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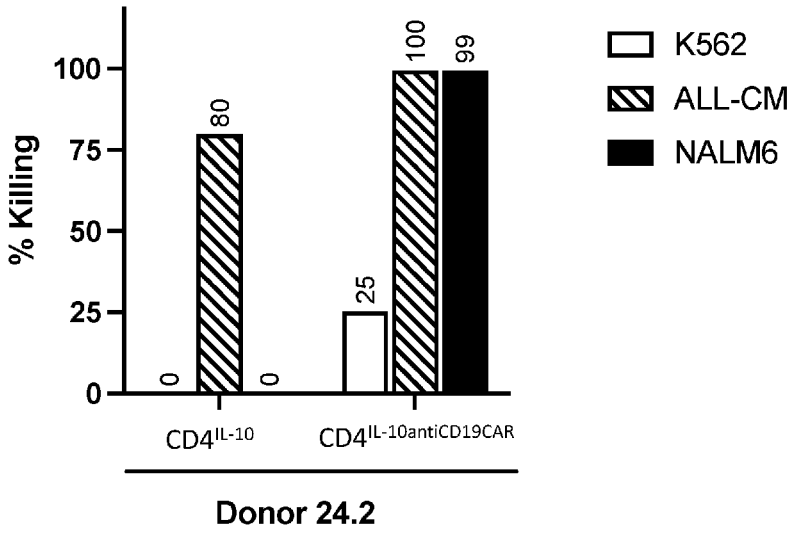


FIG. 10

(57) Abstract: The present disclosure provides a population of CD4$^{IL\text{-}10/CAR}$ cells (autologous or allogeneic single-donor and allogeneic polydonor) generated by genetically modifying CD4$^+$ T cells to express IL-10 and a chimeric antigen receptor. Further provided are methods of generating the CD4$^{IL\text{-}10/CAR}$ cells and methods of using the CD4$^{IL\text{-}10/CAR}$ cells for immune tolerization, treating GvHD, cell and organ transplantation, cancer, and other autoimmune and inflammatory disorders.

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WO 2023/129929 A1

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CD4+ T CELLS EXPRESSING IL-10 AND CHIMERIC ANTIGEN RECEPTORS AND USES THEREOF

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/295,491, filed December 30, 2021, which is hereby incorporated in its entirety by reference.

2. SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing, which has been submitted XML format and is hereby incorporated herein by reference in its entirety. Said XML copy, created on December 24, 2022 is named 37104-50735-Sequence-Listing.xml, and is 95.2 kilobytes (KB) in size.

3. BACKGROUND

[0003] Regulatory T cells belong to a small but important subset of T cells which maintain immunological tolerance to self and non-pathogenic antigens and maintain immune homeostasis. There are two major populations of regulatory T cells – $CD4^+$ $FOXP3^+$ $CD25^+$ T cells ($FOXP3^+$) cells and type 1 regulatory T (Tr1) cells. Both $FOXP3^+$ and Tr1 cells downregulate pathogenic T-cell responses in various preclinical models of organ and pancreatic islet transplantation, graft-versus-host disease (GvHD) and various autoimmune and inflammatory diseases.

[0004] Tr1 cells have been shown to be effective in clinical studies. Administration of cloned, antigen-specific, autologous Tr1 cells to patients with ongoing moderate to severe Crohn's disease resulted in objective, transient remissions (Desreumaux et al., *Gastroenterology.* 2012;143(5):1207-1217.e2.). In addition, adoptive transfer of donor-derived $CD4^+$ T cell populations enriched for allo-specific Tr1 cells to leukemia patients following allogeneic hematopoietic stem cell transplantation (allo-HSCT) resulted in a rapid reconstitution of the immune system and protection against microbial and viral infections, without severe GvHD. In the responder patients, long term remissions and tolerance (> 7 years) resulting in cures were achieved (Bacchetta et al., *Front Immunol.* 2014;5:16).

[0005] Despite these encouraging results, the production of donor-derived or autologous Tr1 cells for large scale therapy for patients with high unmet medical needs is not always feasible, is very cumbersome, and does not allow for the generation of large quantities of pure Tr1 cells.

[0006] Recently, Locafaro and colleagues circumvented some of these problems by transducing purified $CD4^+$ T cells from a single donor with a bidirectional lentiviral vector containing a human IL-10 gene. The resulting single-donor $CD4^{IL\text{-}10}$ populations shared the major functions of naturally occurring Tr1 cells. Like Tr1 cells, single-donor $CD4^{IL\text{-}10}$ cells produce high levels of IL-10 and downregulate the proliferation of both allogeneic $CD4^+$ T cells and allogeneic $CD8^+$ T cells. In addition, they are directly cytotoxic for both normal myeloid cells (including antigen presenting cells, APC) and myeloid leukemia cells. In a humanized xeno-graft-versus-host disease (GvHD) model, these single-donor $CD4^{IL\text{-}10}$ cells were shown to be effective in reducing GvHD while retaining graft-versus-leukemia (GvL) activity. *See* Locafaro et al. Mol Ther. 2017;25(10):2254-2269 and WO 2016/146,542.

[0007] There is a need for means of redirecting the cytotoxic capability of $CD4^{IL\text{-}10}$ cells to non-myeloid cells while simultaneously preserving the immune suppressive and immune homeostasis-maintaining activity of these cells.

4. SUMMARY

[0008] The present disclosure provides a new type of immune cell, $CD4^{IL\text{-}10/CAR}$ cells, which are engineered to express both exogenous IL-10 and a chimeric antigen receptor (CAR). We have discovered that expression of a CAR in addition to IL-10 redirects the cytotoxic properties of these Tr1-like cells to therapeutically useful targets other than myeloid targets, while surprisingly leaving intact the cells' immune-regulatory properties. Also described are methods of treatment using the $CD4^{IL\text{-}10/CAR}$ cells, use of $CD4^{IL\text{-}10/CAR}$ cells for treatment, and use of $CD4^{IL\text{-}10/CAR}$ cells in the manufacture of a medicament for treating various disorders in which the CAR directs the cytotoxic properties of these Tr1-like cells to therapeutically useful targets other than myeloid cells, while leaving intact the cells' immune-regulatory properties.

[0009] The present disclosure provides a genetically modified $CD4^+$ T cell ($CD4^{IL10/CAR}$) comprising: (a) a first exogenous polynucleotide segment encoding a chimeric antigen receptor (CAR); and (b) a second exogenous polynucleotide segment encoding interleukin-10 (IL-10).

[0010] In some embodiments, the first exogenous polynucleotide segment comprises a regulatory element operably linked to a coding sequence of the CAR. In some embodiments, the regulatory element drives constitutive expression of the CAR.

[0011] In some embodiments, the CAR comprises an antigen-binding domain, a hinge region, a transmembrane domain, and an intracellular signaling domain.

[0012] In some embodiments, the antigen-binding domain comprises a single chain antibody fragment. In some embodiments, the single chain antibody fragment comprises a single chain Fv (scFv).

[0013] In some embodiments, the antigen-binding domain targets an antigen associated with an autoimmune disease, inflammatory disorder, or cancer.

[0014] In some embodiments, the antigen is selected from the group consisting of: CD19, CD20, CD22, BCMA, CD27, CD38, B7-H3, CD23, Lyml, Lym2, CLEC5A, CDH179b, FLT3, GCC, Muc, CSF2RA, GFRa4, CD32, CD33, CEA, IL1lRa, IL13Ra, NYBRI, SLea, CD200R, TGFBetaR2, CD276, TROP2, LAMP1, PTK7, DLL3, CDH1, CDH6, CDH17, CDH19, TSHR, tyrosinase, HLA-A*02, HLA-A*24 or citrullinated peptides, insulin, MOG, GAD65, IA2, gliadin, and desmoglein in the context of relevant MHC molecules.

