. In some instances, the terms "specific binding" or "specifically binding," can be used in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, to mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

[0077] In some embodiments, specific binding can be characterized by an equilibrium dissociation constant of at least about $1x10^{-8}$ M or less (*e.g.*, a smaller K_D denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. Moreover, multi-specific antibodies that bind to a first antigen and one or more additional antigens or a bispecific antibody that binds to two different regions of an antigen are nonetheless considered antibodies that "specifically bind," as used herein.

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[0078] Hematologic cancers are cancers originating in the blood or bone marrow. Examples of hematological (or hematogenous) cancers include leukemias, including acute leukemias (such as acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (such as chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma (indolent and high grade forms), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia and myelodysplasia. In a preferred embodiment, the hematological cancer expresses, or over-expresses, CD20. In a preferred embodiment, the hematological cancer expresses, or over-expresses, B cell maturation antigen (BCMA), also known as tumor necrosis factor receptor superfamily member 17 (TNFRSF17).

[0079] "Expression cassette" refers to a nucleic acid comprising expression control sequences operatively linked to a nucleic acid encoding a transcript or polypeptide to be expressed. An expression cassette comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an *in vitro* expression system. Expression cassettes can be a component of a vector such as a cosmid, a plasmid (e.g., naked or contained in a liposome), or a virus (e.g., lentivirus, retrovirus, adenovirus, and adeno-associated virus). An expression cassette can be in a host cell, such as a γδ T cell.

[0080] Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

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Chimeric Antigen Receptor Constructs:

[0081] Aspects of the invention include nucleic acids encoding CARs, and constructs and vectors containing such nucleic acids. In some cases, the nucleic acid is a, e.g., heterologous, component of an expression cassette. In some embodiments, the nucleic acid is a, e.g., heterologous, component of a retroviral vector. In some embodiments, the nucleic acid is a, e.g., heterologous, component of an αβ or γδ T cell, and preferably a γδ T cell. In some embodiments, the nucleic acid is a, e.g., heterologous, component of an γ^+ T cell and/or a δ^+ T cell. In some embodiments, the nucleic acid is a, e.g., heterologous, component of an α^- T cell and/or a β^- T cell.

[0082] Described herein are nucleic acids encoding a CAR binding domain that specifically binds to a tumor associated antigen (TAA) expressed on a surface of a hematological tumor cell. Exemplary TAAs include CD19, CD20, and BCMA. In some embodiments, the binding domain is a CD19 binding domain, such as a CD19 binding domain described in U.S. Patent No. 9,540,445, the contents of which are incorporated by reference in the entirety and for all purposes and in particular for the binding domains, antibodies, antibody fragments, complementarity determining regions, polypeptides containing said complementarity determining regions, nucleic acids encoding for said complementarity determining regions, and epitope specificities and assays for determining epitope specificity described therein. In some embodiments, the binding domain is a CD20 binding domain, such as a CD20 binding domain described in U.S. Patent Appl. No. 2009/0035322, the contents of which are incorporated by reference in the entirety and for all purposes and in particular for the binding domains, antibodies, antibody fragments, complementarity determining regions, polypeptides containing said complementarity determining regions, nucleic acids encoding for said complementarity determining regions, and epitope specificities and assays for determining epitope specificity described therein. In some embodiments, the binding domain is a BCMA binding domain, such as a BCMA binding domain described in WO 2018/133877, or a BCMA binding domain described in U.S. 16/516,028, filed July 18, 2019, the contents of each of which are incorporated by reference in the entirety and for all purposes and in particular for the binding domains, antibodies, antibody fragments, complementarity determining regions, polypeptides containing said complementarity determining regions, nucleic acids encoding for said complementarity determining regions, and epitope specificities and assays for determining epitope specificity described therein. Typically, the region

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encoding the binding domain is 5' of a linker region (*e.g.*, a region encoding a CD8α hinge domain).

[0083] In some embodiments, the binding domain binds the antigen as expressed in a full-length functional polypeptide on the surface of a cell. In some embodiments, the binding domain binds the antigen as presented in an MHC:antigen complex. In some embodiments, the binding domain binds the antigen in an HLA-restricted manner. Binding domains exhibiting specificity for MHC:antigen complexes are described, *e.g.*, in WO/2016/199140 and WO/2016/199141,the contents of each of which are incorporated by reference in the entirety and for all purposes and in particular for the binding domains, antibodies, antibody fragments, complementarity determining regions, polypeptides containing said complementarity determining regions, nucleic acids encoding for said complementarity determining regions, and epitope specificities and assays for determining epitope specificity described therein.

[0084] Exemplary CD20 binding domains include but are not limited to binding domains that selectively bind to an epitope within CD20 bound by, or that competes for binding with, 3B9, 3H7, 2B7, 9C11, or 10F2; or 3B9, 3H7, 2B7, or 9C11; or 3H7. Additionally or alternatively, the CD20 binding domain can comprise the complementary determining regions of an anti-CD20 antibody selected from the group consisting of 3B9, 3H7, 2B7, 9C11, and 10F2; selected from the group consisting of 3B9, 3H7, 2B7, and 9C11; or comprise the complementary determining regions of an anti-CD20 antibody selected from the group consisting of 3H7. The present disclosure also contemplates CD20, CD19, and BCMA binding domains that compete for binding with a sequence provided herein.

[0085] One can determine whether a CD20 binding domain binds to the same epitope as, or competes for binding with, a reference antibody or binding domain by using known methods. For example, to determine if a test antibody binds to the same epitope as a reference binding domain, the reference binding domain can be allowed to bind to CD20 under saturating conditions. Next, the ability of a test binding domain to bind to CD20 molecule can be assessed. If the test binding domain is able to bind to CD20 following saturation binding with the reference binding domain, it can be concluded that the test binding domain binds to a different epitope than the reference binding domain. On the other hand, if the test binding domain is not able to bind to CD20

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following saturation binding with the reference binding domain, then the test binding domain may bind to the same epitope as the epitope bound by the reference binding domain.

[0086] To determine if a binding domain competes for binding with a reference binding domain, the above-described binding methodology is performed in two orientations: In a first orientation, the reference binding domain is allowed to bind to CD20 under saturating conditions followed by assessment of binding of the test binding domain to the CD20 molecule. In a second orientation, the test binding domain is allowed to bind to a CD20 molecule under saturating conditions followed by assessment of binding of the reference binding domain to the CD20 molecule. If, in both orientations, only the first (saturating) binding domain is capable of binding to the CD20 molecule, then it is concluded that the test binding domain and the reference binding domain compete for binding to CD20. As will be appreciated by a person of ordinary skill in the art, a binding domain that competes for binding with a reference binding domain may not necessarily bind to the identical epitope as the reference binding domain, but may sterically block binding of the reference binding domain by binding an overlapping or adjacent epitope. The methods described above to determine competition and epitope binding with an anti-CD20 binding domain can likewise be applied to anti-CD19 binding domains and anti-BCMA binding domains.

[0087] Two binding domains bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one binding domain inhibits binding of the other by at least 50%, for example, 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.*, Junghans *et al.*, Cancer Res. 1990 50:1495-1502). Alternatively, two binding domains have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one binding domain reduce or eliminate binding of the other. Two binding domains have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one binding domain reduce or eliminate binding of the other.

[0088] Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test binding domain is in fact due to binding to the same epitope as the reference binding domain or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can

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be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative binding assay available in the art.

[0089] The present disclosure provides antibodies and CARs with "substantial identity" or "substantial similarity" to the sequences provided herein in the CDR or framework regions. The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with another nucleic acid (or the complementary strand of the other nucleic acid), there is nucleotide sequence identity in %, for example, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or GAP, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[0090] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% sequence identity. In some aspects, residue positions, which are not identical, differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, *e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331, which is herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine,

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alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443 45, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0091] Sequence identity and/or similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA with default or recommended parameters; a program in GCG Version 6.1. FASTA (*e.g.*, FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Sequences also can be compared using the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. Another preferred algorithm when comparing a sequence disclosed herein to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, *e.g.*, Altschul *et al.* (1990) J. Mol. Biol. 215: 403-410 and (1997) Nucleic Acids Res. 25:3389-3402, each of which is herein incorporated by reference.

[0092] Provided herein are anti-CD20, anti-BCMA, or anti-CD19 CARs comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more substitutions (e.g., conservative substitutions). For example, the present disclosure includes anti-CD20 CARs having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 20 or fewer,

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19 or fewer, 18 or fewer, 17 or fewer, 16 or fewer, 15 or fewer, 14 or fewer, 13 or fewer, 12 or fewer, 11 or fewer, 10 or fewer, 9 or fewer, 8 or fewer, 7 or fewer, 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, 2 or fewer, or 1 amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR (e.g., HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, or LCDR3) amino acid sequences disclosed herein. For example, an anti-CD20 CAR can comprise 20, 19, 18, 17, 16, 15, 14 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid substitutions (e.g., conservative amino acid substitutions) relative to any of the HCVR, LCVR, and/or CDR (e.g., HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, or LCDR3) amino acid sequences disclosed herein.

[0093] Similarly, the present disclosure includes anti-BCMA CARs having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 20 or fewer, 19 or fewer, 18 or fewer, 17 or fewer, 16 or fewer, 15 or fewer, 14 or fewer, 13 or fewer, 12 or fewer, 11 or fewer, 10 or fewer, 9 or fewer, 8 or fewer, 7 or fewer, 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, 2 or fewer, or 1 amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR (e.g., HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, or LCDR3) amino acid sequences disclosed herein. For example, an anti-BCMA CAR can comprise 20, 19, 18, 17, 16, 15, 14 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid substitutions (e.g., conservative amino acid substitutions) relative to any of the HCVR, LCVR, and/or CDR (e.g., HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, or LCDR3) amino acid sequences disclosed herein.

[0094] In some embodiments, the isolated nucleic acid encodes an anti-CD20 binding domain having a heavy chain complementary determining region 3 (HCDR3) and a light chain CDR3 (LCDR3), wherein the HCDR3 and LCDR3 are selected from the group consisting of SEQ ID NO:345 (AKDPSYGSGSYHSYYGMDV) and 353 (QQRFNWPLT); 201 (VKDFHYGSGSNYGMDV) and 209 (QQSNDWPLT); and 249 (TKDGSYGHFYSGLDV) and 257 (QQRYYWPLT).

[0095] In some embodiments, the isolated nucleic acid encodes an anti-CD20 binding domain having a heavy chain variable region (HCVR) sequence and a light chain variable region (LCVR) sequence, wherein the HCVR and LCVR sequences are selected from the group consisting of SEQ ID NO: 339 (EEQLVESGGDLVQPGRSLRLSCAASGFTFHDYTMH WVRQAPGKGLEWVSGISWNSGSLGYADSVKGRFTISRDNAKKSLYLQMNSLRAEDTAL YYCAKDPSYGSGSYHSYYGMDVWGQGTTVTVSS) and 347 (EIVLTQSPATLSLSPGE

RATLSCWASQSISRYLVWYQQKCGQAPRLLIYEASKRATGIPVRFSGSGSGTDFTLTISSL ESEDFAVYYCQQRFNWPLTFGGGTKVEIK); 195 (EVQLAESGGDLVQSGRSLRLSCAAS GITFHDYAMHWVRQPPGKGLEWVSGISWNSDYIGYADSVKGRFTISRDNAKKSLYLQM NSLRPDDTALYYCVKDFHYGSGSNYGMDVWGQGTTVTVSP) and 203 (EIVMTQSPATL SMSPGERATLSCRASQSVSRNLAWYQQKVGQAPRLLISGASTRATGIPARFSGSGSGTEF TLTINSLQSEDFAVYYCQQSNDWPLTFGQGTRLEIK); and 243 (EVQLVESGGGLVQPGR SLRLSCAASGFTFYDYAMHWVRQAPGKGLEWVSGISWNSDTIGYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTALYYCTKDGSYGHFYSGLDVWGQGTTVTVSS) and 251 (EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYVASNRATGIPA RFSGSGSGTDFTLTISSLEPDDFAVYYCQQRYYWPLTFGGGTKVEIK).

[0096] In some embodiments, the isolated nucleic acid encodes an anti-CD20 binding domain having a heavy chain complementary determining region 3 (HCDR3) domain and a light chain CDR3 (LCDR3) domain, wherein the HCDR3 domain comprises an amino acid sequence of the formula X1—X2—X3—X4—X5—X6—X7—X8—X9—X10—X11—X12—X13—X14—X15—X16—X17—X18—X19, wherein X1=A, V or T; X2=K; X3=D; X4=P, F or G; X5=S or H; X6=Y; X7=G; X8=S or H; X9=G or F; X10=S or Y; X11=Y, N or S; X12=Y, G or H; X13=G, L or S; X14=Y, M or D; X15=Y, D or V; X16 =G, V or absent; X17=M or absent; X18=D or absent; X19=V or absent (SEQ ID NO: 369); and the LCDR3 domain comprises an amino acid sequence of the formula X1—X2—X3—X4—X5—X6—X7—X8—X9 , wherein X1=Q; X2=Q; X3=R or S; X4=N, Y or F; X5=N, D, or Y; X6=W; X7=P; X8=L; X9=T (SEQ ID NO: 370).

[0097] In some embodiments, the isolated nucleic acid encodes an anti-CD20 binding domain having a heavy chain variable region (HCVR) sequence and a light chain variable region (LCVR) sequence, wherein the HCVR and LCVR sequences are SEQ ID NO: 99 (EVQLVESGGGLVQPGRSLRLSCAASGFTFYDYAMHWVRQAPGKGLEWVSGISWNSGY IGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKDNSYGKFYYGLDVWGQ GTTVTVSS) and 107 (EIVMTQSPATLSVSPGERTTLSCRASQSVSSNLAWYLQKPGQAPR LLIYGASTRATGIPARFSGSGSGTEFILTISSLQSEDFAVYYCQQYNNWPITFGQGTRLEIK).

[0098] In some embodiments, the isolated nucleic acid encodes an anti-CD20 binding domain that binds the same epitope as, competes with, or is an anti-CD20 binding domain having heavy

chain complementarity determining regions (HCDR) and a light chain complementarity determining regions (LCDR), wherein the HCDR and LCDR sequences are the HCDR sequences of SEQ ID NO:99 and the LCDR sequences of SEQ ID NO:107 respectively.