[0015] In some embodiments, the antigen-binding domain comprises an anti-CD19 antigen-binding domain. In some embodiments, the anti-CD19 antigen-binding domain comprises the sequence of SEQ ID NO: 11.

[0016] In some embodiments, the antigen-binding domain is an anti-CD20 antigen-binding domain. In some embodiments, the anti-CD20 antigen-binding domain comprises the sequence of SEQ ID NO: 18.

[0017] In some embodiments, the antigen-binding domain is an anti-BCMA antigen-binding domain. In some embodiments, the anti-BCMA antigen-binding domain comprises the sequence of SEQ ID NOs: 50-53.

[0018] In some embodiments, the hinge region is selected from a human CD8 hinge region, a human CD28 hinge region, a IgG1 hinge region, or a IgG4 hinge region. In some embodiments, the hinge region is derived from human CD8.

[0019] In some embodiments, the transmembrane domain is selected from a TNFRSF 19 transmembrane domain, a CD3zeta transmembrane domain, a CD8α transmembrane domain, a CD4 transmembrane domain, a CD28 transmembrane domain, or an B7-family inducible costimulatory (ICOS) transmembrane domain. In some embodiments, the transmembrane domain is derived from a CD8α transmembrane domain.

[0020] In some embodiments, the CAR further comprises one or more co-stimulatory domains. In some embodiments, the CAR comprises two co-stimulatory domains.

[0021] In some embodiments, the one or more co-stimulatory domains are selected from the group consisting of 4-1BB, CD28, OX40, ICOS, CD27, MYD88-CD40, and KIR2DS2.

[0022] In some embodiments, one of the one or more co-stimulatory domains is derived from CD28.

[0023] In some embodiments, a second co-stimulatory domain is derived from 4-1BB.

[0024] In some embodiments, the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM). In some embodiments, the immunoreceptor tyrosine-based activation motif (ITAM) is derived from CD3zeta.

[0025] In some embodiments, the CAR comprises: an anti-CD19 antigen binding domain, an anti-BCMA antigen binding domain, or an anti-CD20 antigen binding domain; a human CD8 hinge region; a TNFRSF 19 transmembrane region; a 4-1BB co stimulatory domain; and a CD3zeta chain intracellular signaling domain. In some embodiments, the CAR comprises: an anti-CD19 antigen binding domain, an anti-BCMA antigen binding domain, or an anti-CD20 antigen binding domain; a human CD8 hinge region; a CD8α transmembrane region; a 4-1BB co stimulatory domain; and a CD3zeta chain intracellular signaling domain.

[0026] In some embodiments, the CAR comprises the sequence of SEQ ID NOs: 9, 16, 22, 34, 41-49, or 54.

[0027] In some embodiments, the first exogenous polynucleotide segment comprises the sequence of SEQ ID NOs: 10, 17, 23, 35, or 55.

[0028] In some embodiments, the first exogenous polynucleotide segment is integrated into the T cell nuclear genome.

[0029] In some embodiments, the first exogenous polynucleotide segment is not integrated into the T cell nuclear genome.

[0030] In some embodiments, the first exogenous polynucleotide segment is in a vector.

[0031] In some embodiments, the second exogenous polynucleotide segment comprises a regulatory element operably linked to a coding sequence of the IL-10. In some embodiments, the IL-10 is a human IL-10. In some embodiments, the IL-10 is a viral IL-10.

[0032] In some embodiments, the IL-10 is a protein having the sequence of SEQ ID NO: 1. In some embodiments, the second exogenous polynucleotide segment comprises the sequence of SEQ ID NO: 2.

[0033] In some embodiments, the regulatory element drives constitutive expression of the IL-10.

[0034] In some embodiments, the second exogenous polynucleotide segment is integrated into the T cell nuclear genome.

[0035] In some embodiments, the second exogenous polynucleotide segment is not integrated into the T cell nuclear genome.

[0036] In some embodiments, the second exogenous polynucleotide segment is in a vector.

[0037] In some embodiments, the first exogenous polynucleotide segment and the second exogenous polynucleotide segment are in the same vector. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a lentiviral vector.

[0038] In some embodiments, the $CD4^+$ T cell constitutively expresses at least 100pg IL-10 per 10^6 of the $CD4^+$ T cells/mL of culture medium.

[0039] In some embodiments, the $CD4^+$ T cell constitutively expresses at least 100 pg, 200 pg, 500pg, 1ng, 5ng, 10ng, or 50ng IL-10 per 10^6 of the $CD4^+$ T cells/mL.

[0040] In some embodiments, the $CD4^+$ T cells expresses at least 1ng IL-10 per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies.

[0041] In some embodiments, the $CD4^+$ T cells expresses at least 2ng, 5ng, 10ng, 100ng, 200ng, or 500ng IL-10 per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies.

[0042] In some embodiments, the $CD4^+$ T cell expresses IL-10 at a level at least 5-fold higher than unmodified $CD4^+$ T cells. In some embodiments, the $CD4^+$ T cell expresses IL-10 at a level at least 10-fold higher than unmodified $CD4^+$ T cells.

[0043] In some embodiments, the $CD4^+$ T cell expresses at least 100 pg IL-5 per 10^6 of the $CD4^+$ T cells/mL. In some embodiments, the $CD4^+$ T cell expresses at least 100 pg, 200 pg, 500pg, 1ng, 5ng, 10ng, or 50ng IL-5 per 10^6 of the $CD4^+$ T cells/mL.

[0044] In some embodiments, the $CD4^+$ T cell expresses at least 1ng IL-5 per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies.

[0045] In some embodiments, the $CD4^+$ T cells expresses at least 2ng, 5ng, 10ng, 100ng, 200ng, or 500ng IL-5 per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies.

[0046] In some embodiments, the $CD4^+$ T cell expresses at least 100 pg IFN-γ per 10^6 of the $CD4^+$ T cells/mL

[0047] In some embodiments, the $CD4^+$ T cell expresses at least 100 pg, 200 pg, 500pg, 1ng, 5ng, 10ng, or 50ng IFN-γ per 10^6 of the $CD4^+$ T cells/mL.

[0048] In some embodiments, the $CD4^+$ T cell expresses at least 1ng IFN-γ per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies.

[0049] In some embodiments, the $CD4^+$ T cells expresses at least 2ng, 5ng, 10ng, 100ng, 200ng, or 500ng IFN-γ per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies.

[0050] In some embodiments, the $CD4^+$ T cell expresses at least 25 pg IL-4 per 10^6 of the $CD4^+$ T cells/mL.

[0051] In some embodiments, the $CD4^+$ T cell expresses at least 25 pg, 50 pg, 75 pg, 100 pg, 200 pg, 500pg, 1ng, 5ng, 10ng, or 50ng IL-4 per 10^6 of the $CD4^+$ T cells/mL.

[0052] In some embodiments, the $CD4^+$ T cell expresses at least 100 pg IL-4 per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies.

[0053] In some embodiments, the $CD4^+$ T cells expresses at least 100 pg, 200 pg, 300 pg, 400 pg, 500 pg, 600 pg, 700 pg, 800 pg, 900 pg, 1000 pg, 2ng, 5ng, 10ng, 100ng, 200ng, or 500ng IL-4 per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies.

[0054] In some embodiments, the expression one or more of IL-10, IL-4, IFN-γ, and IL-5 is stable after one or more re-stimulations.

[0055] In some embodiments, the first exogenous polynucleotide segment or the second exogenous polynucleotide segment, further comprises a sequence encoding a selection marker.

[0056] In some embodiments, the selection marker is ΔNGFR. In some embodiments, the ΔNGFR has the sequence of SEQ ID NO: 3. In some embodiments, the second exogenous polynucleotide segment comprises a sequence of SEQ ID NO: 4.

[0057] In some embodiments, the selection marker is a truncated form of an EGFR polypeptide.

[0058] In some embodiments, the $CD4^+$ T cell is capable of in vitro cytotoxicity to a $CD19^+$ target cell.

[0059] In some embodiments, the $CD4^+$ T cell is capable of in vivo cytotoxicity to a $CD19^+$ target cell.

[0060] In some embodiments, the $CD19^+$ target cell is an autoantibody producing B cell.

[0061] In some embodiments, the $CD19^+$ target cell is a $CD19^+$ cancer cell.

[0062] In some embodiments, the $CD4^+$ T cell is capable of in vitro cytotoxicity to a myeloid target cell.

[0063] In some embodiments, the $CD4^+$ T cell is capable of in vivo cytotoxicity to a myeloid target cell.

[0064] In some embodiments, the $CD4^+$ T cell is capable of cytotoxicity to a $CD19^+$ target cell and a myeloid target cell.

[0065] In some embodiments, the myeloid target cell express one or more of Class I MHC, CD13, CD54 and CD112.

[0066] In some embodiments, the cytotoxicity to a $CD19^+$ target cell is maintained after one or more in vitro restimulations. In some embodiments, the cytotoxicity to a $CD19^+$ target cell is maintained after one or more in vitro expansions.

[0067] In some embodiments, the cytotoxicity to a myeloid target cell is maintained after one or more vitro restimulations. In some embodiments, the cytotoxicity to a myeloid target cell is maintained after one or more in vitro expansions.

[0068] In some embodiments, the $CD4^+$ T cell is capable of suppressing allogeneic $CD4^+$ T cell proliferation.

[0069] In some embodiments, the $CD4^+$ T cell is capable of suppressing allogeneic $CD8^+$ T cell proliferation.

[0070] In some embodiments, the $CD4^+$ T cell is capable of suppressing allogeneic $CD4^+$ T cell proliferation, allogenic $CD8^+$ T cell proliferation, and PBMC proliferation.

[0071] In some embodiments, the suppressive property is maintained after one or more restimulations.

[0072] In another aspect, the present disclosure features a population of $CD4^+$ T cells comprising any of the genetically modified $CD4^+$ cells provided herein.

[0073] In some embodiments, the $CD4^+$ T cells before the genetic modification were obtained from two, three, four, five, six, seven, eight, nine, or ten different T cell donors and pooled.

[0074] In some embodiments, the $CD4^+$ T cells in the population collectively have six, seven, eight, nine, ten, eleven, twelve, or more different HLA haplotypes.

[0075] In some embodiments, all the $CD4^+$ T cells in the population have at least 1/10, 2/10, 3/10, 4/10, 5/10, 6/10, 7/10, 8/10, or 9/10 match at the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci to each other.

[0076] In some embodiments, all the $CD4^+$ T cells in the population have at least 1/8, 2/8, 3/8, 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci to each other.

[0077] In some embodiments, all the $CD4^+$ T cells in the population have 2/2 match at the HLA-A locus to each other.

[0078] In some embodiments, all the $CD4^+$ T cell in the population have 2/2 match at the HLA-B locus to each other.

[0079] In some embodiments, all the $CD4^+$ T cell in the population have 2/2 match at the HLA-C locus to each other.

[0080] In some embodiments, all the $CD4^+$ T cells in the population have at least 3/4 or 4/4 match at the HLA-DRB1 and HLA-DQB1 loci with each other.

[0081] In some embodiments, all the $CD4^+$ T cells in the population have less than 5/10, 6/10, 7/10, 8/10, or 9/10 match at the HLA-A, HLA-B, HLA C, HLA-DRB1, and HLA-DQB1 loci to each other.

[0082] In some embodiments, all the $CD4^+$ T cells in the population have less than 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA C, and HLA DRB1 loci to each other.

[0083] In some embodiments, all the $CD4^+$ T cells in the population have less than 2/2 match at the HLA-A locus to each other.

[0084] In some embodiments, all the $CD4^+$ T cell in the population have less than 2/2 match at the HLA-B locus to each other.

[0085] In some embodiments, all the $CD4^+$ T cell in the population have less than 2/2 match at the HLA-C locus to each other.

[0086] In some embodiments, all the $CD4^+$ T cells in the population have less than 4/2, 3/4 or 4/4 match at the HLA-DRB1 and HLA DQB1 loci with each other.

[0087] In some embodiments, all the $CD4^+$ T cells in the population have an A*02 allele or are A*02 negative.

[0088] In some embodiments, all the $CD4^+$ T cells in the population have an A*24 allele or are A*24 negative.

[0089] In some embodiments, at least 30% of the $CD4^+$ T cells within the population of $NGFR^+$ cells express the CAR.

[0090] In some embodiments, at least 60% of the $CD4^+$ T cells within the population of $NGFR^+$ cells express the CAR.

[0091] In some embodiments, at least 90% of the $CD4^+$ T cells within the population of $NGFR^+$ cells express the CAR.

[0092] In some embodiments, the $CD4^+$ T cells are in a frozen suspension.

[0093] In some embodiments, the $CD4^+$ T cells are in a liquid suspension.

[0094] In some embodiments, the liquid suspension has previously been frozen.

[0095] In another aspect, this disclosure features any of the pharmaceutical compositions provided herein comprising any of the $CD4^+$ T cells provided herein or any of the populations of $CD4^+$ T cells provided herein.

[0096] In another aspect, this disclosure features a method of making $CD4^{IL10/CAR}$ cells, comprising the steps of: (a) obtaining primary $CD4^+$ T cells from one or more T cell donors; and (b) modifying the $CD4^+$ T cells by introducing (i) a first exogenous polynucleotide segment encoding a chimeric antigen receptor (CAR), and (ii) a second exogenous polynucleotide segment encoding IL-10.

[0097] In some embodiments, in step (a), the primary $CD4^+$ T cells are obtained from two, three, four, five, six, seven, eight, nine, or ten different T cell donors.

[0098] In some embodiments, the method further comprising pooling the genetically modified $CD4^+$ T cells from step (b).

[0099] In some embodiments, wherein in step (a), the primary $CD4^+$ T cells are autologous to the patient.

[00100] In some embodiments, the method further comprising the step, after step (a) and before step (b), or after step (b) of: incubating the $CD4^+$ T cells in the presence of an anti-CD3 antibody, and anti-CD28 antibody or anti-CD3 antibody and CD28 antibody coated beads.

[00101] In some embodiments, the method further incubating the $CD4^+$ T cells further in the presence of IL-2.

[00102] In some embodiments, the first exogenous polynucleotide segment, second exogenous polynucleotide segment, or both are introduced into the primary $CD4^+$ T cells using one or more viral vectors. In some embodiments, the viral vector is a lentiviral vector.

[00103] In some embodiments, the first exogenous polynucleotide segment comprises a coding sequence of a CAR.

[00104] In some embodiments, the CAR is specific to a target antigen associated with an autoimmune disease, inflammatory disorder or cancer.

[00105] In some embodiments, the target antigen is associated with an autoimmune disease, inflammatory disorder or cancer.

[00106] In some embodiments, the CAR is an anti-CD19 CAR, an anti-CD20 CAR, an anti-CD22 CAR, an anti BCMA CAR, an anti-B7-H3 CAR, an anti-CD27 CAR, or an anti-CD38 CAR.

[00107] In some embodiments, the CAR comprises the sequence of SEQ ID NOs: 9, 16, 22, 34, 41-49, or 54.

[00108] In some embodiments, the first exogenous polynucleotide segment comprises the sequence of SEQ ID NOs: 10, 17, 23, 35, or 55.