[0099] In some embodiments, the isolated nucleic acid encodes an anti-CD20 binding domain that binds the same epitope as, competes with, or is an anti-CD20 binding domain having an HCDR1 that is or comprises SEQ ID NO: 101 (GFTFYDYA), an HCDR2 that is or comprises SEQ ID NO: 103 (ISWNSGYI), and/or an HCDR3 that is or comprises SEQ ID NO: 105 (AKDNSYGKFYYGLDV). In some embodiments, the isolated nucleic acid encodes an anti-CD20 binding domain that binds the same epitope as, competes with, or is an anti-CD20 binding domain having an LCDR1 that is or comprises SEQ ID NO: 109 (QSVSSN), an LCDR2 that is or comprises SEQ ID NO: 111 (GAS), and/or an LCDR3 that is or comprises SEQ ID NO: 113 (QQYNNWPIT). In some embodiments, the isolated nucleic acid encodes an anti-CD20 binding domain that binds the same epitope as, competes with, or is an anti-CD20 binding domain having an HCDR1 that is or comprises SEQ ID NO:101, an HCDR2 that is or comprises SEQ ID NO:103, an HCDR3 that is or comprises SEQ ID NO: 105, an LCDR1 that is or comprises SEQ ID NO: 109, an LCDR2 that is or comprises SEQ ID NO: 111, and an LCDR3 that is or comprises SEQ ID NO: 113. In some embodiments, the isolated nucleic acid encodes an anti-CD20 binding domain having an HCDR1 comprising SEQ ID NO:101, an HCDR2 comprising SEQ ID NO:103, an HCDR3 comprising SEQ ID NO: 105, an LCDR1 comprising SEQ ID NO: 109, an LCDR2 comprising SEQ ID NO: 111, and an LCDR3 comprising SEQ ID NO: 113.

[00100] Exemplary BCMA binding domains include but are not limited to binding domains that selectively bind to an epitope within BCMA bound by, or that competes for binding with a BCMA binding domain described in WO 2018/133877, or a BCMA binding domain described in U.S. 16/516,028, filed July 18, 2019. Additionally or alternatively, the BCMA binding domain can comprise the complementary determining regions of an anti-BCMA antibody selected from the group consisting of an anti-BCMA antibody or chimeric antigen receptor described in WO 2018/133877, and an anti-BCMA antibody or chimeric antigen receptor described in U.S. 16/516,028, filed July 18, 2019.

[00101] Exemplary BMCA binding domains include but are not limited to binding domains that selectively bind to an epitope within BCMA bound by, or that competes for binding with, anti-

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BCMA-CAR 16716P, anti-BCMA-CAR 16747P, and/or anti-BCMA-CAR 21587N. Additionally or alternatively, the BCMA binding domain can comprise the complementary determining regions of an anti-BCMA CAR selected from the group consisting of anti-BCMA-CAR 16716P, anti-BCMA-CAR 16747P, and anti-BCMA-CAR 21587N.

[00102] In some embodiments, the isolated nucleic acid encodes an anti-BCMA binding domain having a heavy chain complementary determining region 3 (HCDR3) and a light chain CDR3 (LCDR3), wherein the HCDR3 and LCDR3 are selected from the group consisting of SEQ ID NO:21 (RAGDNWNWFDP) and SEQ ID NO:22 (QQAKSVPFT); SEQ ID NO:23 (EGGNYGMDV) and SEQ ID NO:24 (QQANSFPPT); and SEQ ID NO:25 (FAEYCGGNICYYYGMDV) and SEQ ID NO:26 (QQCGGSPWT).

[00103] In some embodiments, the isolated nucleic acid encodes an anti-BCMA binding domain having a heavy chain variable region (HCVR) sequence and a light chain variable region (LCVR) sequence, wherein the HCVR and LCVR sequences are selected from the group consisting of 16716P binding domain HCVR SEQ ID NO: 27 (MSVPTQVLGLLLLWLTDARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYVMSWV RQAPGKGLEWVSAIIGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC AKRAGDNWNWFDPWGQGTLVTV) and 16716P binding domain LCVR SEQ ID NO: 28 (DIQMTQSPSSVSASLGDRVTITCRASQGISSWLAWYQRKPGKAPKLLIYAASSLQSGVPS RFSGSGSGADFTLTISSLQPEDFATYYCQQAKSVPFTFGPGTKVDIK); 16747P binding domain HCVR SEQ ID NO: 29 (MSVPTQVLGLLLLWLTDARCQVQLVESGGGLV KPGGSLRLSCAASGFTFSDYYISWIRQAPGKGLEWVSYISSSGSSIKYADSVKGRFTISRD NAKNSLYLQMNSLRAEDTAVYYCAREGGNYGMDVWGQGTTVTV) and 16747P binding domain LCVR SEQ ID NO: 30 (DIQMTQSPSSVSASVGDRVTITCRASQGINNW LVWYQQKPGKAPKLLIYAATSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAN SFPPTFGQGTKLEIK); and 21587N binding domain HCVR SEQ ID NO: 31 (MSVPTQVLGLLLLWLTDARCQVQLQESGPGLVKPSETLSLTCTVSGGSINYYYWNWIR QPPGKGLEWIGYISYSGNTNYNPSLKSRVTISVATSRNQFSLTLSSVTAADTAVYYCARF AEYCGGNICYYYGMDVWGQGTTVTV) and 21587N binding domain LCVR SEQ ID NO:32 (EIVLTQSPGTLSLSPGERATFSCRASQSVGSSFLAWYQQKPGQAPRRLMYGASNRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQCGGSPWTFGQGTKVEIK).

[00104] Provided herein are anti-BCMA CARs comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more substitutions (e.g., conservative substitutions). For example, the present disclosure includes anti-BCMA CARs having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 20 or fewer, 19 or fewer, 18 or fewer, 17 or fewer, 16 or fewer, 15 or fewer, 14 or fewer, 13 or fewer, 12 or fewer, 11 or fewer, 10 or fewer, 9 or fewer, 8 or fewer, 7 or fewer, 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, 2 or fewer, or 1 amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR (e.g., HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, or LCDR3) amino acid sequences disclosed herein. For example, an anti-BCMA CAR can comprise 20, 19, 18, 17, 16, 15, 14 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid substitutions (e.g., conservative amino acid substitutions) relative to any of the HCVR, LCVR, and/or CDR (e.g., HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, or LCDR3) amino acid sequences disclosed herein.

[00105] Exemplary binding domains described herein typically comprise, in order from the amino to carboxy terminus, a heavy chain region followed by a light chain region (VH-VL). Where a certain order of of VH and VL region in the binding domain is explicitly or implicitly described, the present disclosure is also understand to describe the alternate embodiment in which the order of VH and VL regions are reversed, e.g., in an scFV or a CAR comprising an scFv binding domain. Thus, description of a VH-VL order also describes the alternate VL-VH order, e.g., in an scFV or a CAR comprising an scFv binding domain. Moreover, description of a VL-VH order also describes the alternate VH-VL order, e.g., in an scFV or a CAR comprising an scFv binding domain.

[00106] Generally, the CAR encoding nucleic acids described herein include an extracellular linker portion that encodes a peptide linker that links the binding domain to a transmembrane domain. Exemplary linker portions include, without limitation, a linker portion that encodes the CD8α hinge domain, *e.g.*, SEQ ID NO:1 (PTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIY) or SEQ ID NO:2 (TTTPAPRP PTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIY). Typically, the region encoding the peptide linker (*e.g.*, CD8α hinge domain) is 3' of the region encoding the binding domain and 5' of a region encoding a transmembrane domain.

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[00107] The CAR encoding nucleic acids described herein include a transmembrane domain. The transmembrane domain can link an extracellular antigen binding domain, *e.g.*, and hinge, to one or more intracellular signaling components. For example, the transmembrane domain can link an antigen binding domain, *e.g.*, and hinge, to a CD3ζ signaling domain and optionally with one or two costimulation endodomains. Exemplary transmembrane domains include without limitation a CD8α transmembrane domain, *e.g.*, SEQ ID NO:3 (IWAPLAGTCGVLLLSLVITLYC). Typically, the region encoding the transmembrane domain (*e.g.*, CD8α transmembrane domain) is 3' of the region encoding the peptide linker (*e.g.*, CD8α hinge domain) and 5' of a region encoding one or more cytoplasmic domains.

[00108] In some embodiments, the isolated nucleic acid encodes a cytoplasmic region containing one or more cytoplasmic domains. The region encoding the cytoplasmic region is typically 3' of the region encoding the transmembrane domain. The cytoplasmic domains are typically signaling domains that provide an activating signal for γδ T cell proliferation, cytotoxic activity, and/or pro-inflammatory cytokine expression (*e.g.*, TNF-α or IFNγ). An exemplary cytoplasmic domain is a CD3ζ signaling domain. In some embodiments, the CD3ζ signaling domain is or comprises SEQ ID NO:4 (RVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR). In some embodiments, the CD3ζ signaling domain is or comprises SEQ ID NO:5 (RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPR). In some embodiments, the cytoplasmic region contains multiple (*e.g.*, 2, 3, 4, 5, or 6) signaling domains, such as multiple (*e.g.*, 2, 3, 4, 5, or 6) CD3ζ signaling domains, *e.g.*, each independently selected from SEQ ID NO: 4 and 5. In some embodiments, the cytoplasmic region contains multiple (*e.g.*, 2, 3, 4, 5, or 6) non- CD3ζ signaling domains and a CD3ζ signaling domain. In some embodiments, the cytoplasmic region contains a non- CD3ζ signaling domain and multiple (*e.g.*, 2, 3, 4, 5, or 6) CD3ζ signaling domains.

[00109] The cytoplasmic region can contain one or more costimulation endodomains. A region encoding one or more costimulation endodomains can be 5' or 3' of a region encoding a signaling domain. In some embodiments, the region encoding one or more costimulation endodomains is 5' of the region encoding a signaling domain. In some embodiments, a region encoding one or more costimulation endodomains is 5' of a signaling domain and an additional region encoding

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one or more costimulation endodomains is 3' of the signaling domain. Exemplary costimulation endodomains include, without limitation, CD28; CD137 (4-1BB); CD278 (ICOS); CD27; CD134 (OX40); Dap10; Dap12; DNAm-1; 2B4; a SLAM domain; and TLR2 costimulation endodomains, and combinations thereof.

[00110] In some embodiments, the construct encodes at least one 4-1BB costimulation endodomain, and optionally a second costimulation endodomain selected from a 4-1BB, 2B4, ICOS, CD28, and CD27 costimulation endodomain. In some embodiments, the construct encodes at least two 4-1BB costimulation endodomains, or two 4-1BB costimulation endodomains in combination with one, two, three, or four, or more, costimulation endodomains selected from a 4-1BB, ICOS, CD28, and CD27. In some embodiments, the 4-1BB costimulation endodomain comprises SEQ ID NO: 6 (KRGRKKLLYIFKQPFMRPVQTT QEEDGCSCRFPEEEEGGCEL).

[00111] In some embodiments, the construct encodes one CD27 costimulation endodomain, and optionally a second costimulation endodomain selected from a 4-1BB, ICOS, CD28, and CD27 costimulation endodomain. In some embodiments, the construct encodes a CD27 costimulation endodomain, and a 4-1BB costimulation endodomain. In some embodiments, the construct encodes two CD27 costimulation endodomains. In some embodiments, the CD27 costimulation endodomain comprises SEQ ID NO: 7 (QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQED YRKPEPACSP).

[00112] In some embodiments, the construct encodes a secretion signal, e.g., SEQ ID NO: 33 (MALPVTALLLPLALLLHAARP) operably linked to facilitate secretion of a C-terminal polypeptide, such as a cytokine that supports the activation, cytotoxicity, and/or persistence of a T cell (e.g., CAR-T cell). In some embodiments, the construct encodes a secretion signal, e.g., SEQ ID NO: 33 operably linked to facilitate secretion of a common gamma chain cytokine such as IL-15 or an active fragment thereof, e.g., SEQ ID NO: 34 (NWVNVISDLKKIED LIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSS NGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS). Exemplary common gamma chain cytokines include IL-2 and IL-15. In some embodiments, the common gamma chain cytokine is selected from IL-2, IL-7, and IL-15. In some embodiments, the common gamma chain cytokine is IL-15. IL-15 sequences, including codon optimized nucleic acid sequences encoding sIL15, are disclosed herein and in WO 2007/037780.

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[00113] In some embodiments, the construct encodes one or more multi-cistronic linker regions, e.g., between a signaling domain and/or costimulation endodomain and a secretion signal operably linked to facilitate secretion of a cytokine. A multi-cistronic linker region is a region of polypeptide sequence or RNA sequence that facilitates the production of multiple discrete polypeptides from a single transcription product. In some embodiments, the multi-cistronic linker region encodes a cleavage sequence. Suitable cleavage sequences include self-cleavage sequences such as a P2A, F2A, E2A, or T2A cleavage sequence and/or sequences that are cleaved by an endogenous protease, such as furin.

[00114] In some embodiments, the cleavage sequence is a P2A cleavage sequence. In some embodiments, the cleavage sequence is a furin cleavage sequence. In some embodiments, the cleavage sequences are a P2A and a furin cleavage sequence. In some embodiments, the cleavage sequence is the P2A cleavage sequence of SEQ ID NO: 43 (SGSGATNFSLLKQAGDVEENPGP). In some embodiments, the cleavage sequence is a furin cleavage sequence of SEQ ID NO: 44 (RAKR). In some embodiments, the cleavage sequence is a P2A+furin cleavage sequence of SEQ ID NO: 45 (RAKRSGSGATNFSLLKQAG DVEENPGP).

[00115] In some embodiments, the cleavage sequence is or comprises a P2A cleavage sequence of SEQ ID NO: 52 (ATNFSLLKQAGDVEENPGP). In some embodiments, the cleavage sequence is or comprises an F2A cleavage sequence of SEQ ID NO: 53 (VKQTLNNFDLLKLAGDVESNPGP). In some embodiments, the cleavage sequence is or comprises an E2A cleavage sequence of SEQ ID NO: 54 (QCTNYALLKLAGDVESNPGP). In some embodiments, the cleavage sequence is or comprises an T2A cleavage sequence of SEQ ID NO: 55 (EGRSLLTCGDVEENPGP). In certain aspects, multiple self-cleavage sequences can be encoded carboxy terminal to a signaling and/or costimulatory domain and amino-terminal to an encoded secreted cytokine (e.g., common gamma chain cytokine such as IL-15), preferably wherein the multiple self cleavage sequences are independently selected from the group consisting of a P2A cleavage sequence, a T2A cleavage sequence, an E2A cleavage sequence, and an F2A cleavage sequence. In certain aspects, one or more self-cleavage sequences and one or more sequences cleaved by an endogenous protease are encoded in a construct described herein. In certain embodiments, a endogenous protease recognition site is encoded amino terminal to a self cleavage sequence.