[00109] In some embodiments, the IL-10 comprises the sequence of SEQ ID NO: 1. In some embodiments, the second exogenous polynucleotide segment comprises the sequence of SEQ ID NO: 2.

[00110] In some embodiments, the first exogenous polynucleotide segment or the second exogenous polynucleotide segment, further comprising a segment encoding a selection marker.

[00111] In some embodiments, the encoded selection marker is ΔNGFR. In some embodiments, the encoded selection marker has the sequence of SEQ ID NO: 3.

[00112] In some embodiments, the selection marker is a truncated form of EGFR polypeptide.

[00113] In some embodiments, the method further comprising the step, after step (b), of: isolating the genetically-modified $CD4^+$ T cells expressing the selection marker, thereby generating an enriched population of genetically-modified $CD4^+$ T cells.

[00114] In some embodiments, at least 30% or at least 60% of the genetically-modified $CD4^+$ T cells in the enriched population express IL-10 and the CAR.

[00115] In some embodiments, at least 90% of the genetically-modified $CD4^+$ T cells in the enriched population express IL-10 and the CAR.

[00116] In some embodiments, at least 40% of the genetically-modified $CD4^+$ T cells in the enriched population express the selection marker.

[00117] In some embodiments, at least 75% of the genetically-modified $CD4^+$ T cells in the enriched population express the selection marker.

[00118] In some embodiments, at least 90% of the genetically-modified $CD4^+$ T cells in the enriched population express the selection marker.

[00119] In some embodiments, the method further comprising the step of incubating the enriched population of genetically-modified $CD4^+$ T cells.

[00120] In some embodiments, the step of incubating the enriched population of genetically-modified $CD4^+$ T cells is performed in the presence of anti-CD3 antibody and anti-CD28 antibody or CD3 antibody and CD28 antibody coated beads in the presence of IL-2.

[00121] In some embodiments, the method further comprising the later step of freezing the genetically-modified $CD4^+$ T cells.

[00122] In some embodiments, the at least two T cell donors have at least 1/10, 2/10, 3/10, 4/10, 5/10, 6/10, 7/10, 8/10, or 9/10 match at the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci to each other.

[00123] In some embodiments, the at least two T cell donors have at least 1/8, 2/8, 3/8, 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci to each other.

[00124] In some embodiments, the at least two T cell donors have 2/2 match at the HLA-A locus to each other. In some embodiments, the at least two T cell donors have 2/2 match

at the HLA-B locus to each other. In some embodiments, the at least two T cell donors have 2/2 match at the HLA-C locus to each other.

[00125] In some embodiments, the at least two T cell donors have at least 3/4 or 4/4 match at the HLA-DRB1 and HLA-DQB1 loci to each other.

[00126] In some embodiments, the at least two T cell donors have less than 5/10, 6/10, 7/10, 8/10, or 9/10 match at the HLA-A, HLA-B, HLA C, HLA-DRB1, and HLA-DQB1 loci to each other.

[00127] In some embodiments, the at least two T cell donors have less than 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA C, and HLA DRB1 loci to each other.

[00128] In some embodiments, the at least two T cell donors have less than 2/2 match at the HLA-A locus to each other. In some embodiments, the at least two T cell donors have less than 2/2 match at the HLA-B locus to each other. In some embodiments, the at least two T cell donors have less than 2/2 match at the HLA-C locus to each other.

[00129] In some embodiments, the at least two T cell donors have less than 4/2, 3/4 or 4/4 match at the HLA-DRB1 and HLA DQB1 loci with each other.

[00130] In some embodiments, each of the at least two T cell donors has an A*02 allele or are A*02 negative. In some embodiments, each of the at least two T cell donors has an A*02 allele or are A*02 negative.

[00131] In some embodiments, in step (a), the primary $CD4^+$ T cells are obtained from one or more frozen stocks.

[00132] In some embodiments, in step (a), the primary $CD4^+$ T cells are obtained from unfrozen peripheral blood mononuclear cells of the at least two different T cell donors.

[00133] In some embodiments, the method further comprising the step of isolating $CD4^+$ T cells from the peripheral blood mononuclear cells.

[00134] In another aspect, this disclosure features a method for treating a hematological cancer, comprising: administering to a hematological cancer patient a therapeutically effective amount of any of the $CD4^{IL\text{-}10/CAR}$ cells provided, any of the population of $CD4^{IL\text{-}10/CAR}$ cells provided herein, or any of the pharmaceutical compositions provided herein sufficient to induce anti-cancer effect.

[00135] In some embodiments, the method further comprising the step of administering allo HSCT graft to the patient prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$.

[00136] In another aspect, this disclosure features a method of treating a patient with a malignancy, comprising: administering an allo-HSCT graft to the patient, and administering a therapeutically effective amount of any of the $CD4^{IL\text{-}10/CAR}$ cells provided, any of the population of $CD4^{IL\text{-}10/CAR}$ cells provided herein, or any of the pharmaceutical compositions provided herein.

[00137] In some embodiments, the amount of $CD4^{IL\text{-}10/CAR}$ cells is further sufficient to suppress or prevent graft versus host disease (GvHD) without suppressing graft versus leukemia (GvL) or graft versus tumor (GvT) efficacy of the allo HSCT.

[00138] In some embodiments, the malignancy or hematological cancer is a myeloid leukemia.

[00139] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells that express CD13.

[00140] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells that express HLA-class I.

[00141] In some embodiments, the myeloid leukemia is acute myeloid leukemia (AML).

[00142] In some embodiments, the malignancy or hematological cancer is a $CD19^+$, $CD20^+$, $CD22^+$, $CD27^+$, $CD38^+$, BCMA+, or B7-$H3^+$ hematological cancer.

[00143] In some embodiments, the $CD19^+$, $CD20^+$, $CD22^+$, $CD27^+$, $CD38^+$, BCMA+, or B7-$H3^+$ hematological cancer is selected from chronic lymphocytic leukemia, acute lymphoblastic leukemia (ALL), and non-Hodgkin's lymphomas.

[00144] In some embodiments, the allo-HSCT graft is obtained from a related or unrelated donor with respect to the patient.

[00145] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are non-autologous to the patient.

[00146] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are autologous to the patient.

[00147] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are allogeneic to the patient.

[00148] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are not anergized to host allo-antigens prior to administration to the patient.

[00149] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are Tr1-like cells.

[00150] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are polyclonal.

[00151] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are polyclonal and non-autologous to the patient.

[00152] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are polyclonal and autologous to the patient.

[00153] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are isolated from at least two donors prior to being genetically modified.

[00154] In some embodiments, none of the at least two donors is the same donor as the allo-HSCT donor.

[00155] In some embodiments, the allo-HSCT graft is obtained from a matched or mismatched donor with respect to the patient.

[00156] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells target and kill cells that express CD19, CD20, or BCMA. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells target and kill cells that express CD54. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells that express HLA-class I and CD54. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells that express CD112 and CD155. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells that express CD58.

[00157] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells in the patient.

[00158] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells target and kill solid tumor cells in the patient.

[00159] In another aspect, this disclosure features a method of treating a hematological cancer by allogeneic hematopoietic stem cell transplant (allo-HSCT), comprising:

administering allo-HSCT graft to a patient;

administering to the patient an amount of $CD4^{IL\text{-}10/CAR}$ cells sufficient to suppress or prevent graft-versus-host disease (GvHD) without suppressing graft-versus-leukemia (GvL) or graft-versus-tumor (GvT) efficacy of the allo-HSCT graft,

wherein the $CD4^{IL\text{-}10/CAR}$ cells comprise $CD4^{+}$ T cells genetically modified by vector-mediated gene transfer of one or more vectors comprising the coding sequence of human IL-10

under control of a constitutive promoter or inducible promoter and the coding sequence of a CAR under control of a constitutive promoter or inducible promoter;

wherein the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells in the patient;

wherein the wherein the $CD4^{IL\text{-}10/CAR}$ cells are not anergized to host allo-antigens prior to administration to the patient; and

wherein the $CD4^{IL\text{-}10/CAR}$ cells are non-autologous to the patient, and are Tr1-like.