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[00116] In some embodiments, the multi-cistronic linker region encodes an internal ribosome entry site. An exemplary internal ribosome entry site is encoded by SEQ ID NO: 56 (CTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGT TATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGT CTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCT GTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTC TGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCG GCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCAC GTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAAC AAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCC TCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCCCCCC GAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATA).

[00117] Another exemplary internal ribosome entry site is encoded by SEQ ID NO: 60 (AGCAGGTTTCCCCAACTGACACAAAACGTGCAACTTGAAACTCCGCCTGGTCTTTC CAGGTCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGC GAGTGTTAGTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGGAACTCC TCCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCCACACGGGCCCGTCATG TGTGCAACCCCAGCACGGCGACTTTACTGCGAAACCCACTTTAAAGTGACATTGAAA CTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCCGAGGTAA CACGCGACACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGCGCTCGGTT TAAAAAGCTTCTATGCCTGAATAGGTGACCGGAGGTCGGCACCTTTCCTTTGCAATT ACTGACCAC).

[00118] Further suitable internal ribosome entry sites include, but are not limited to, those described in Nucleic Acids Res. 2010 Jan;38(Database issue):D131-6. doi: 10.1093/nar/gkp981. Epub 2009 Nov 16, those described at iresite.org, those described in WO 2018/215787, the sequence described in GenBank accession No. KP019382.1, and the IRES element disclosed in GenBank accession No. LT727339.1.

[00119] Additional multi-cistronic linker regions, including cleavage self-cleavage, and IRES elements, are disclosed in US 2018/0360992 and U.S. 8,865,467.

[00120] In some embodiments, the isolated nucleic acid encodes SEQ ID NO:8 (MSVPTQVLGLLLLWLTDARCEIVMTQSPATLSVSPGERTTLSCRASQSVSSNLAWYLQ KPGQAPRLLIYGASTRATGIPARFSGSGSGTEFILTISSLQSEDFAVYYCQQYNNWPITFG QGTRLEIKGGGGSGGGGSGGGGEVQLVESGGGLVQPGRSLRLSCAASGFTFYDYAMH WVRQAPGKGLEWVSGISWNSGYIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAL YYCAKDNSYGKFYYGLDVWGQGTTVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPA AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCQRRKYRSNKGESPVEPAEPC HYSCPREEEGSTIPIQEDYRKPEPACSPRVKFSRSADAPAYQQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPPR), a 3H7 – CD8 – CD27z polypeptide comprising the following domains in order: a 3H7 binding domain, a CD8α hinge and transmembrane domain, a CD27 costimulation endodomain, and a CD3ζ signaling domain.

[00121] In some embodiments, the isolated nucleic acid encodes SEQ ID NO: 9 (MSVPTQVLGLLLLWLTDARCEIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQ KPGQAPRLLIYGTSTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNNWPLTFG GGTKVEIKGGGGSGGGGSGGGGEVQLVESGGGLVQPGRSLRLSCVASGFTFNDYAMH WVRQAPGKGLEWVSVISWNSDSIGYADSVKGRFTISRDNAKNSLYLQMHSLRAEDTAL YYCAKDNHYGSGSYYYYQYGMDVWGQGTTVTVSSTTTPAPRPPTPAPTIASQPLSLRPE ACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPF MRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR), a 3B9-CD8-BBz polypeptide comprising the following domains in order: a 3B9 binding domain, a CD8α hinge and transmembrane domain, a 4-1BB costimulation endodomain, and a CD3ζ signaling domain.

[00122] In some embodiments, the isolated nucleic acid encodes SEQ ID NO: 10 (MSVPTQVLGLLLLWLTDARCEIVMTQSPATLSVSPGERTTLSCRASQSVSSNLAWYLQ KPGQAPRLLIYGASTRATGIPARFSGSGSGTEFILTISSLQSEDFAVYYCQQYNNWPITFG QGTRLEIKGGGGSGGGGSGGGGEVQLVESGGGLVQPGRSLRLSCAASGFTFYDYAMH WVRQAPGKGLEWVSGISWNSGYIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAL YYCAKDNSYGKFYYGLDVWGQGTTVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPA AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQ

TTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD
KRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR), a 3H7-CD8-BBz polypeptide comprising the following domains in order: a 3H7 binding domain, a CD8α hinge and transmembrane domain, a 4-1BB costimulation endodomain, and a CD3ζ signaling domain.

[00123] In some embodiments, the isolated nucleic acid encodes SEQ ID NO: 11 (MSVPTQVLGLLLLWLTDARCEIVLTQSPATLSLSPGERAALSCRASQSVSNYLAWYQQ
KPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPLTFG
GGTKVEIRGGGGSGGGGSGGGGEVQLVESGGGLVQPGRSLRLSCAASGFTFRDYTMH
WVRQGPGKGLEWVSGISWNSDYIGYADSVKGRFTISRDNAKNSLYLQMNSLRVEDTAL
YYCAKLSGTYRDYFYGVDVWGQGTTVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPA
AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQ
TTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD
KRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR), a 2B7-CD8-BBz polypeptide comprising the following domains in order: a 2B7 binding domain, a CD8α hinge and transmembrane domain, a 4-1BB costimulation endodomain, and a CD3ζ signaling domain.

[00124] In some embodiments, the isolated nucleic acid encodes SEQ ID NO: 12 (MSVPTQVLGLLLLWLTDARCEIVVTQSPATLSLSPGERATLSCRTSQTTTSYLAWYRQK
PGQAPRLLIYDASNRAAGIPARFSGSGSGTDFTLTINSLEPEDFAVYYCQLRTNWITFGQG
TRLEIKGGGGSGGGGSGGGGQVQLVESGGDSVKPGGSLRLSCAASGFTFSDSYMTWIR
QAPGKGLEWVSFISSSGSTIYYADSVKGRFTISRDNVKKSLYLQMNRLRAEDTAVYYCA
REEPGNYVYYGMDVWGQGTTVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGA
VHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE
DGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR
DPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT
KDTYDALHMQALPPR), a 9C11-CD8-BBz polypeptide comprising the following domains in order: a 9C11 binding domain, a CD8α hinge and transmembrane domain, a 4-1BB costimulation endodomain, and a CD3ζ signaling domain.

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[00125] In some embodiments, the isolated nucleic acid encodes SEQ ID NO: 20 (M S V P T Q V L G L L L L W L T D A R C E I V M T Q S P A T L S V S P G E R T T L S C R A S Q S V S S N L A W Y L Q K P G Q A P R L L I Y G A S T R A T G I P A R F S G S G S G T E F I L T I S S L Q S E D F A V Y Y C Q Q Y N N W P I T F G Q G T R L E I K G G G G S G G G G S G G G G G E V Q L V E S G G G L V Q P G R S L R L S C A A S G F T F Y D Y A M H W V R Q A P G K G L E W V S G I S W N S G Y I G Y A D S V K G R F T I S R D N A K N S L Y L Q M N S L R A E D T A L Y Y C A K D N S Y G K F Y Y G L D V W G Q G T T V T V S S T T T P A P R P P T P A P T I A S Q P L S L R P E A C R P A A G G A V H T R G L D F A C D I Y I W A P L A G T C G V L L L S L V I T L Y C R V K F S R S A D A P A Y Q Q G Q N Q L Y N E L N L G R R E E Y D V L D K R R G R D P E M G G K P Q R R K N P Q E G L Y N E L Q K D K M A E A Y S E I G M K G E R R R G K G H D G L Y Q G L S T A T K D T Y D A L H M Q A L P P R) , a 3H7-CD3z polypeptide comprising the following domains in order: a 3H7 binding domain, a CD8α hinge and transmembrane domain, and a CD3ζ signaling domain.

[00126] In some embodiments, the isolated nucleic acid encoding a 3H7-CD8-27z polypeptide comprises the sequence of SEQ ID NO:13 (ATGTCCGTGCCTACCCAGGTGCTGGGCCTGCTGCTGCTGTGGCTGACCGACGCCAG ATGCGAAATAGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAG AACCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAACTTAGCCTGGTACCT TCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCACCAGGGCCAC TGGTATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGAGTTCATTCTCACCAT CAGCAGCCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCAGCAGTATAATAACTG GCCGATCACCTTCGGCCAAGGGACACGGCTGGAGATTAAAGGTGGAGGTGGATCTG GAGGAGGAGGATCCGGTGGAGGAGGTGAAGTGCAACTGGTGGAGTCTGGGGGAGG CTTGGTACAGCCTGGCAGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTT TATGATTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGT CTCAGGTATTAGTTGGAATAGTGGTTACATAGGCTATGCGGACTCTGTGAAGGGCCG ATTCACCATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCT GAGAGCTGAGGACACGGCCTTGTATTACTGTGCAAAAGATAACAGCTATGGAAAGT TCTACTACGGTTTGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAACCA CGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTG TCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGG

GCTGGACTTCGCCTGTGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGT
CCTTCTCCTGTCACTGGTTATCACCCTTTACTGCCAACGACGCAAGTACCGCTCCAAT
AAAGGAGAGTCACCAGTAGAACCCGCCGAACCTTGTCACTATTCATGTCCACGCGA
AGAGGAGGGTTCAACGATCCCTATTCAGGAAGATTACAGAAAGCCGGAACCTGCTT
GTAGCCCCAGAGTGAAGTTCAGCCGCAGCGCCGACGCCCCTGCCTACCAGCAGGGC
CAGAACCAGCTGTATAACGAGCTGAACCTGGGCAGGCGGGAGGAATACGACGTGCT
GGACAAGCGCAGAGGCCGGGACCCTGAGATGGGCGGCAAGCCCCAGAGGCGGAAG
AACCCCCAGGAAGGCCTGTATAACGAACTGCAGAAAGACAAGATGGCCGAGGCCTA
CAGCGAGATCGGCATGAAGGGCGAGCGGCGACGCGGCAAGGGCCACGACGGCCTG
TACCAGGGCCTGTCCACCGCCACCAAGGACACCTACGACGCCCTGCACATGCAGGC
CCTGCCTCCCCGTTAG).

[00127] In some embodiments, the isolated nucleic acid encoding a 3H7-CD8-BBz polypeptide comprises the sequence of SEQ ID NO:14 (ATGTCCGTGCCTACCCAGGTGCTGGGCCTGCTGCTGCTGTGGCTGACCGACGCCAG
ATGCGAAATAGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAG
AACCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAACTTAGCCTGGTACCT
TCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCACCAGGGCCAC
TGGTATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGAGTTCATTCTCACCAT
CAGCAGCCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCAGCAGTATAATAACTG
GCCGATCACCTTCGGCCAAGGGACACGGCTGGAGATTAAAGGTGGAGGTGGATCTG
GAGGAGGAGGATCCGGTGGAGGAGGTGAAGTGCAACTGGTGGAGTCTGGGGGAGG
CTTGGTACAGCCTGGCAGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTT
TATGATTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGT
CTCAGGTATTAGTTGGAATAGTGGTTACATAGGCTATGCGGACTCTGTGAAGGGCCG
ATTCACCATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCT
GAGAGCTGAGGACACGGCCTTGTATTACTGTGCAAAAGATAACAGCTATGGAAAGT
TCTACTACGGTTTGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAACCA
CGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTG
TCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGG
GCTGGACTTCGCCTGTGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGT
CCTTCTCCTGTCACTGGTTATCACCCTTTACTGCAAACGGGGCAGAAAGAAACTCCT

GTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATG
GCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAG
TTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAA
CGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCC
GGGACCCTGAGATGGGGGGAAAGCCGCAGAGAAGGAAGAACCCTCAGGAAGGCCT
GTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGA
AAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACA
GCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAA).

[00128] In some embodiments, the isolated nucleic acid encoding a 3B9-CD8-BBz polypeptide comprises the sequence of SEQ ID NO:15 (ATGAGCGTTCCAACCCAAGTTCTGGGACTGCTTCTGCTCTGGTTGACTGACGCTAGG
TGCGAAATAGTAATGACCCAATCCCCAGCCACTCTCTCCGTTAGCCCAGGTGAAAGA
GCCACTCTTAGTTGCAGGGCTAGTCAATCCGTATCTAGCAACCTGGCCTGGTACCAG
CAAAAGCCCGGACAAGCGCCGCGGTTGTTGATCTATGGGACGAGCACACGAGCTAC
GGGTATTCCGGCCAGGTTCTCAGGGTCTGGCTCCGGAACCGAATTTACATTGACGAT
CAGTAGTCTGCAATCAGAGGATTTCGCCGTTTACTATTGCCAACAGTACAATAATTG
GCCGCTCACATTCGGGGGAGGAACCAAGGTCGAGATTAAGGGAGGTGGGGGTAGTG
GGGGCGGGGGGTCAGGAGGTGGAGGAGAGGTACAGTTGGTAGAAAGCGGCGGGGG
GTTGGTTCAACCTGGACGGAGTCTGAGATTGTCTTGCGTGGCTTCCGGCTTTACTTTC
AATGATTACGCCATGCACTGGGTACGCCAGGCGCCTGGAAAGGGTCTGGAGTGGGT
TTCCGTGATATCCTGGAATAGTGATAGTATAGGCTATGCCGATAGTGTAAAAGGAAG
GTTTACAATCTCTAGGGATAACGCTAAGAACAGCCTGTACCTTCAAATGCATAGTCT
CCGGGCTGAGGACACAGCCTTGTACTATTGTGCTAAGGACAATCATTATGGAAGCG
GGTCATATTATTACTATCAATATGGGATGGATGTGTGGGGTCAGGGAACGACCGTTA
CGGTATCCTCAACCACCACCCCTGCACCAAGGCCCCCGACTCCCGCGCCCACCATCG
CGTCACAGCCTCTTAGCCTGCGACCGGAAGCATGCAGACCAGCTGCCGGGGGGGCC
GTGCATACGAGAGGTTTGGACTTCGCCTGCGATATCTACATCTGGGCGCCCTTGGCC
GGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCCTTTACTGCAAACGGGGC
AGAAAGAAACTCCTGTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACT
CAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTG
AACTGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAG

AACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGA
CAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGCAGAGAAGGAAGAAC
CCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAG
TGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACC
AGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTG
CCCCCTCGCTAA).

[00129] In some embodiments, the isolated nucleic acid encoding a 2B7-CD8-BBz polypeptide comprises the sequence of SEQ ID NO:16 (ATGTCCGTACCTACCCAGGTGCTGGGCCTGCTGCTGCTGTGGCTGACCGACGCCAG
ATGCGAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGAAAG
AGCCGCCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAACTACTTAGCCTGGTACCA
ACAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGATGCATCCAACAGGGCCA
CTGGCATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCA
TCAGCAGCCTAGAGCCTGAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAGCAACT
GGCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAGAGGTGGAGGTGGATCT
GGAGGAGGAGGATCCGGTGGAGGAGGTGAAGTGCAGCTGGTGGAGTCTGGGGGAG
GCTTGGTACAGCCTGGCAGGTCCCTGCGACTCTCCTGTGCAGCCTCTGGATTCACCT
TTCGAGATTATACCATGCACTGGGTCCGGCAAGGTCCAGGGAAGGGCCTGGAATGG
GTCTCAGGTATTAGTTGGAATAGTGATTACATAGGCTATGCGGACTCTGTGAAGGGC
CGATTCACCATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGT
CTGAGAGTTGAGGACACGGCCTTGTATTACTGTGCAAAGCTCAGTGGGACCTACAG
GGACTACTTCTACGGAGTGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTC
AACCACCACCCCTGCACCAAGGCCCCCGACTCCCGCGCCCACCATCGCGTCACAGCC
TCTTAGCCTGCGACCGGAAGCATGCAGACCAGCTGCCGGGGGGGCCGTGCATACGA
GAGGTTTGGACTTCGCCTGCGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTG
GGGTCCTTCTCCTGTCACTGGTTATCACCCTTTACTGCAAACGGGGCAGAAAGAAAC
TCCTGTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAG
ATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTG
AAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTA
TAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTG
GCCGGGACCCTGAGATGGGGGGAAAGCCGCAGAGAAGGAAGAACCCTCAGGAAGG

CCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGA
TGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGT
ACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAA
).

[00130] In some embodiments, the isolated nucleic acid encoding a 9C11-CD8-BBz polypeptide comprises the sequence of SEQ ID NO:17 (ATGTCCGTGCCTACCCAGGTGCTGGGCCTGCTGCTGCTGTGGCTGACCGACGCCAG
ATGCGAAATTGTGGTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGAAAG
AGCCACCCTCTCCTGCAGGACCAGTCAGACTACTACCAGCTACTTAGCCTGGTACCG
ACAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGATGCATCCAACAGGGCCG
CTGGCATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCA
TCAACAGCCTGGAGCCTGAAGATTTTGCAGTTTATTACTGTCAGCTGCGTACCAACT
GGATCACCTTCGGCCAAGGGACACGACTGGAGATTAAAGGTGGAGGTGGATCTGGA
GGAGGAGGATCCGGTGGAGGAGGTCAGGTGCAGCTGGTGGAGTCTGGGGGAGACTC
GGTCAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAG
TGACTCCTACATGACTTGGATCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTTC
ATTCATTAGTAGTAGTGGAAGTACCATATATTATGCAGACTCTGTGAAGGGCCGATT
CACCATTTCCAGGGACAACGTCAAGAAGTCATTGTATCTGCAGATGAACAGACTGA
GAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGAAGAACCAGGAAACTACGTC
TATTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAACCACC
ACCCCTGCACCAAGGCCCCCGACTCCCGCGCCCACCATCGCGTCACAGCCTCTTAGC
CTGCGACCGGAAGCATGCAGACCAGCTGCCGGGGGGGCCGTGCATACGAGAGGTTT
GGACTTCGCCTGCGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCT
TCTCCTGTCACTGGTTATCACCCTTTACTGCAAACGGGGCAGAAAGAAACTCCTGTA
TATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCT
GTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAGTTC
AGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGA
GCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGG
ACCCTGAGATGGGGGGAAAGCCGCAGAGAAGGAAGAACCCTCAGGAAGGCCTGTA
CAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAA

GGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGC CACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAA).

[00131] In some embodiments, the isolated nucleic acid comprises a codon optimized sequence encoding a CD8α hinge region. Exemplary codon optimized CD8α hinge region nucleic acid sequences include, without limitation, SEQ ID NO: 18 (ACCACCACCCCTGCACCAAGGCCCCCGACTCCCGCGCCCACCATCGCGTCA CAGCCTCTTAGCCTGCGACCGGAAGCATGCAGACCAGCTGCCGGGGGGGCCGTGCA TACGAGAGGTTTGGACTTCGCCTGCGAT). In some embodiments, the CD8α hinge region is encoded by the following sequence SEQ ID NO:19 (ACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAGCC CCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGA GGGGGCTGGACTTCGCCTGTGAT).

[00132] In some embodiments, the isolated nucleic acid encodes a 3B9 binding domain and comprises the following sequence encoding a CD8α hinge domain SEQ ID NO:18. In some embodiments, the isolated nucleic acid encodes a 2B7 binding domain and comprises the following sequence encoding a CD8α hinge domain SEQ ID NO:18. In some embodiments, the isolated nucleic acid encodes a 9C11 binding domain and comprises the following sequence encoding a CD8α hinge domain SEQ ID NO:18. In some embodiments, the isolated nucleic acid encodes a 3H7 binding domain and comprises the following sequence encoding a CD8α hinge domain SEQ ID NO: 19.

[00133] In some embodiments, the isolated nucleic acid encodes SEQ ID NO: 35 (MSVPTQVLGLLLLWLTDARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYVMSWV RQAPGKGLEWVSAIIGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC AKRAGDNWNWFDPWGQGTLVTVSSGGGGSGGGGSGGGGDIQMTQSPSSVSASLGDRV TITCRASQGISSWLAWYQRKPGKAPKLLIYAASSLQSGVPSRFSGSGSGADFTLTISSLQP EDFATYYCQQAKSVPFTFGPGTKVDIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGA VHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE DGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR DPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT KDTYDALHMQALPPR), an anti-BCMA-CAR polypeptide comprising the following domains

in order: a 16716P binding domain, a CD8α hinge and transmembrane domain, a 4-1BB costimulation endodomain, and a CD3ζ signaling domain.

[00134] In some embodiments, the isolated nucleic acid encodes SEQ ID NO: 36 (MSVPTQVLGLLLLWLTDARCQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYISWIR QAPGKGLEWVSYISSSGSSIKYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCA REGGNYGMDVWGQGTTVTVSSGGGGSGGGGSGGGGDIQMTQSPSSVSASVGDRVTIT CRASQGINNWLVWYQQKPGKAPKLLIYAATSLQSGVPSRFSGSGSGTDFTLTISSLQPED FATYYCQQANSFPPTFGQGTKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDG CSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR), an anti-BCMA-CAR polypeptide comprising the following domains in order: a 16747P binding domain, a CD8α hinge and transmembrane domain, a 4-1BB costimulation endodomain, and a CD3ζ signaling domain.

[00135] In some embodiments, the isolated nucleic acid encodes SEQ ID NO: 37 (MSVPTQVLGLLLLWLTDARCQVQLQESGPGLVKPSETLSLTCTVSGGSINYYYWNWIR QPPGKGLEWIGYISYSGNTNYNPSLKSRVTISVATSRNQFSLTLSSVTAADTAVYYCARF AEYCGGNICYYYGMDVWGQGTTVTVSSGGGGSGGGGSGGGGEIVLTQSPGTLSLSPGE RATFSCRASQSVGSSFLAWYQQKPGQAPRRLMYGASNRATGIPDRFSGSGSGTDFTLTIS RLEPEDFAVYYCQQCGGSPWTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPA AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQ TTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR), an anti-BCMA-CAR polypeptide comprising the following domains in order: a 21587N binding domain, a CD8α hinge and transmembrane domain, a 4-1BB costimulation endodomain, and a CD3ζ signaling domain.

[00136] In some embodiments, the isolated nucleic acid encodes SEQ ID NO: 38 (MSVPTQVLGLLLLWLTDARCQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYISWIR QAPGKGLEWVSYISSSGSSIKYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCA REGGNYGMDVWGQGTTVTVSSGGGGSGGGGSGGGGDIQMTQSPSSVSASVGDRVTIT

CRASQGINNWLVWYQQKPGKAPKLLIYAATSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQANSFPPTFGQGTKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH
TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDG
CSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP
EMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD
TYDALHMQALPPRRAKRSGSGATNFSLLKQAGDVEENPGPMALPVTALLLPLALLLHA
ARPNWVVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDA
SIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS*), an anti-BCMA-CAR polypeptide comprising the following domains in order: a 16747P binding domain, a CD8α hinge and transmembrane domain, a 4-1BB costimulation endodomain, a CD3ζ signaling domain, a furin+P2A cleavage domain, a secretion signal, and a sIL15 domain.

[00137] In some embodiments, the isolated nucleic acid encoding an anti-BCMA CAR 16716P polypeptide comprises the sequence of SEQ ID NO: 39 (ATGAGCGTGCCTACCCAGGTGCTGGGACTGCTGCTGCTGTGGCTGACAGACGCAAG
GTGCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGACTGGTGCAGCCAGGAGGATCCC
TGAGGCTGTCTTGCGCCGCCAGCGGCTTCACCTTTAGCTCCTACGTGATGTCCTGGGT
GCGCCAGGCACCTGGCAAGGGACTGGAGTGGGTGTCTGCCATCATCGGCTCTGGCG
GCAGCACATACTATGCCGACAGCGTGAAGGGCCGGTTCACCATCTCCAGAGATAAC
TCTAAGAATACACTGTATCTGCAGATGAACAGCCTGAGGGCAGAGGACACCGCCGT
GTACTATTGCGCCAAGAGAGCCGGCGACAACTGGAATTGGTTTGATCCATGGGGCC
AGGGCACCCTGGTGACAGTGTCTAGCGGAGGAGGAGGATCTGGAGGAGGAGGAAG
CGGCGGAGGAGGCGACATCCAGATGACACAGTCCCCATCCTCTGTGAGCGCCTCCC
TGGGCGATAGGGTGACCATCACATGTCGCGCCTCTCAGGGCATCAGCTCCTGGCTGG
CATGGTACCAGAGGAAGCCAGGCAAGGCCCCTAAGCTGCTGATCTATGCAGCATCT
AGCCTGCAGAGCGGAGTGCCTTCCCGGTTCTCTGGAAGCGGATCCGGAGCAGACTTT
ACCCTGACAATCTCCTCTCTGCAGCCAGAGGATTTCGCCACCTACTATTGTCAGCAG
GCCAAGTCCGTGCCATTCACCTTTGGCCCCGGCACAAAGGTGGATATCAAGACCACC
ACCCCTGCACCAAGGCCCCCGACTCCCGCGCCCACCATCGCGTCACAGCCTCTTAGC
CTGCGACCGGAAGCATGCAGACCAGCTGCCGGGGGGGCCGTGCATACGAGAGGTTT
GGACTTCGCCTGCGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCT
TCTCCTGTCACTGGTTATCACCCTTTACTGCAAACGGGGCAGAAAGAAACTCCTGTA

TATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCT
GTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAGTTC
AGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGA
GCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGG
ACCCTGAGATGGGGGGAAAGCCGCAGAGAAGGAAGAACCCTCAGGAAGGCCTGTA
CAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAA
GGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGC
CACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAA).

[00138] In some embodiments, the isolated nucleic acid encoding an anti-BCMA CAR 16747P polypeptide comprises the sequence of SEQ ID NO: 40 (ATGAGCGTGCCTACCCAGGTGCTGGGACTGCTGCTGCTGTGGCTGACAGACGCAAG
GTGCCAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGAAGCCAGGAGGAAGC
CTGAGGCTGTCCTGCGCCGCCTCTGGCTTCACCTTTAGCGACTACTATATCTCCTGGA
TCAGGCAGGCACCTGGCAAGGGACTGGAGTGGGTGTCCTACATCAGCTCCTCTGGC
AGCTCCATCAAGTATGCCGACTCTGTGAAGGGCCGGTTCACCATCTCCAGAGATAAC
GCCAAGAATTCTCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACAGCCGT
GTACTATTGCGCCAGAGAGGGCGGCAATTATGGCATGGACGTGTGGGGCCAGGGCA
CCACAGTGACCGTGTCTAGCGGCGGCGGCGGCTCTGGAGGAGGAGGAAGCGGCGG
AGGAGGCGACATCCAGATGACACAGAGCCCATCCAGCGTGAGCGCCAGCGTGGGCG
ATAGGGTGACCATCACATGTCGCGCCTCCCAGGGCATCAACAATTGGCTGGTGTGGT
ACCAGCAGAAGCCAGGCAAGGCCCCCAAGCTGCTGATCTATGCAGCCACCTCCCTG
CAGTCTGGAGTGCCTAGCCGGTTCTCCGGATCTGGAAGCGGAACCGACTTTACCCTG
ACAATCAGCTCCCTGCAGCCAGAGGATTTTGCCACATACTATTGTCAGCAGGCCAAC
TCCTTCCCCCCTACCTTTGGCCAGGGCACAAAGCTGGAGATCAAGACCACCACCCCT
GCACCAAGGCCCCCGACTCCCGCGCCCACCATCGCGTCACAGCCTCTTAGCCTGCGA
CCGGAAGCATGCAGACCAGCTGCCGGTGGGGCGGTGCATACGAGAGGTTTGGACTT
CGCCTGCGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCT
GTCACTGGTTATCACCCTTTACTGCAAACGGGGCAGAAAGAAACTCCTGTATATATT
CAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCT
GCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAGTTCAGCAG
GAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCA

ATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCT
GAGATGGGGGGAAAGCCGCAGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATG
AACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGA
GCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCA
AGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAA).