[00160] In another aspect, this disclosure features method of treating a hematological cancer by allogeneic hematopoietic stem cell transplant (allo-HSCT), comprising:

administering allo-HSCT graft to a patient;

administering to the patient an amount of $CD4^{IL\text{-}10/CAR}$ cells, optionally wherein the amount is sufficient to suppress or prevent graft-versus-host disease (GvHD) without suppressing graft-versus-leukemia (GvL) or graft-versus-tumor (GvT) efficacy of the allo-HSCT graft,

wherein the $CD4^{IL\text{-}10/CAR}$ cells comprise $CD4^+$ T cells genetically modified by vector-mediated gene transfer of one or more vectors comprising the coding sequence of human IL-10 under control of a constitutive promoter or inducible promoter and the coding sequence of a CAR under control of a constitutive promoter or inducible promoter;

wherein the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells in the patient;

wherein the wherein the $CD4^{IL\text{-}10/CAR}$ cells are not anergized to host allo-antigens prior to administration to the patient; and

wherein the $CD4^{IL\text{-}10/CAR}$ cells are autologous to the patient, and are Tr1-like.

[00161] In another aspect, this disclosure features a method for preventing relapse of a $CD19^+$, $CD20^+$, $CD22^+$, $CD27^+$, $CD38^+$, $BCMA^+$ or B7-$H3^+$ hematological cancer in a patient, comprising:

administering to a patient, identified as having a $CD19^+$, $CD20^+$, $CD22^+$, BCMA+, or B7-$H3^+$ hematological cancer or at risk of having a relapse of a $CD19^+$, $CD20^+$, $CD22^+$, $BCMA^+$ or B7-$H3^+$ hematological cancer, a therapeutically effective amount of any of the $CD4^{IL\text{-}10/CAR}$ cells provided, any of the population of $CD4^{IL\text{-}10/CAR}$ cells provided herein, or any of the pharmaceutical compositions provided herein sufficient to induce anti-cancer effect. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are administered after administration of HSCT.

[00162] In another aspect, this disclosure features a method for preventing relapse of a B7-H3$^+$ cancer in a patient, comprising: administering to a patient, identified as having a B7-H3$^+$ cancer, a therapeutically effective amount of any of the CD4$^{IL\text{-}10/CAR}$ cells provided, any of the population of CD4$^{IL\text{-}10/CAR}$ cells provided herein, or any of the pharmaceutical compositions provided herein sufficient to induce anti-cancer effect.

[00163] In some embodiments, the B7-H3$^+$ cancer is solid tumor.

[00164] In some embodiments, the solid tumor is selected from the group consisting of: breast cancer, brain cancer, lung cancer, liver cancer, stomach cancer, spleen cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, uterine cancer, skin cancer, head cancer, neck cancer, sarcomas, neuroblastomas and ovarian cancer.

[00165] In another aspect, this disclosure features a method for treating a patient with minimal residual disease, comprising: administering to a patient, identified as having minimal residual disease or at risk of having minimal residual disease, a therapeutically effective amount of any of the CD4$^{IL\text{-}10/CAR}$ cells provided, any of the population of CD4$^{IL\text{-}10/CAR}$ cells provided herein, or any of the pharmaceutical compositions provided herein sufficient to induce anti-cancer effect.

[00166] In another aspect, this disclosure features a method of treating a patient, comprising: administering a therapeutically effective amount of any of the CD4$^{IL\text{-}10/CAR}$ cells provided, any of the population of CD4$^{IL\text{-}10/CAR}$ cells provided herein, or any of the pharmaceutical compositions provided herein, to a patient in need of immune tolerization.

[00167] In some embodiments, the method further comprising the preceding step of thawing a frozen suspension of CD4$^{IL\text{-}10/CAR}$ cells or the population of CD4$^{IL\text{-}10/CAR}$ cells.

[00168] In some embodiments, the patient has an inflammatory or autoimmune disease.

[00169] In some embodiments, the inflammatory or autoimmune disease is selected from the group consisting of: autoimmune uveitis, psoriasis, vitiligo, alopecis areata, psoriatic arthritis, inflammatory bowel disease, Hashimoto's thyroiditis, autoimmune vasculitis, ulcerative colitis, bullous diseases, scleroderma, celiac disease, graves disease, systemic sclerosis, myasthenia gravis, anti-NMDA encephalitis, pemphigoid diseases (vulgaris and foliaceus), epidermolysis bullosa acquisita, thrombotic thrombocytopenic purpura, ididopathic thrombocytic purpora, autoantibody induced vascular inflammation, autoantibody induced carditis, rheumatoid arthritis, autoantibody induced rheumatoid arthritis, neuromyelitis optica spectrum disorders,

systemic lupus erythematosus (SLE), multiple sclerosis (MS), sjögren's syndrome, autoimmune myopathies, type I diabetes, addison disease, pernicious anemia, autoimmune hepatitis, primary biliary cholangitis (PBC), autoimmune pancreatitis, goodpasture's disease, primary membranous nephropathy, ovarian insufficiency, autoimmune orchitis, dry eye disease, aplastic anemia, autoimmune neutropenia, and idiopathic interstitial pneumonias.

[00170] In some embodiments, the inflammatory or autoimmune disease is Crohn's disease, ulcerative colitis, celiac disease, type-1 diabetes, lupus, psoriasis, psoriatic arthritis, alkylosing spondylitis, or rheumatoid arthritis.

[00171] In some embodiments, the patient has a disease or disorder involving hyperactivity of NLPR3 inflammasome.

[00172] In some embodiments, the patient has type 2 diabetes, neurodegenerative disease, cardiovascular disease or inflammatory bowel disease.

[00173] In some embodiments, the patient has a disease or disorder involving increased IL-1β production by activated monocytes, macrophages or dendritic cells

[00174] In some embodiments, the patient has a disease or disorder involving increased IL-18 production by activated monocytes, macrophages or dendritic cells.

[00175] In some embodiments, the patient has a disease or disorder involving increased mature caspase 1 production by activated monocytes, macrophages or dendritic cells.

[00176] In some embodiments, the patient has an allergic or atopic disease.

[00177] In some embodiments, wherein the allergic or atopic disease is selected from the group consisting of: asthma, atopic dermatitis, and rhinitis.

[00178] In some embodiments, the patient has a food allergy.

[00179] In some embodiments, the patient has a solid tumor.

[00180] In some embodiments, the solid tumor is selected from the group consisting of: breast cancer, brain cancer, lung cancer, liver cancer, stomach cancer, spleen cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, uterine cancer, skin cancer, head cancer, neck cancer, sarcomas, neuroblastomas and ovarian cancer.

[00181] In some embodiments, the method further comprising the step of organ transplantation to the patient, either prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition. In some

embodiments, the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition prevents or reduces severity of host rejection of the organ transplantation.

[00182] In some embodiments, the method further comprising the step of transplanting iPS cell-derived cells or tissues to the patient, either prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition prevents or reduces severity of host rejection of the cell transplantation.

[00183] In some embodiments, the method further comprising the step of administering a recombinant AAV to the patient, either prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells, the population of $^{CD4IL\text{-}10/CAR}$ cells, or the pharmaceutical composition reduces immune responses against the recombinant AAV.

[00184] In some embodiments, the method further comprising the step of administering a recombinant viral vector other than AAV to the patient, either prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells, the population of $^{CD4IL\text{-}10/CAR}$ cells, or the pharmaceutical composition reduces immune responses against the recombinant viral vector other than AAV.

[00185] In some embodiments, the method further comprises the step of administering an immunogenic therapeutic protein to the patient, either prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition reduces immune responses against the immunogenic therapeutic protein. In some embodiments, the immunogenic therapeutic protein is selected from a therapeutic antibody, a factor VIII replacement, a cytokine, and a cytokine mutein.

[00186] In some embodiments, the patient has an excessive immune response against viral or bacterial infection. In some embodiments, the patient has a coronavirus infection. In some embodiments, the patient has organ and/or tissue damage.

[00187] In some embodiments, the method further comprising the step of detecting the selection marker in a biological sample obtained from the patient, thereby detecting presence or absence of $CD4^{IL\text{-}10/CAR}$ cells.

[00188] In some embodiments, the biological sample is a biopsy or blood from the patient.

[00189] In another aspect, this disclosure features a method of treating or inhibiting autoimmune disease, allergic disease, or inflammatory disease in a patient, comprising: administering to a patient, identified as having autoimmune disease, allergic disease, or inflammatory disease, a therapeutically effective amount of any of the $CD4^{IL\text{-}10/CAR}$ cells provided, any of the population of $CD4^{IL\text{-}10/CAR}$ cells provided herein, or any of the pharmaceutical compositions provided herein sufficient to treat or inhibit the autoimmune disease, allergic disease, or inflammatory disease.

[00190] In another aspect, this disclosure features a method for reducing transplant rejection in a patient transplanted with hematopoietic stem cells, bone marrow cells, cord blood cells, tissue stem cells or a solid organ, comprising: administering to a patient, identified as having rejection of transplanted hematopoietic stem cells, bone marrow cells, tissue stem cells or a solid organ, a therapeutically effective amount of any of the $CD4^{IL\text{-}10/CAR}$ cells provided, any of the population of $CD4^{IL\text{-}10/CAR}$ cells provided herein, or any of the pharmaceutical compositions provided herein sufficient to reduce transplant rejection.

[00191] In another aspect, this disclosure features a method for treating graft-versus-host disease (GvHD) in a patient, comprising: administering to a patient, identified as having graft-versus-host disease (GvHD) or at risk of having graft-versus-host disease (GvHD), a therapeutically effective amount of any of the $CD4^{IL\text{-}10/CAR}$ cells provided, any of the population of $CD4^{IL\text{-}10/CAR}$ cells provided herein, or any of the pharmaceutical compositions provided herein sufficient to suppress or prevent GvHD.

[00192] In some embodiments, the graft-versus-host disease (GvHD) comprises acute GvHD.

[00193] In some embodiments, the graft-versus-host disease (GvHD) comprises chronic GvHD.

[00194] In another aspect, this disclosure features a method for treating tissue or organ damage in a patient, comprising: administering to a patient, identified as having tissue or organ

damage or at risk of having tissue or organ damage, a therapeutically effective amount of any of the $CD4^{IL\text{-}10/CAR}$ cells provided herein, any of the populations of $CD4^{IL\text{-}10/CAR}$ cells provided herein, or the pharmaceutical composition provided herein sufficient to induce repair of tissue or organ damage.

[00195] In another aspect, this disclosure features a polynucleotide construct comprising: (a) a first polynucleotide segment encoding a chimeric antigen receptor (CAR); and (b) a second polynucleotide segment encoding interleukin-10 (IL-10).

[00196] In some embodiments, the first polynucleotide segment comprises a regulatory element operably linked to a coding sequence of the CAR. In some embodiments, the regulatory element drives constitutive expression of the CAR.

[00197] In some embodiments, the second polynucleotide segment comprises a regulatory element operably linked to a coding sequence of the IL-10. In some embodiments, the regulatory element drives constitutive expression of the IL-10.

[00198] In some embodiments, the method further comprising an internal ribosome entry site (IRES) or a self-cleaving peptide between the first polynucleotide segment and the second polynucleotide segment. In some embodiments, the self-cleaving peptide is selected from the group consisting of F2A, P2A, T2A and E2A.

[00199] In some embodiments, the CAR comprises an antigen-binding domain, a hinge region, a transmembrane domain, and an intracellular signaling domain.

[00200] In some embodiments, the antigen-binding domain comprises a single chain antibody fragment. In some embodiments, the single chain antibody fragment comprises a single chain Fv (scFv).

[00201] In some embodiments, the antigen-binding domain targets an antigen associated with an autoimmune disease, inflammatory disorder, or cancer.

[00202] In some embodiments, the antigen is selected from the group consisting of: CD19, CD20, CD22, BCMA, B7-H3, CD27, CD38, CEA. BCMA, CD23, Lyml, Lym2, CLEC5A, CDH179b, FLT3, GCC, Muc, CSF2RA, GFRa4, CD32, CD33, IL1lRa, IL13Ra, NYBRI, SLea, CD200R, TGFBetaR2, CD276, TROP2, LAMP1, PTK7, DLL3, CDH1, CDH6, CDH17, CDH19, TSHR, tyrosinase, HLA-A2, citrullinated peptides, insulin, GAD65, IA2, gliadin, and desmoglein.

[00203] In some embodiments, the antigen-binding domain is an anti-CD19 antigen binding domain. In some embodiments, the anti-CD19 antigen-binding domain comprises the sequence of SEQ ID NO: 11.

[00204] In some embodiments, the antigen-binding domain is an anti-CD20 antigen binding domain. In some embodiments, the anti-CD20 antigen-binding domain comprises the sequence of SEQ ID NO: 18.

[00205] In some embodiments, the antigen-binding domain is an anti-BCMA antigen binding domain. In some embodiments, the anti-BCMA antigen-binding domain comprises the sequence of SEQ ID NOs: 50-53.

[00206] In some embodiments, the hinge region is selected from a human CD8 hinge region, a human CD28 hinge region, a IgG1 hinge region, or a IgG4 hinge region. In some embodiments, the hinge region is derived from human CD8.

[00207] In some embodiments, the transmembrane domain is selected from a TNFRSF 19 transmembrane domain, a CD3zeta transmembrane domain, a CD8α transmembrane domain, a CD4 transmembrane domain, a CD28 transmembrane domain, or an B7-family inducible costimulatory (ICOS) transmembrane domain. In some embodiments, the transmembrane domain is derived from CD8α.

[00208] In some embodiments, the CAR further comprises one or more co-stimulatory domains.

[00209] In some embodiments, the CAR comprises two co-stimulatory domains.

[00210] In some embodiments, the one or more co-stimulatory domains are selected from the group consisting of 4-1BB, CD28, OX40, ICOS, CD27, MYD88-CD40, and KIR2DS2.

[00211] In some embodiments, one of the one or more co-stimulatory domain is derived from CD28.

[00212] In some embodiments, a second co-stimulatory domain is derived from 4-1BB.

[00213] In some embodiments, the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM). In some embodiments, the immunoreceptor tyrosine-based activation motif (ITAM) is derived from CD3zeta.

[00214] In some embodiments, the CAR comprises: an anti-CD19 antigen binding domain, a BCMA or an anti-CD20 antigen-binding domain; a human CD8 hinge region; a CD8

transmembrane region; a 4 a CD28 co-stimulatory domain; and a CD3zeta chain intracellular signaling domain.

[00215] In some embodiments, the CAR comprises the sequence of SEQ ID NOs: 9, 16, 22, or 34.

[00216] In some embodiments, the first polynucleotide segment comprises the sequence of SEQ ID NOs: 10, 17, 23, 35, or 55.

[00217] In some embodiments, the IL-10 is a human IL-10. In some embodiments, the IL-10 is a viral IL-10.