[00139] In some embodiments, the isolated nucleic acid encoding an anti-BCMA CAR 21587N polypeptide comprises the sequence of SEQ ID NO: 41 (ATGAGCGTGCCTACCCAGGTGCTGGGACTGCTGCTGCTGTGGCTGACAGACGCAAG
GTGCCAGGTGCAGCTGCAGGAGTCTGGCCCTGGCCTGGTGAAGCCATCCGAGACCC
TGTCTCTGACCTGCACAGTGAGCGGCGGCTCCATCAATTACTATTACTGGAACTGGA
TCAGGCAGCCACCTGGCAAGGGACTGGAGTGGATCGGCTACATCAGCTATTCCGGC
AACACCAATTACAACCCTTCTCTGAAGAGCAGGGTGACCATCAGCGTGGCCACATC
CCGCAATCAGTTCAGCCTGACACTGAGCTCCGTGACCGCAGCAGACACAGCCGTGT
ATTACTGCGCAAGGTTTGCAGAGTACTGCGGAGGCAACATCTGTTATTACTATGGCA
TGGACGTGTGGGGCCAGGGCACCACAGTGACCGTGTCTAGCGGCGGCGGCGGCTCT
GGAGGAGGAGGAAGCGGAGGAGGAGGAGAGATCGTGCTGACCCAGTCCCCAGGCA
CACTGTCTCTGAGCCCTGGAGAGAGGGCCACATTCTCTTGTCGCGCCTCCCAGTCTG
TGGGCTCCTCTTTTCTGGCCTGGTACCAGCAGAAGCCAGGACAGGCACCACGGAGA
CTGATGTATGGAGCATCCAATAGGGCAACCGGAATCCCAGACAGATTCAGCGGCTC
CGGCTCTGGCACAGACTTCACCCTGACAATCAGCAGACTGGAGCCAGAGGACTTCG
CCGTGTACTATTGCCAGCAGTGTGGAGGATCCCCATGGACCTTTGGCCAGGGAACAA
AGGTGGAGATCAAGACCACCACCCCTGCACCAAGGCCCCCGACTCCCGCGCCCACC
ATCGCGTCACAGCCTCTTAGCCTGCGACCGGAAGCATGCAGACCAGCTGCCGGGGG
GGCCGTGCATACGAGAGGTTTGGACTTCGCCTGCGATATCTACATCTGGGCGCCCTT
GGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCCTTTACTGCAAACG
GGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTATGAGACCAGTACAAA
CTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGA
TGTGAACTGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGG
CCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTT
TGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGCAGAGAAGGAA
GAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCT

ACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCT
TTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGG
CCCTGCCCCCTCGCTAA).

[00140] In some embodiments, the isolated nucleic acid encoding an anti-BCMA CAR 16747P+sIL15 polypeptide comprises the sequence of SEQ ID NO: 42 (ATGAGCGTGCCTACCCAGGTGCTGGGACTGCTGCTGCTGTGGCTGACAGACGCAAG
GTGCCAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGAAGCCAGGAGGAAGC
CTGAGGCTGTCCTGCGCCGCCTCTGGCTTCACCTTTAGCGACTACTATATCTCCTGGA
TCAGGCAGGCACCTGGCAAGGGACTGGAGTGGGTGTCCTACATCAGCTCCTCTGGC
AGCTCCATCAAGTATGCCGACTCTGTGAAGGGCCGGTTCACCATCTCCAGAGATAAC
GCCAAGAATTCTCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACAGCCGT
GTACTATTGCGCCAGAGAGGGCGGCAATTATGGCATGGACGTGTGGGGCCAGGGCA
CCACAGTGACCGTGTCTAGCGGCGGCGGCGGCTCTGGAGGAGGAGGAAGCGGCGG
AGGAGGCGACATCCAGATGACACAGAGCCCATCCAGCGTGAGCGCCAGCGTGGGCG
ATAGGGTGACCATCACATGTCGCGCCTCCCAGGGCATCAACAATTGGCTGGTGTGGT
ACCAGCAGAAGCCAGGCAAGGCCCCCAAGCTGCTGATCTATGCAGCCACCTCCCTG
CAGTCTGGAGTGCCTAGCCGGTTCTCCGGATCTGGAAGCGGAACCGACTTTACCCTG
ACAATCAGCTCCCTGCAGCCAGAGGATTTTGCCACATACTATTGTCAGCAGGCCAAC
TCCTTCCCCCCTACCTTTGGCCAGGGCACAAAGCTGGAGATCAAGACCACCACCCCT
GCACCAAGGCCCCCGACTCCCGCGCCCACCATCGCGTCACAGCCTCTTAGCCTGCGA
CCGGAAGCATGCAGACCAGCTGCCGGTGGGGCGGTGCATACGAGAGGTTTGGACTT
CGCCTGCGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCT
GTCACTGGTTATCACCCTTTACTGCAAACGGGGCAGAAAGAAACTCCTGTATATATT
CAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCT
GCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAGTTCAGCAG
GAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCA
ATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCT
GAGATGGGGGGAAAGCCGCAGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATG
AACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGA
GCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCA
AGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCCGCGCGAAGCGA

TCAGGCAGCGGGGCGACAAATTTCAGCCTTCTGAAACAAGCAGGCGACGTGGAAGA
AAACCCCGGTCCAATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCT
GCTCCACGCCGCCAGGCCGAACTGGGTGAATGTAATAAGTGATTTGAAAAAAATTG
AAGATCTTATTCAATCTATGCATATTGATGCTACTTTATATACGGAAAGTGATGTTCA
CCCCAGTTGCAAAGTAACAGCAATGAAGTGCTTTCTCTTGGAGTTACAAGTTATTTC
ACTTGAGTCCGGAGATGCAAGTATTCATGATACAGTAGAAAATCTGATCATCCTAGC
AAACAACAGTTTGTCTTCTAATGGGAATGTAACAGAATCTGGATGCAAAGAATGTG
AGGAACTGGAGGAAAAAAATATTAAAGAATTTTTGCAGAGTTTTGTACATATTGTCC
AAATGTTCATCAACACTTCTTGA).

[00141] In some embodiments, the isolated nucleic acid encodes SEQ ID NO: 46 (MSVPTQVLGLLLLWLTDARCEIVMTQSPATLSVSPGERTTLSCRASQSVSSNLAWYLQ
KPGQAPRLLIYGASTRATGIPARFSGSGSGTEFILTISSLQSEDFAVYYCQQYNNWPITFG
QGTRLEIKGGGGSGGGGSGGGGEVQLVESGGGLVQPGRSLRLSCAASGFTFYDYAMH
WVRQAPGKGLEWVSGISWNSGYIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAL
YYCAKDNSYGKFYYGLDVWGQGTTVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPA
AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQ
TTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD
KRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPRGSGATNFSLLKQAGDVEENPGPMALPVTALLLPLALLL
HAARPNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG
DASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS*),
an anti-CD20-CAR polypeptide comprising the following domains in order: a 3H7 binding domain, a CD8α hinge and transmembrane domain, a 4-1BB costimulation endodomain, a CD3ζ signaling domain, a P2A cleavage domain (GSGATNFSLLKQAGDVEENPGP, SEQ ID NO: 47), a secretion signal, and a sIL15 domain.

[00142] In some embodiments, the isolated nucleic acid encodes SEQ ID NO: 48 (MSVPTQVLGLLLLWLTDARCEIVMTQSPATLSVSPGERTTLSCRASQSVSSNLAWYLQ
KPGQAPRLLIYGASTRATGIPARFSGSGSGTEFILTISSLQSEDFAVYYCQQYNNWPITFG
QGTRLEIKGGGGSGGGGSGGGGEVQLVESGGGLVQPGRSLRLSCAASGFTFYDYAMH
WVRQAPGKGLEWVSGISWNSGYIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAL
YYCAKDNSYGKFYYGLDVWGQGTTVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPA

AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQ
TTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD
KRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPRGSGATNFSLLKQAGDVEENPGPMRISKPHLRSISIQCYL
CLLLNSHFLTEAGIHVFILGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESD
VHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEL
EEKNIKEFLQSFVHIVQMFINTS*), an anti-CD20-CAR polypeptide comprising the following domains in order: a 3H7 binding domain, a CD8α hinge and transmembrane domain, a 4-1BB costimulation endodomain, a CD3ζ signaling domain, a P2A cleavage domain of SEQ ID NO: 47, a secretion signal of SEQ ID NO: 49 (MRISKPHLRSISIQCYLCLLLNSHFLTEAG IHVFILGCFSAGLPKTEA), and a sIL15 domain.

[00143] In some embodiments, the isolated nucleic acid encoding an anti-CD20 CAR + sIL15 polypeptide comprises the sequence of SEQ ID NO: 50 (ATGTCCGTGCCTACCCAGGTGCTGGGCCTGCTGCTGCTGTGGCTGACCGACGCCAG
ATGCGAAATAGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAG
AACCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAACTTAGCCTGGTACCT
TCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCACCAGGGCCAC
TGGTATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGAGTTCATTCTCACCAT
CAGCAGCCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCAGCAGTATAATAACTG
GCCGATCACCTTCGGCCAAGGGACACGGCTGGAGATTAAAGGTGGAGGTGGATCTG
GAGGAGGAGGATCCGGTGGAGGAGGTGAAGTGCAACTGGTGGAGTCTGGGGGAGG
CTTGGTACAGCCTGGCAGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTT
TATGATTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGT
CTCAGGTATTAGTTGGAATAGTGGTTACATAGGCTATGCGGACTCTGTGAAGGGCCG
ATTCACCATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCT
GAGAGCTGAGGACACGGCCTTGTATTACTGTGCAAAAGATAACAGCTATGGAAAGT
TCTACTACGGTTTGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAACCA
CGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTG
TCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGG
GCTGGACTTCGCCTGTGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGT
CCTTCTCCTGTCACTGGTTATCACCCTTTACTGCAAACGGGGCAGAAAGAAACTCCT

GTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATG
GCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAG
TTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAA
CGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCC
GGGACCCTGAGATGGGGGGAAAGCCGCAGAGAAGGAAGAACCCTCAGGAAGGCCT
GTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGA
AAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACA
GCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCGGTAGC
GGGGCTACGAACTTCTCCCTTCTTAAACAAGCGGGAGACGTGGAAGAAAATCCCGG
ACCTATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCC
GCCAGGCCGAACTGGGTGAATGTAATAAGTGATTTGAAAAAAATTGAAGATCTTAT
TCAATCTATGCATATTGATGCTACTTTATATACGGAAAGTGATGTTCACCCCAGTTGC
AAAGTAACAGCAATGAAGTGCTTTCTCTTGGAGTTACAAGTTATTTCACTTGAGTCC
GGAGATGCAAGTATTCATGATACAGTAGAAAATCTGATCATCCTAGCAAACAACAG
TTTGTCTTCTAATGGGAATGTAACAGAATCTGGATGCAAAGAATGTGAGGAACTGG
AGGAAAAAAATATTAAAGAATTTTTGCAGAGTTTTGTACATATTGTCCAAATGTTCA
TCAACACTTCTTGA).

[00144] In some embodiments, the isolated nucleic acid encoding an anti-CD20 CAR + sIL15 polypeptide comprises the sequence of SEQ ID NO: 51 (ATGTCCGTGCCTACCCAGGTGCTGGGCCTGCTGCTGCTGTGGCTGACCGACGCCAG
ATGCGAAATAGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAG
AACCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAACTTAGCCTGGTACCT
TCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCACCAGGGCCAC
TGGTATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGAGTTCATTCTCACCAT
CAGCAGCCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCAGCAGTATAATAACTG
GCCGATCACCTTCGGCCAAGGGACACGGCTGGAGATTAAAGGTGGAGGTGGATCTG
GAGGAGGAGGATCCGGTGGAGGAGGTGAAGTGCAACTGGTGGAGTCTGGGGGAGG
CTTGGTACAGCCTGGCAGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTT
TATGATTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGT
CTCAGGTATTAGTTGGAATAGTGGTTACATAGGCTATGCGGACTCTGTGAAGGGCCG
ATTCACCATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCT

GAGAGCTGAGGACACGGCCTTGTATTACTGTGCAAAAGATAACAGCTATGGAAAGT
TCTACTACGGTTTGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAACCA
CGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTG
TCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGG
GCTGGACTTCGCCTGTGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGT
CCTTCTCCTGTCACTGGTTATCACCCTTTACTGCAAACGGGGCAGAAAGAAACTCCT
GTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATG
GCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAG
TTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAA
CGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCC
GGGACCCTGAGATGGGGGGAAAGCCGCAGAGAAGGAAGAACCCTCAGGAAGGCCT
GTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGA
AAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACA
GCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCGGTAGC
GGGGCTACGAACTTCTCCCTTCTTAAACAAGCGGGAGACGTGGAAGAAAATCCCGG
ACCTATGAGAATTTCGAAACCACATTTGAGAAGTATTTCCATCCAGTGCTACTTGTG
TTTACTTCTAAACAGTCATTTTCTAACTGAAGCTGGCATTCATGTCTTCATTTTGGGC
TGTTTCAGTGCAGGGCTTCCTAAAACAGAAGCCAACTGGGTGAATGTAATAAGTGAT
TTGAAAAAAATTGAAGATCTTATTCAATCTATGCATATTGATGCTACTTTATATACG
GAAAGTGATGTTCACCCCAGTTGCAAAGTAACAGCAATGAAGTGCTTTCTCTTGGAG
TTACAAGTTATTTCACTTGAGTCCGGAGATGCAAGTATTCATGATACAGTAGAAAAT
CTGATCATCCTAGCAAACAACAGTTTGTCTTCTAATGGGAATGTAACAGAATCTGGA
TGCAAAGAATGTGAGGAACTGGAGGAAAAAAATATTAAAGAATTTTTGCAGAGTTT
TGTACATATTGTCCAAATGTTCATCAACACTTCTTGA).

[00145] In some embodiments, the isolated nucleic acid encodes SEQ ID NO: 57 (MSVPTQVLGLLLLWLTDARCEIVMTQSPATLSVSPGERTTLSCRASQSVSSNLAWYLQ
KPGQAPRLLIYGASTRATGIPARFSGSGSGTEFILTISSLQSEDFAVYYCQQYNNWPITFG
QGTRLEIKGGGGSGGGGSGGGGEVQLVESGGGLVQPGRSLRLSCAASGFTFYDYAMH
WVRQAPGKGLEWVSGISWNSGYIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAL
YYCAKDNSYGKFYYGLDVWGQGTTVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPA
AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQ

TTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD
KRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR*), an anti-CD20-CAR polypeptide comprising the following domains in order: a 3H7 binding domain, a CD8α hinge and transmembrane domain, a 4-1BB costimulation endodomain, and a CD3ζ signaling domain; and, via an internal ribosome entry site (e.g., encoded by SEQ ID NO: 56) 3'of the region encoding SEQ ID NO: 57, the isolated nucleic acid further encodes SEQ ID NO: 58 (MALPVTALLLPLALLLHAARPNWVNVISDLKKIEDLIQSMHIDATLYTESDVHP
SCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEK
NIKEFLQSFVHIVQMFINTS*), a secretion signal of SEQ ID NO: 33 and a sIL15 domain.