[00218] In some embodiments, the IL-10 is a protein having the sequence of SEQ ID NO: 1. In some embodiments, the second polynucleotide segment comprises the sequence of SEQ ID NO: 2.

[00219] In some embodiments, the first polynucleotide segment or the second polynucleotide segment, further comprising a sequence encoding a selection marker.

[00220] In some embodiments, the selection marker is ΔNGFR. In some embodiments, the ΔNGFR has the sequence of SEQ ID NO: 3. In some embodiments, the polynucleotide construct comprises a sequence of SEQ ID NO: 4.

[00221] In some embodiments, the selection marker is a truncated form of an EGFR polypeptide.

[00222] In some embodiments, the construct is a vector. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a lentiviral vector.

[00223] In another aspect, this disclosure features a polynucleotide construct, comprising: a first polynucleotide segment having the sequence of SEQ ID NOs: 10, 17, 23, 35, or 55; and a second polynucleotide segment having the sequence of SEQ ID NO: 2.

[00224] In another aspect, this disclosure features a polynucleotide construct, comprising: a first polynucleotide segment having the sequence of SEQ ID NOs: 10, 17, 23, 35, or 55; a second polynucleotide segment having the sequence of SEQ ID NO: 2; and a third polynucleotide segment having the sequence of SEQ ID NO: 33 in between the first polynucleotide segment and the second polynucleotide segment.

[00225] In another aspect, this disclosure features a polynucleotide construct, comprising: a first polynucleotide segment having the sequence of SEQ ID NOs: 10, 17, 23, 35,

or 55; a second polynucleotide segment having the sequence of SEQ ID NO: 2; a third polynucleotide segment having the sequence of SEQ ID NO: 27 in between the first polynucleotide segment and the second polynucleotide segment; and a fourth polynucleotide segment having the sequence of SEQ ID NO: 4.

[00226] In another aspect, this disclosure features $CD4^{IL\text{-}10}$ cells from a single donor or multiple donors, where the IL-10 is viral IL-10. The viral IL-10 having the sequence of SEQ ID NO: 6 or 18. In some embodiments, the viral IL-10 is encoded by a polynucleotide having the sequence of SEQ ID NO: 7. In some embodiments, the IL-10 is human IL-10 where one, two, three, four, five, six, seven, eight, nine or ten amino-acid from human IL-10 are replaced by the corresponding amino-acid sequence from viral IL-10. In some embodiments, the $CD4^+$ T cells are transduced with exogenous viral IL-10 under the control of constitutive promoter. In some embodiments, the expression control elements drive expression of viral IL-10 in activated $CD4^+$ T cells. In some embodiments, the exogenous polynucleotide encoding viral IL-10 is integrated into the T cell nuclear genome. In some embodiments, the exogenous polynucleotide encoding viral IL-10 is not integrated into the T cell nuclear genome. In some embodiments, the exogenous polynucleotide encoding viral IL-10 has the sequence of SEQ ID NO: 7.

[00227] In another aspect, this disclosure features $CD4^{IL\text{-}10}$ cells from a single donor or multiple donors, where the IL-10 is IL-10 of a *Mus musculus*, "MOUSE" (SEQ ID NO: 58); *Rattus norvegicus*, "RAT" (SEQ ID NO: 59); *Macaca mulatta*, "MACMU" (SEQ ID NO: 60); Gorilla gorilla, "GORILLA" (SEQ ID NO: 61); *Macaca fascicularis*, "CYNO" (SEQ ID NO: 62); *Papio Anubis*, "OLIVE BABOON" (SEQ ID NO: 63); *Pan paniscus*, "BONOBO" (SEQ ID NO: 64); *Pan troglodytes*, "CHIMP" (SEQ ID NO: 65); and EBVB9 (SEQ ID NO: 66). In some embodiments, the IL-10 is a protein having at least 90%, 95%, 98%, or 99% sequence identity to IL-10 of a *Mus musculus*, "MOUSE" (SEQ ID NO: 58); *Rattus norvegicus*, "RAT" (SEQ ID NO: 59); *Macaca mulatta*, "MACMU" (SEQ ID NO: 60); Gorilla gorilla, "GORILLA" (SEQ ID NO: 61); *Macaca fascicularis*, "CYNO" (SEQ ID NO: 62); *Papio Anubis*, "OLIVE BABOON" (SEQ ID NO: 63); *Pan paniscus*, "BONOBO" (SEQ ID NO: 64); *Pan troglodytes*, "CHIMP" (SEQ ID NO: 65); and EBVB9 (SEQ ID NO: 66).

[00228] In another aspect, this disclosure features $CD4^{IL\text{-}10}$ cells from a single donor or multiple donors, where the IL-10 is a variant of human IL-10. The variant of human IL-10 having the sequence of SEQ ID NO: 67 or SEQ ID NO: 68. In some embodiments, the IL-10 is human IL-10 where one, two, three, four, five, six, seven, eight, nine or ten amino-acid from

human IL-10 are replaced by the corresponding amino-acid sequence from IL-10 of another species (e.g., IL-10 of a *Mus musculus*, "MOUSE" (SEQ ID NO: 58); *Rattus norvegicus*, "RAT" (SEQ ID NO: 59); *Macaca mulatta*, "MACMU" (SEQ ID NO: 60); Gorilla gorilla, "GORILLA" (SEQ ID NO: 61); *Macaca fascicularis*, "CYNO" (SEQ ID NO: 62); *Papio Anubis*, "OLIVE BABOON" (SEQ ID NO: 63); *Pan paniscus*, "BONOBO" (SEQ ID NO: 64); *Pan troglodytes*, "CHIMP" (SEQ ID NO: 65); and EBVB9 (SEQ ID NO: 66). In some embodiments, the $CD4^+$ T cells are transduced with exogenous the IL-10 variant under the control of constitutive promoter. In some embodiments, the expression control elements drive expression of the IL-10 variant in activated $CD4^+$ T cells. In some embodiments, the exogenous polynucleotide encoding the IL-10 variant is integrated into the T cell nuclear genome. In some embodiments, the exogenous polynucleotide encoding the IL-10 variant is not integrated into the T cell nuclear genome.

[00229] In yet another aspect, the present disclosure provides a method of making viral IL-10 $CD^{4IL\text{-}10}$, comprising the steps of:

(i) obtaining primary $CD4^+$ T cells from a single T cell donor; and

(ii) modifying the donor $CD4^+$ T cells by introducing an exogenous polynucleotide encoding viral IL-10,

thereby obtaining the genetically-modified $CD4^+$ T cells.

[00230] In some embodiments, the method further comprises the step, after step (i), or after step (ii), of: incubating the primary $CD4^+$ T cells in the presence of an anti-CD3 antibody, and anti-CD28 antibody or anti-CD3 antibody and CD28 antibody coated beads.

[00231] In some embodiments, the method comprises incubating the primary $CD4^+$ T cells further in the presence of IL-2. In some embodiments, the exogenous polynucleotide encoding viral IL-10 using a vector.

[00232] In some embodiments, the exogenous polynucleotide encoding viral IL-10 comprises a segment encoding a selection marker. In some embodiments, the encoded selection marker is ΔNGFR. In some embodiments, the encoded selection marker has the sequence of SEQ ID NO:3. In some embodiments, the encoded selection marker is a truncated EGFR polypeptide. In some embodiments, the encoded selection marker is a truncated human EGFR polypeptide.

[00233] In some embodiments, the method further comprises the step, after step (ii), of: isolating the genetically-modified $CD4^+$ T cells expressing the selection marker, thereby generating an enriched population of genetically-modified $CD4^+$ T cells.