[00146] In some embodiments, the isolated nucleic acid encoding an anti-CD20 CAR + sIL15 polypeptide comprises the sequence of SEQ ID NO: 59 (ATGTCCGTGCCTACCCAGGTGCTGGGCCTGCTGCTGCTGTGGCTGACCGACGCCAG
ATGCGAAATAGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAG
AACCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAACTTAGCCTGGTACCT
TCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCACCAGGGCCAC
TGGTATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGAGTTCATTCTCACCAT
CAGCAGCCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCAGCAGTATAATAACTG
GCCGATCACCTTCGGCCAAGGGACACGGCTGGAGATTAAAGGTGGAGGTGGATCTG
GAGGAGGAGGATCCGGTGGAGGAGGTGAAGTGCAACTGGTGGAGTCTGGGGGAGG
CTTGGTACAGCCTGGCAGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTT
TATGATTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGT
CTCAGGTATTAGTTGGAATAGTGGTTACATAGGCTATGCGGACTCTGTGAAGGGCCG
ATTCACCATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCT
GAGAGCTGAGGACACGGCCTTGTATTACTGTGCAAAAGATAACAGCTATGGAAAGT
TCTACTACGGTTTGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAACCA
CGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTG
TCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGG
GCTGGACTTCGCCTGTGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGT
CCTTCTCCTGTCACTGGTTATCACCCTTTACTGCAAACGGGGCAGAAAGAAACTCCT
GTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATG

GCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAG
TTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAA
CGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCC
GGGACCCTGAGATGGGGGGAAAGCCGCAGAGAAGGAAGAACCCTCAGGAAGGCCT
GTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGA
AAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACA
GCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAGAGT
ACTGCGGCCGCTACGTAAATTCCGCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGC
CGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATAT
TGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCA
TTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGA
AGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTT
GCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGT
GTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGAT
AGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGG
ATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCT
TTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCCCCCCGAACCACGGGGAC
GTGGTTTTCCTTTGAAAAACACGATGATATTAATTAAGCCACCGCCATGGCCTTACC
AGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGAACTG
GGTGAATGTAATAAGTGATTTGAAAAAAATTGAAGATCTTATTCAATCTATGCATAT
TGATGCTACTTTATATACGGAAAGTGATGTTCACCCCAGTTGCAAAGTAACAGCAAT
GAAGTGCTTTCTCTTGGAGTTACAAGTTATTTCACTTGAGTCCGGAGATGCAAGTAT
TCATGATACAGTAGAAAATCTGATCATCCTAGCAAACAACAGTTTGTCTTCTAATGG
GAATGTAACAGAATCTGGATGCAAAGAATGTGAGGAACTGGAGGAAAAAAATATTA
AAGAATTTTTGCAGAGTTTTGTACATATTGTCCAAATGTTCATCAACACTTCTTGA).

[00147] In some embodiments, the isolated nucleic acid is a linear nucleic acid. In some embodiments, the isolated nucleic acid is a circular nucleic acid. In some embodiments, the isolated nucleic acid is a vector, such as a plasmid vector, an adenoviral vector, an adeno-associated viral vector, a viral vector, a retroviral vector, or a lentiviral vector. In some embodiments, the isolated nucleic acid, or an, *e.g.*, contiguous, portion thereof containing the binding domain transmembrane domain and one or more signaling and/or costimulation

endodomains is integrated into the genome of a host cell, such as a host γδ T cell. In an exemplary embodiment, the isolated nucleic acid is retroviral vector.

γδ T Cells:

[00148] Aspects of the invention include γδ T cells that functionally express an isolated nucleic acid described herein, and thereby expresses a CAR on the surface of the γδ T cell.

[00149] Aspects of the invention can additionally or alternatively include γδ T cells having *in vitro* or *in vivo* cytotoxic activity against a hematological tumor cell that exhibits cell surface expression of the tumor associated antigen (TAA). In some cases, the cytotoxic activity is innate activity. In some cases, the cytotoxicity is at least in part, significantly (> about 25%), or entirely, due to the presence of a CAR construct having a binding domain that specifically binds the TAA expressed on the surface of the hematological tumor cell. In some cases, the γδ T cells exhibit hematological tumor cell killing activity of said γδ T cell is greater than an innate level of *in vitro* and/or *in vivo* hematological tumor cell killing activity in a control γδ T cell. In some cases, the control γδ T cell does not comprise a CAR construct. In some cases, the control γδ T cell comprises a CAR construct lacking a binding domain described herein, a hinge region described herein, a transmembrane domain described herein, a signaling domain described herein, and/or a costimulation endodomain described herein.

[00150] In some cases, the cytotoxicity is at least in part, significantly (> about 25%), or entirely, due to the presence of a CAR construct having a binding domain that specifically binds CD20 or an epitope within CD20. In some cases, the γδ T cells functionally express a CD20 specific CAR encoded by an isolated nucleic acid described herein.

[00151] In some embodiments, γδ T cells described herein can exhibit HLA-restricted (*e.g.*, HLA class I restricted) cytotoxicity. In other embodiments, most (>50%), substantially all (>90%), or all of the cytotoxic activity is not HLA-restricted (*e.g.*, HLA class I restricted). HLA-restricted cytotoxic activity can be assessed by comparing *in vitro* cytotoxicity against an HLA (*e.g.*, HLA class I) (null) tumor cell line versus *in vitro* cytotoxicity against an HLA+ (*e.g.*, HLA class I^+) tumor cell line. In some embodiments, the HLA-restricted cytotoxic activity is at least in part, significantly (>25%), or entirely, provided by the use of a T cell Receptor-like binding

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domain. T cell receptor like binding domains are binding domains that specifically recognize the antigen when presented on the surface of a cell in complex with an MHC molecule. T cell Receptor-like binding domains are further described, *e.g.*, in WO 2016/199141.

[00152] γδ T cells described herein can exhibit robust and/or persistent hematological tumor cell killing activity. In some cases, the hematological tumor cell killing activity can persist for at least about 6 days to 120 days, or for at least about 6 days to 180 days, from first contact with a hematological tumor cell. In some cases, the hematological tumor cell killing activity of a γδ T cell described herein, or a progeny thereof, can persist for at least about 6 days to 120 days, or for at least about 6 days to 180 days, from first contact with a hematological tumor cell, or from administration of the γδ T cell described herein. This persistent hematological tumor cell killing activity can be exhibited *in vitro*, *in vivo*, or both *in vitro* and *in vivo*.

[00153] Aspects of the invention can additionally or alternatively include γδ T cells that proliferate in response to contact with cells that exhibit cell surface expression, or overexpression, of the tumor associated antigen (TAA). The cells that exhibit cell surface expression of the tumor associated antigen (TAA) can be normal hematological cells, such as normal B cells. The cells that exhibit cell surface expression, or overexpression, of the tumor associated antigen (TAA) can be hematological tumor cells. In some cases, the proliferation is an innate activity. In some cases, the proliferation is at least in part, significantly (> about 20% or > about 25%), or entirely, due to the presence of a CAR construct having a binding domain that specifically binds the TAA expressed on the surface of the hematological cell or hematological tumor cell. In some cases, the γδ T cells exhibit a greater level of *in vitro* and/or *in vivo* proliferation as compared to a control γδ T cell. In some cases, the control γδ T cell does not comprise a CAR construct. In some cases, the control γδ T cell comprises a CAR construct lacking a binding domain described herein, a hinge region described herein, a transmembrane domain described herein, a signaling domain described herein, and/or a costimulation endodomain described herein.

[00154] In some cases, the proliferation is at least in part, significantly (> about 20 or > about 25%), or entirely, due to the presence of a CAR construct having a binding domain that specifically binds CD20 or an epitope within CD20. In some cases, γδ T cells exhibiting proliferation in response to contact with a hematological cell or hematological tumor cell that exhibits cell surface

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expression of CD20 functionally express a CD20 specific CAR encoded by an isolated nucleic acid described herein.

[00155] γδ T cells described herein can exhibit robust and/or persistent proliferation in a host organism that comprises the hematological cell or hematological tumor cell that exhibits cell surface expression, or overexpression, of the tumor associated antigen (TAA). In some cases, the proliferation can persist for at least about 6 days to 120 days, or for at least about 6 days to 180 days, from first contact with a hematological tumor cell or from a date of administration of the γδ T cell to the host organism. In some cases, the proliferation of a γδ T cell described herein, or a progeny thereof, in the host organism that comprises the hematological cell or hematological tumor cell that exhibits cell surface expression, or overexpression, of the tumor associated antigen (TAA) can persist for at least about 6 days to 120 days, or for at least about 6 days to 180 days, from first contact with a hematological cell or hematological tumor cell or from the date of first administration of the γδ T cell to the host organism. In some cases, the proliferation in the host organism is at least in part, significantly (> about 20% or > about 25%), or entirely, due to the presence of a CAR construct having a binding domain that specifically binds CD20 or an epitope within CD20. In some cases, γδ T cells exhibiting proliferation in the host organism comprising a hematological cell or hematological tumor cell that exhibits cell surface expression of CD20 functionally express a CD20 specific CAR encoded by an isolated nucleic acid described herein.

[00156] In some embodiments, the γδ T cells described herein express, or persistently express, pro-inflammatory cytokines such as tumor necrosis factor alpha or interferon gamma after contact with the hematological cell or hematological tumor cell. In some embodiments, the γδ T cells described herein, or progeny thereof, express, or persistently express, pro-inflammatory cytokines such as tumor necrosis factor alpha or interferon gamma after contact with the hematological cell or hematological tumor cell, *e.g.*, in a host organism comprising the hematological cell or hematological tumor cell.

[00157] In some embodiments, the γδ T cell, or a pharmaceutical composition containing the γδ T cell, exhibits essentially no, or no graft versus host response when introduced into an allogeneic host. In some embodiments, the γδ T cell, or a pharmaceutical composition containing the γδ T cell, exhibits a clinically acceptable level of graft versus host response when introduced into an allogeneic host. In some embodiments, a clinically acceptable level is an amount of graft

versus host response that does not require cessation of a γδ T cell treatment to achieve a therapeutically effective treatment. In some embodiments, a clinically acceptable level of graft versus host response (GvHD) is an acute response that is less severe than Grade C according to an applicable IBMTR grading scale. The severity of acute graft versus host response is determined by an assessment of the degree of involvement of the skin, liver, and gastrointestinal tract. The stages of individual organ involvement are combined to produce an overall grade, which has prognostic significance. Grade I(A) GvHD is characterized as mild disease, grade II(B) GvHD as moderate, grade III(C) as severe, and grade IV(D) life-threatening. The IBMTR grading system defines the severity of acute GvHD as follows (Rowlings *et al.*, Br J Haematol 1997; 97:855):

- Grade A – Stage 1 skin involvement alone (maculopapular rash over <25 percent of the body) with no liver or gastrointestinal involvement
- Grade B – Stage 2 skin involvement; Stage 1 to 2 gut or liver involvement
- Grade C – Stage 3 involvement of any organ system (generalized erythroderma; bilirubin 6.1 to 15.0 mg/dL; diarrhea 1500 to 2000 mL/day)
- Grade D – Stage 4 involvement of any organ system (generalized erythroderma with bullous formation; bilirubin >15 mg/dL; diarrhea >2000 mL/day OR pain OR ileus).

See also, Schoemans et al., *Bone Marrow Transplantation* volume 53, pages1401–1415 (2018), e.g., at Tables 1 and 2, which discloses criteria for assessing and grading acute GvHD.

[00158] In some embodiments, the γδ T cell, or a pharmaceutical composition containing the γδ T cell, exhibits reduced or substantially reduced graft versus host response when introduced into an allogeneic host as compared to a graft versus host response exhibited by control αβ T cells, or a control pharmaceutical composition comprising the control αβ T cells, administered to an allogeneic host. In some cases, the control αβ T cell is an allogeneic non-engineered control αβ T cell. In some cases, the control αβ T cell does not comprise a CAR or does not comprise the same CAR as a reference γδ T cell.

[00159] The γδ T cells described herein can be δ1, δ2, δ3, or δ4 γδ T cells, or combinations thereof. In some cases, the γδ T cells are mostly (>50%), substantially (>90%), essentially all, or entirely $\delta 2^-$ γδ T cells. In some cases, the γδ T cells are mostly (>50%), substantially (>90%), essentially all, or entirely δ1 γδ T cells.

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[00160] γδ T cells can be obtained from an allogeneic or an autologous donor. The γδ T cells can be, partially or entirely purified, or not purified, and expanded *ex vivo*. Methods and compositions for *ex vivo* expansion include, without limitation, those described in WO 2017/197347. The expansion may be performed before or after, or before and after, a CAR construct is introduced into the γδ T cell(s).

[00161] γδ T cells described herein can be stored, *e.g.*, cryopreserved, for use in adoptive cell transfer.

Methods of Inhibiting or Killing Tumor Cells

[00162] One or multiple non-engineered, γδ T-cell populations, engineered, γδ T- cell populations, and/or admixtures thereof, having cytotoxic activity against a hematological tumor cell can be administered to a subject in any order or simultaneously. If simultaneously, the multiple non-engineered, γδ T-cell population, engineered, γδ T-cell population, and/or admixtures thereof, of the invention can be provided in a single, unified form, such as an intravenous injection, or in multiple forms, for example, as multiple intravenous infusions, s.c, injections or pills. The non-engineered, γδ T-cell population, engineered, γδ T-cell population, and/or admixtures thereof, of the invention can be packed together or separately, in a single package or in a plurality of packages. One or all of the non-engineered γδ T-cell population, engineered γδ T-cell population, and/or admixtures thereof, of the invention can be given in multiple doses. If not simultaneous, the timing between the multiple doses may vary to as much as about a week, a month, two months, three months, four months, five months, six months, or about a year. In some cases, a non-engineered, enriched γδ T-cell population, an engineered, enriched γδ T-cell population, and/or admixtures thereof, of the invention can proliferate within a subject's body, *in vivo*, after administration to a subject. One or more non-engineered γδ T-cell populations, one or more engineered γδ T-cell populations, and/or admixtures thereof, can be frozen to provide cells for multiple treatments with the same cell preparation. One or more non-engineered γδ T-cell populations, one or more engineered γδ T-cell populations, and/or admixtures thereof, of the disclosure, and pharmaceutical compositions comprising the same, can be packaged as a kit. A kit may include instructions (e.g., written instructions) on the use of the non-engineered γδ T-cell population, the engineered γδ T-cell population, and/or admixtures thereof, and compositions comprising the same.

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[00163] In some cases, a method of treating a hematological cancer comprises administering to a subject a therapeutically-effective amount of a non-engineered γδ T-cell population, an engineered γδ T-cell population, and/or admixtures thereof, wherein the administration treats the hematological cancer. In some embodiments the therapeutically-effective amount of the non-engineered, γδ T-cell population, the engineered γδ T-cell population, and/or admixtures thereof, is administered for at least about 10 seconds, 30 seconds, 1 minute, 10 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, δ hours, 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, δ days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, or 1 year. In some embodiments the therapeutically-effective amount of the non-engineered γδ T-cell population, the engineered γδ T-cell population, and/or admixtures thereof, is administered for at least one week. In some embodiments the therapeutically-effective amount of the non- engineered γδ T-cell population, the engineered γδ T-cell population, and/or admixtures thereof, is administered for at least two weeks.

[00164] A non-engineered γδ T-cell population, an engineered γδ T-cell population, and/or admixtures thereof, described herein can be administered before, during, or after the occurrence of a disease or condition, and the timing of administering a pharmaceutical composition containing the γδ T-cell population can vary. For example, the γδ T-cell population can be used as a prophylactic and can be administered continuously to subjects with a propensity to conditions or diseases in order to lessen a likelihood of the occurrence of the disease or condition. The initial administration can be via any route practical, such as by any route described herein using any formulation described herein. In some examples, the administration of a γδ T-cell population of the disclosure is an intravenous administration. One or multiple dosages of the γδ T-cell population can be administered as soon as is practicable after the onset of a hematological cancer and for a length of time necessary for the treatment of the immune disease, such as, for example, from about 24 hours to about 48 hours, from about 48 hours to about 1 week, from about 1 week to about 2 weeks, from about 2 weeks to about 1 month, from about 1 month to about 3 months. In some embodiments, one or multiple dosages of the γδ T-cell population can be administered years after onset of the cancer and before or after other treatments.

[00165] In some embodiments, the γδ T-cell population is administered simultaneously or sequentially with one or more methods to elevate common gamma chain cytokine(s). As used herein, "one or more methods to elevate common gamma chain cytokine(s): refers to a method, or

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combination of methods, that alters the physiological state of a subject, such that at least one common gamma chain cytokine level is elevated in the subject. In some embodiments, the method elevates the level of one or more common gamma chain cytokine(s) selected from the group consisting of IL-2, IL-7, and IL-15, preferably wherein the method elevates the level of IL-15 in the subject. In some embodiments, the method comprises lymphodepletion. In some embodiments, the method comprises administering one or more common gamma chain cytokine(s) to the subject. In some cases, IL-2, IL-7, and/or IL-15, preferably IL-15, are administered. In some embodiments, the method comprises secreting common gamma chain cytokine(s) from an administered, e.g., γδ T cell. In some cases, IL-2, IL-7, and/or IL-15, preferably IL-15, are secreted.

[00166] In some embodiments, the administering one or more methods to elevate common gamma chain cytokine(s) comprises lymphodepletion before introducing the γδ T cell(s). In some embodiments, the administering one or more methods to elevate common gamma chain cytokine(s) comprises administering simultaneously with introducing the γδ T cell(s) or sequentially an amount of common gamma chain cytokine(s) effective to increase proliferation, cytotoxic activity, persistence, or the combination thereof of the introduced γδ T cell(s), preferably wherein the method comprises administering IL-2 or one or more mimetics thereof, more preferably wherein the method comprises administering IL-15 or one or more mimetics thereof. The amount of administered common gamma chain cytokine(s) can be an amount effective to increase proliferation, cytotoxic activity, persistence, or the combination thereof of the introduced γδ T cell(s) before and/or after introducing the γδ T cell(s). Exemplary amounts of IL-15 include, without limitation between 0.01 – 10 μg/kg/dose every 24 hours for IL-15. Exemplary amounts of IL-2 include, without limitation, between about $3x10^6$ and about $22x10^6$ units every 8 - 48 hours. For example, the dosing regimen for IL2 in RCC is 600,000 International Units/kg (0.037 mg/kg) IV q8hr infused over 15 minutes for a maximum 14 doses.

[00167] In some embodiments, the administering one or more methods to elevate common gamma chain cytokine(s) comprises lymphodepletion before administering the γδ T cell(s) and administering simultaneously with introducing the γδ T cell(s) or sequentially an amount of common gamma chain cytokine(s) effective to increase proliferation, cytotoxic activity, persistence, or the combination thereof of the introduced γδ T cell(s).

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EXAMPLES

[00168] **Example 1**

[00169] Human PBMCs at $1x10^6$/mL were activated in a modified cell culture media on pre-coated anti-Vδ1 antibody D1-08 or D1-35 (see, WO 2017/197347) for 5 days in the presence of IL-2 (100 U/mL) in 24-well plates (Costar). On day 5, cell cultures were transduced with γ-retroviral constructs encoding chimeric antigen receptors (2B7-5.1, SEQ ID NO:11; 3B9-5.1, SEQ ID NO:9; 3H7-5.1, SEQ ID NO:10; 9C11-5.1, SEQ ID NO:12) in the presence of retronectin. On day 6 cells were returned to the modified cell culture media and further expanded with feeding and IL-2 replacement as needed. On days 17, 18 or 19, cells were harvested, and remaining αβ T cells were depleted using AutoMACS® kit (Miltenyi Biotec). Purity of γδ cell population and transduction efficiency was assessed by FACS. In parallel, untransduced cell cultures were expanded in the same manner, without adding the retroviral supernatant. As shown in FIG. 3, untransduced expanded Vδ1 cells from multiple donors are not cytotoxic to normal B cells from an allogeneic donor. Introduction of CD20 CAR into Vδ1 cells conferred robust cytotoxicity to these cells against normal B cells. Cytotoxicity was measured as % Annexin V+ cells by flow cytometry in 4hr assay.

[00170] **Example 2**

[00171] Vδ1 cells were activated, transduced and expanded in the same manner as described above. 3H7 CAR construct SEQ ID NO:10 was used to demonstrate cytotoxicity against two CD20+ cell lines – Daudi and Raji. As shown in FIG. 4, introduction of the CAR potentiated innate cytotoxicity of unengineered Vδ1 cells.

[00172] **Example 3**

[00173] Vδ1 cells were activated, transduced and expanded in the same manner as described above. Four different constructs (SEQ ID NOs:9, 10, 11, 12) were introduced into Vδ1 cells during expansion and tested against Raji-Luc cells. Cytotoxicity was determined by total luminescence measurement after adding luminescent substrate D-Luciferin (Perkin Elmer) after 18 hr co-incubation at varying E/T ratios. As shown in FIG. 5, anti-CD20 CAR cells comprising a 4-1BB costimulation endodomain described herein exhibited robust cell killing activity against Raji cells.

[00174] **Example 4**

[00175] CARs constructs were made with several different domains and CD20 binding domains (3H7-CD3z, SEQ ID NO:20; 3H7-5.1, SEQ ID NO:10; and 3H7-CD27z, SEQ ID NO:8). CAR constructs were introduced as describe above and cytotoxicity was tested against Raji-Luc cells as described in previous example (18 hr cytotoxicity at varying E/T ratios). FIG. 6 illustrates robust cell killing activity against Raji cells with different signaling and/or costimulation endodomain(s).

[00176] **Example 5**

[00177] Various CAR constructs were introduced into expanded Vδ1 cells and tested in long-term cytotoxicity assay with target cell re-challenging (Serial Killing) using IncuCyte® instrument. Briefly, Raji cells were labeled with NucRed reagent and total fluorescence of cells was recorded over time. Cells were co-incubated at E/T ratio of 3 for 72 hours in growth media without any cytokine addition. At the end of 72 h, cultures were re-challenged with another dose of Raji cells, and monitored for killing. This procedure was repeated with cultures where Raji cells were cleared by 144 h. FIG. 7. 3H7-ICOSz is a construct in which 4-1BB costimulation endodomain is replaced with an ICOS endodomain (WLTKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTL (SEQ ID NO: 354)).

[00178] **Example 6**

[00179] Raji cells were subcutaneously implanted into NSG mice (Jackson Labs). When tumors reached about 100 mm^3 size, animals were treated with $5x10^6$ Vδ1 CD20 CAR+ cells to compare efficacy of various costimulation endodomains ("co-stim" or "costim") in vivo. Animals were dosed concomitantly with IL-2 (60,000 U/dose) 3 times a week throughout the study. As shown in FIG. 8, the tested constructs exhibited robust in vivo efficacy in treating hematological tumors in NSG mice. Without wishing to be bound by theory, it is hypothesized that the optimized CAR constructs of 3H7-5.1, 3H7-CD3z, and 3H7-CD27z exhibit superior in vivo tumor control, proliferation, activation, persistence, and/or cytoxicity as compared to non-optimized CAR constructs.

[00180] **Example 7**

[00181] Raji cells were subcutaneously implanted into NSG mice (Jackson Labs). When tumors reached about 100 mm^3 animals were treated with $5x10^6$ Vδ1 CD20 CAR+ cells that were pre-

labeled with CellTrace Violet. On Day2 and Day 6 tumors and various other organs were extracted, digested and the resulting cell suspension analyzed for (A) presence of γδ T cells and Raji cells (FIG. 9) and (B) proliferation of γδ cells as evidenced by CellTrace Violet dye dilution (FIG. 10). Animals received concomitant IL-2 (60,000 U/dose) 3 times a week until Day 6. As shown in FIG. 9, the introduced γδ T cells robustly increased in the intratumoral milieu, and facilitated a significant decrease in the ratio of tumor cells to γδ T cells from day 2 to day 6. As shown in Fig. 10, γδ T cells proliferated robustly and preferentially in the intratumoral space.

[00182] **Example 8**

[00183] NSG mice were inoculated with Raji-Luc cells (0.5 mln/animal). On Day 4 animals were treated with $8.7x10^6$ Vδ1 CAR+ cells (SEQ ID NO: 10) or $6.8x10^5$ αβ T cells transduced with the same construct. Survival of animals was monitored over the period of 140 days. All animals received 3 doses of IL-2 (60,000/animal) on Day 0, Day 1, and Day 2. FIG. 11. As shown in FIG. 11, administration of γδ T cells described herein increased the survival of subjects having a hematological cancer.

[00184] **Example 9**

[00185] SRG-15 mice (Herndler-Brandstetter et al., PNAS, 2017) expressing human IL-15 were inoculated with Raji-Luc cells ($0.5x10^6$/animal). On Day 4 animals were treated with $20.2x10^6$ of Vδ1 CAR+ cells (SEQ ID NO: 10) or $1.9x10^6$ of αβ T cells transduced with the same construct. Survival of animals was monitored over the period of 70 days. FIG. 12. As shown in FIG. 12, the introduced γδ T cells did not elicit a GvHD response. In contrast, introduced αβ T cells elicited a GvHD response.

[00186] **Example 10**

[00187] NSG mice were inoculated with Raji cells (1×10^6 /animal) subcutaneously in right hind flank. When tumor volumes reached ~100 mm^3 mice were randomized and treated with 5×10^6 Vδ1 CAR T cells encoding CD20 CAR or CD20 CAR and soluble IL-15. Animals were concomitantly dosed with IL-2 (60,000 U/dose, Peprotech, 3x week) throughout the study. Four animals from CD20 + sIL15 CAR T group that had no measurable tumor at Day 62 were re-challenged with 1×10^6 Raji cells subcutaneously on opposite (left) flank. A control group of animals was also included to demonstrate tumor growth kinetics. Results are illustrated in FIG.

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14. As shown in FIG. 14, administration of γδ CAR-T cells having a nucleic acid construct that encodes a heterologous soluble IL-15 produced a persistent anti-tumor effect that lasted beyond 60 days (e.g., from 60 to 110 days).

[00188] Example 11

[00189] Human PBMCs at 1 x 10^6 /mL in growth media were activated in a 24-well plate (Costar) pre-coated with anti-Vδ1 antibody D1-08 or D1-35 for 5 days in the presence of IL-2 (100 U/mL). On day 5, cell cultures were transduced with γ-retroviral constructs encoding BCMA chimeric antigen receptor (SEQ ID NOs: 35-38) in the presence of retronectin. On day 6 cells were returned to growth media and further expanded with feeding and IL-2 replacement as needed. On days 17, 18 or 19, cells were harvested, and remaining αβ T cells were depleted using AutoMACS® kit (Miltenyi Biotec). Purity of γδ cell population and transduction efficiency was assessed by FACS (FIG. 15). Briefly, CAR-T cells were stained by incubating cells with 1 μg/mL of soluble recombinant biotinylated BCMA (Acro Biosystems). Detection of binding was performed using streptavidin-BV421 at the manufacturer-suggested dilution of 1:500. In parallel, untransduced cell cultures were expanded in the same manner, without adding the retroviral supernatant. Expanded cells were tested in the in vitro cytotoxicity assay on BCMA positive cell lines. As shown in FIG. 16 and FIG. 17, untransduced expanded Vδ1 cells elicited some degree of cytotoxicity against multiple myeloma and Burkitt lymphoma cell lines that are known to express BCMA to various degrees. This cytotoxicity was potentiated by introduction of BCMA CAR constructs. Cytotoxicity was determined by total luminescence measurement in 96-well plates, by adding luminescent substrate D-Luciferin (Perkin Elmer) after 18 hr co-incubation at indicated E/T ratios. A BCMA negative SCABER cell line was used as control.

[00190] Example 12

[00191] NCI-H929 multiple myeloma cells (1 x 10^6 /animal) were subcutaneously implanted into NSG mice (Jackson Labs). When tumors reached about 200 mm^3 size, animals were treated with 5 x 10^6 Vδ1 BCMA CAR+ cells to compare efficacy of 16716P and 16747P scFv derived CAR constructs in vivo. Animals were dosed concomitantly with IL-2 (13,000 IU/dose, Proleukin®) 3 times a week throughout the study. Results are illustrated in FIG. 18. As shown in FIG. 19, anti-BCMA CAR+ cells exhibited robust in vivo tumor burden control.

* * *

[00192] The preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles and aspects of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary aspects shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims.

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Claims:

1. An isolated nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises

a. a binding domain that specifically binds to a tumor associated antigen (TAA) expressed on a surface of a hematological tumor cell;

b. a CD8α hinge domain;

c. a CD8α transmembrane domain;

d. a costimulatory signaling region, optionally wherein the costimulatory signaling region is selected from a 4-1BB (CD137) costimulatory signaling region and a CD27 costimulatory signaling region; and

e. a CD3ζ signaling domain.

2. The isolated nucleic acid sequence of claim 1, wherein the (a)-(e) are in 5' to 3' order.

3. The isolated nucleic acid sequence of claim 1 or 2, wherein the binding domain specifically binds to CD20.

4. The isolated nucleic acid sequence of claim 3, wherein

a. the binding domain selectively binds to an epitope within CD20 bound by, or competes for binding with, an anti-CD20 antibody selected from the group consisting of 3B9, 3H7, 2B7, and 9C11, preferably 3H7; and/or

b. the binding domain comprises the complementary determining regions of an anti-CD20 antibody selected from the group consisting of 3B9, 3H7, 2B7, and 9C11, preferably 3H7.

5. The isolated nucleic acid sequence of any one of claims 1 to 4, wherein the binding domain encodes:

a. a heavy chain variable region (HCVR) sequence and a light chain variable region (LCVR) sequence, wherein the HCVR and LCVR sequences are SEQ ID NO:99 and 107 respectively;

b. a heavy chain complementarity determining region 1, 2, and 3 sequence of SEQ ID NOs: 101, 103, and 105 respectively, and a light chain

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complementarity determining region 1, 2, and 3 sequence of SEQ ID NOs: 109, 111, and 113 respectively;

c. a heavy chain complementary determining region 3 (HCDR3) and a light chain CDR3 (LCDR3), wherein the HCDR3 and LCDR3 are selected from the group consisting of SEQ ID NO:345 and 353; 201 and 209; and 249 and 257;

d. a heavy chain variable region (HCVR) sequence and a light chain variable region (LCVR) sequence, wherein the HCVR and LCVR sequences are selected from the group consisting of SEQ ID NO: 339 and 347; 195 and 203; and 243 and 251; and/or

e. a heavy chain complementary determining region 3 (HCDR3) domain and a light chain CDR3 (LCDR3) domain, wherein the HCDR3 domain comprises an amino acid sequence of the formula X1—X2—X3—X4—X5—X6—X7—X8—X9—X10—X11—X12—X13—X14—X15—X16—X17—X18—X19, wherein X1=A, V or T; X2=K; X3=D; X4=P, F or G; X5=S or H; X6=Y; X7=G; X8=S or H; X9=G or F; X10=S or Y; X11=Y, N or S; X12=Y, G or H; X13=G, L or S; X14=Y, M or D; X15=Y, D or V; X16 =G, V or absent; X17=M or absent; X18=D or absent; X19=V or absent (SEQ ID NO: 369); and the LCDR3 domain comprises an amino acid sequence of the formula X1—X2—X3—X4—X5—X6—X7—X8—X9, wherein X1=Q; X2=Q; X3=R or S; X4=N, Y or F; X5=N, D, or Y; X6=W; X7=P; X8=L; X9=T (SEQ ID NO: 370).

6. The isolated nucleic acid sequence of claim 1 or 2, wherein the binding domain specifically binds to CD19 or BCMA.

7. The isolated nucleic acid sequence of claim 6, wherein the binding domain specifically binds to BCMA.

8. The isolated nucleic acid sequence of claim 7, wherein:

a. the binding domain selectively binds to an epitope within BCMA bound by, or competes for binding with, an anti-BCMA binding region having a sequence selected from the group consisting of SEQ ID NO: 27 and 28; SEQ ID NO: 29 and 30; and SEQ ID NO: and 31 and 32; and/or

b. the binding domain comprises the complementarity determining regions of an anti-BCMA binding region having a sequence selected from the group consisting of SEQ ID NO: 27 and 28; SEQ ID NO: 29 and 30; and SEQ ID NO: and 31 and 32.

9. The isolated nucleic acid sequence of any one of claims 1 to 8, wherein the CAR comprises:

a. a CD8α hinge domain comprising SEQ ID NO:1 (PTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIY) or SEQ ID NO:2 (TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIY);

b. a CD8α transmembrane domain comprising SEQ ID NO:3 (IWAPLAGTCGVLLLSLVITLYC); and/or

c. a CD3ζ signaling domain comprising:

(i) SEQ ID NO:4 (RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA LHMQALPPR); or

(ii) SEQ ID NO:5 (RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR).

10. The isolated nucleic acid sequence of claim 9, wherein the CAR comprises:

a. a 4-1BB costimulatory signaling region comprising SEQ ID NO:6 (KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL); or

b. a CD27 costimulatory signaling region comprising SEQ ID NO:7. (QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSP), or

wherein the isolated nucleic acid encodes the 4-1BB costimulatory signaling region comprising SEQ ID NO:6 and the CD27 costimulatory signaling region comprising SEQ ID NO:7.

11. The isolated nucleic acid sequence of any one of claims 1 to 10, wherein the nucleic acid further encodes:

a. a secreted cytokine; or

b. a secreted common gamma chain interleukin; or

c. a secreted IL-15, preferably wherein the IL-15 comprises the sequence of SEQ ID NO:34, more preferably wherein the IL-15 comprises the sequence of SEQ ID NO: 34 operably linked to a secretion signal sequence of SEQ ID NO:33, or wherein the IL-15 comprises the sequence of SEQ ID NO: 34 operably linked to a secretion signal sequence of SEQ ID NO: 49; or

d. a secreted common gamma chain interleukin, preferably IL-15, and a multi-cistronic linker region amino terminal to the interleukin or interleukin secretion signal, preferably wherein the multicistronic linker region comprises a sequence of any one of SEQ ID NOs: 43-45, 47, or 52-55 or a combination thereof, or encodes an internal ribosome entry site, e.g., SEQ ID NO: 56 or 60.

12. The isolated nucleic acid sequence of any one of claims 1 to 5, 9 to 10, or 11, wherein the binding domain specifically binds to CD20 and the nucleic acid encodes SEQ ID NO:8, 9, 10, 11, 12, 20, 46, 48, or 57 and 58.

13. The isolated nucleic acid sequence of claim 12, wherein the nucleic acid comprises the sequence of SEQ ID NO: 13, 14, 15, 16, 17, 50, 51, or 59.

14. The isolated nucleic acid sequence of any one of claims 1 to 2, 6 to 10, or 11, wherein the binding domain specifically binds to BCMA and the nucleic acid encodes SEQ ID NO: 35, 36, 37, or 38.

15. The isolated nucleic acid sequence of claim 14, wherein the nucleic acid comprises the sequence of SEQ ID NO: 39, 40, 41, or 42.

16. A polypeptide comprising a chimeric antigen receptor comprising an amino acid sequence encoded by any one of the preceding isolated nucleic acids of claims 1 to 15.

17. A γδ T cell comprising a polypeptide according to claim 16 or comprising a nucleic acid encoding a CAR construct according to any one of claims 1 to 15, wherein the γδ T cell functionally expresses a binding domain of the polypeptide or the nucleic acid encoded CAR on the surface of the γδ T cell.

18. The γδ T cell of claim 17, wherein the γδ T cell exhibits *in vitro* and/or *in vivo* cell killing activity against a hematological tumor cell that exhibits cell surface expression of the tumor associated antigen (TAA).

19. The γδ T cell of claim 18, wherein the hematological tumor cell killing activity of said γδ T cell is greater than an innate level of *in vitro* and/or *in vivo* hematological tumor cell killing activity in a control γδ T cell that does not comprise a CAR construct.

20. The γδ T cell of claim 19, wherein the γδ T cell exhibits the increased hematological tumor cell killing activity against HLA class I^+ hematological tumor cells.

21. The γδ T cell of any one of claims 18 to 20, wherein the hematological tumor cell killing activity or increased hematological tumor cell killing activity persists for, for about, for at least, or for at least about, 6 days to 180 days after first contact with the hematological tumor cell.

22. The γδ T cell of any one of claims 18 to 21, wherein the γδ T cell proliferates in response to contact with a hematological tumor cell that exhibits cell surface expression of the tumor associated antigen (TAA).

23. The γδ T cell of any one of claims 18 to 22, wherein the γδ T cell exhibits increased proliferation in response to contact with a hematological tumor cell that exhibits cell surface expression of the tumor associated antigen (TAA) as compared to a control γδ T cell that does not functionally express the nucleic acid encoded CAR on the surface of the γδ T cell.

24. The γδ T cell of any one of claims 18 to 23, wherein the γδ T cell proliferates in a host organism that comprises the hematological tumor cell that exhibits cell surface expression of the tumor associated antigen (TAA).

25. The γδ T cell of any one of claims 22 to 24, wherein the γδ T cell proliferation or increased γδ T cell proliferation persists for, for about, for at least, or for at least about, 6 days to 180 days after first contact with the hematological tumor cell.

26. The γδ T cell of any one of claims 17 to 25, wherein the γδ T cell expresses pro-inflammatory cytokines comprising tumor necrosis factor alpha or interferon gamma after contact with the hematological tumor cell.

27. The γδ T cell of any one of claims 17 to 26, wherein the γδ T cell exhibits reduced, substantially reduced, essentially none, or no graft versus host response when introduced into an allogeneic host in comparison to a graft versus host response exhibited by an αβ T cell administered to an allogeneic host.

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28. The γδ T cell of any one of claims 17 to 27, wherein the γδ T cell is a δ1, a δ2, a δ3, or a δ4 γδ T cell, preferably a $\delta 2^-$ γδ T cell, more preferably a δ1 γδ T cell.

29. A plurality of γδ T cells according to any one of claims 17 to 28.

30. The plurality of γδ T cells of claim 29, wherein the plurality comprises at least about 10^8 γδ T cells, preferably from about 10^8 γδ T cells to about 10^{11} γδ T cells.

31. The plurality of γδ T cells of claim 29 or 30, wherein the plurality comprises a composition that is at least 60%, 80%, or from about 60% or 80% to about 90% or 95% δ1, δ2, δ3, or δ4 γδ T cells, preferably δ1 or δ2 γδ T cells, more preferably $\delta 2^-$ γδ T cells, most preferably δ1 γδ T cells.

32. A method of making the γδ T cell of any one of claims 17 to 28 or a plurality of γδ T cells of any one of claims 29 to 31, wherein the method comprises transfecting γδ T cell(s) with a construct comprising an isolated nucleic acid sequence according to any one of claims 1 to 15.

33. The method of claim 32, wherein the method comprises retroviral transduction.

34. The method of claim 32 or 33, wherein the method comprises *ex vivo* expansion of the γδ T cell(s), wherein the *ex vivo* expansion is performed before transfection and/or after transfection of the isolated nucleic acid sequence.

35. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and a γδ T cell of any one of claims 17 to 28 or a plurality of γδ T cells of any one of claims 29 to 31.

36. A method of killing a hematological tumor cell, the method comprising contacting the hematological tumor cell with a tumor cell killing effective amount of a γδ T cell of any one of claims 17 to 28; a plurality of γδ T cells of any one of claims 29 to 31; or a pharmaceutical composition of claim 35.

37. The method of claim 36, wherein the method comprises introducing a therapeutically effective amount of the γδ T cell(s) or the pharmaceutical composition into a host organism comprising the hematological tumor cell.

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38. The method of claim 37, wherein the method comprises introducing into a host organism comprising the hematological tumor cell a therapeutically effective amount of the γδ T cell(s) or the pharmaceutical composition and simultaneously or sequentially administering one or more methods to elevate common gamma chain cytokine(s).

39. The method of claim 38, wherein the administering one or more methods to elevate common gamma chain cytokine(s) comprises administering simultaneously with introducing the γδ T cell(s) or sequentially an amount of common gamma chain cytokine(s) effective to increase proliferation, cytotoxic activity, persistence, or the combination thereof of the introduced γδ T cell(s), preferably wherein the method comprises administering IL-2, more preferably wherein the method comprises administering IL-15.

40. The method of claim 39, wherein the one or more methods to elevate common gamma chain cytokine(s) comprise administering an amount of common gamma chain cytokine(s) effective to increase proliferation, cytotoxic activity, persistence, or the combination thereof of the introduced γδ T cell(s) before and/or after introducing the γδ T cell(s).

41. The method of any one of claims 38 to 40, wherein the one or more methods to elevate common gamma chain cytokine(s) comprises lymphodepletion before introducing the γδ T cell(s).

42. The method of any one of claims 38 to 40, wherein the one or more methods to elevate common gamma chain cytokine(s) comprises secretion of one or more common gamma chain cytokine(s) from the introduced γδ T cell(s).

43. The method of any one of claims 37 to 42, wherein the method reduces the *in vivo* tumor burden in the host organism, and/or increases the mean survival time of the host organism as compared to a control organism, wherein the control organism is not treated with the γδ T cell(s) or the pharmaceutical composition.

44. The method of any one of claims 36 to 43, wherein the method is a method of treating cancer in a subject in need thereof.

45. Use of a tumor cell killing effective amount of a γδ T cell of any one of claims 17 to 28; a plurality of γδ T cells of any one of claims 29 to 31; or a pharmaceutical composition of claim 35 in the manufacture of a medicament for the treatment of a hematological tumor cell cancer in a subject in need thereof.

46. A method of treating cancer in a subject in need thereof, the method comprising:

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a. administering a therapeutically effective amount of γδ T cells, wherein the cancer comprises hematological tumor cells that exhibit cell surface expression of CD20; or

b. administering a therapeutically effective amount of γδ T cells, wherein the cancer comprises hematological tumor cells that exhibit cell surface expression of BCMA.

47. The method of claim 46, wherein the method comprises simultaneously with the administering of γδ T cells or sequentially, administering one or more methods to elevate common gamma chain cytokine(s).

48. The method of claim 46 or 47, wherein the method comprises performing a plurality of administrations of the γδ T cells, wherein the interval between the plurality of administrations is at least about a week, preferably at least about 2, 3, 4, 5, 6, 7, 8, or 12 weeks, and/or no more than once every 6 or 12 months.

49. A pharmaceutical composition for use in any one of the methods of claims 46 to 48.

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