[00234] In some embodiments, the method further comprises the step of incubating the enriched population of genetically-modified $CD4^+$ T cells. In some embodiments, the step of incubating the enriched population of genetically-modified $CD4^+$ T cells is performed in the presence of anti-CD3 antibody and anti-CD28 antibody or CD3 antibody and CD28 antibody coated beads in the presence of IL-2.

[00235] In some embodiments, in step (i), the primary $CD4^+$T cells are obtained from frozen stock. In some embodiments, in step (i), the primary $CD4^+$T cells are obtained from unfrozen peripheral blood mononuclear cells of the single T cell donor.

[00236] In some embodiments, the method further comprises the step of isolating $CD4^+$ T cells from the peripheral blood mononuclear cells. In some embodiments, the peripheral blood mononuclear cells are obtained from buffy coat or apheresis.

5. BRIEF DESCRIPTION OF THE DRAWINGS

[00237] **FIG. 1** is a non-limiting illustration of structure for various chimeric antigen receptors (CARs) provided herein.

[00238] **FIG. 2A** illustrates the partial structure of an anti-CD19 CAR coding sequence that includes a scFv from a fully-human anti-CD19 monoclonal antibody FMC63, a CD8α hinge, a CD8α transmembrane domain, a CD28 costimulatory domain, and a CD3ζ activation domain, that is used in the lentiviral vector for delivering the anti-CD19 CAR into $CD4^+$ T cells to produce $CD4^{IL\text{-}10anti\text{-}CD19\ CAR}$ cells.

[00239] **FIG. 2B** illustrates a lentiviral vector (pLV anti-CD19 CAR) used for delivering the anti-CD19 CAR into $CD4^+$ T cells to produce $CD4^{IL\text{-}10antiCD19CAR}$ cells.

[00240] **FIGs. 3A-3B** illustrate a bidirectional plasmid (pLVIL-10) used to generate the lentiviral vector (LVV; $LVV^{IL\text{-}10\text{-}NGFR}$) used for delivering human IL-10 and ΔNGFR coding sequences into $CD4^+$ T cells to produce $CD4^{IL\text{-}10}$ cells or $CD4^{IL\text{-}10\ antiCD19CAR}$ cells. **FIG. 3A** illustrates the partial structure of a bidirectional lentiviral vector for delivering human IL-10 and ΔNGFR coding sequences into CD4+ T cells from multiple donors to produce polydonor CD4IL-10 cells. **FIG. 3B** illustrates the complete and circular structure of a bidirectional lentiviral vector (hPGK.IL10.WPRE.mhCMV. ΔNGFR.SV40PA) for delivering human IL-10 and ΔNGFR coding sequences into $CD4^+$ T cells from multiple donors to produce polydonor $CD4^{IL\text{-}10}$ cells.

[00241] **FIG. 4** shows for illustrative purposes antigen expression during natural B-cell differentiation.

[00242] **FIGs. 5A-5C** show schematics for non-limiting examples of methods of generating $CD4^{IL\text{-}10antiCD19CAR}$ cells. In each method, human $CD4^+$ T cells were activated with anti-CD3/anti-CD28 beads (Miltenyi) (3:1 cells:beads ratio), in complete culture medium (X-vivo supplemented with 5% human AB serum and rhIL-2 (50U/mL). **FIG. 5A** shows the activated $CD4^+$ T cells were transduced with a lentiviral vector encoding an anti-CD19 CAR ($LVV^{CD19\text{-}CAR}$) 48 hours after activation and transduced 24 hours later (i.e., 72 hours after activation) with a bidirectional lentiviral vector encoding human IL-10 and a truncated form of the human NGF receptor ($LVV^{IL\text{-}10\text{-}NGFR}$). Both LVVs were used at a multiplicity of infection (MOI) of 20. **FIG. 5B** shows activated $CD4^+$ T cells were transduced with the $LVV^{IL\text{-}10\text{-}NGFR}$ 48 hours after activation and transduced 24 hours later (i.e., 24 hours after activation) with $LVV^{CD19\text{-}CAR}$. **FIG. 5C** shows the activated $CD4^+$ T cells were transduced simultaneously with $LVV^{IL\text{-}10\text{-}NGFR}$ and $LVV^{CD19\text{-}CAR}$ 48 hours after activation. Controls (do not express the CAR) were generated by transducing activated $CD4^+$ T cells 48 hours after activation with $LVV^{IL\text{-}10\text{-}NGFR}$ only. For each of **FIGs. 5A-5C**, the transductions were conducted in the presence of polybrene (8 µg/mL). Additionally, for each of **FIGs. 5A-5C**,10-28 days after transduction, transduced cells (i.e., ΔNGFR+ cells) were purified using anti-CD271 microbeads (Miltenyi) and anti-CD19 CAR microbeads (Miltenyi). Purified cells were re-stimulated every 14 days as previously described (Andolfi et al. Mol. Ther. 20(9):1778-1790 (2012) and Locafaro et al. Mol. Ther. 25(10): 2254-2269 (2017)). After the second (TF2) and third (TF3) re-stimulation the resulting cells ($CD4^{IL\text{-}10antiCD19CAR}$ cells or control $CD4^{IL\text{-}10}$ cells) were characterized in vitro and in vivo. Culture medium was refreshed every 2-3 days, as needed through the entire culture period.

[00243] **FIG. 6A** shows flow cytometry plots (anti-CD19 CAR expression on x-axis and NGFR expression a proxy for IL-10, on y-axis) for control (left panel) and $CD4^{IL\text{-}10antiCD19\ CAR}$ (right panel) produced according to the methods described in **FIG. 5A**. **FIG. 6B** shows flow cytometry plots (CD19 on x-axis and NGFR on y-axis) for control (left panel) and $CD4^{IL\text{-}10antiCD19CAR}$ (right panel) produced according to the methods described in **FIG. 5B**. **FIG. 6C** shows flow cytometry plots (CD19 on x-axis and NGFR on y-axis) for control (left panel) and $CD4^{IL\text{-}10antiCD19\ CAR}$ (right panel) produced according to the methods described in **FIG. 5C**.

[00244] **FIG. 7** shows flow cytometry plots (anti-CD19 CAR expression on x-axis and NGFR expression on y-axis) for $CD4^{IL\text{-}10}$ T cells (control) and $CD4^{IL\text{-}10anti\text{-}CD19\ CAR}$ T cells. Top panel shows $CD4^{IL\text{-}10}$ T cells (control) and $CD4^{IL\text{-}10antiCD19\ CAR}$ T cells generated from donor 26.1 according to the method shown in **FIG. 5C**. Bottom panel shows $CD4^{IL\text{-}10}$ T cells (control) and $CD4^{IL\text{-}10antiCD19CAR}$ T cells generated from donor 26.2 according to the methods shown in **FIG. 5C**.

[00245] **FIGs. 8A-8D** shows cytokine production profiles for $CD4^{IL\text{-}10}$ T cells (control) and $CD4^{IL\text{-}10/\ CAR}$ T cells generated from $CD4^+$ T cells isolated from three different donors (26.1, 26.2, and 26.3). Cytokine production profile includes IL-10 (**FIG. 8A**), IFN-γ (**FIG. 8B**), IL-4 (**FIG. 8C**), and IL-5 (**FIG. 8D**).

[00246] **FIG. 9** shows cytokine production profiles for $CD4^{IL\text{-}10}$ T cells (control) and $CD4^{IL\text{-}10/CAR}$ T cells generated from $CD4^+$ T cells isolated from donor 24.2.

[00247] **FIG. 10** shows that $CD4^{IL\text{-}10antiCD19CAR}$ cells generated from $CD4^+$ T cells isolated from donor 24.2 killed the CD19 expressing NALM6 cells in vitro. ALL-CM is a myeloid cell line and K562, a cell line sensitive to NK killing, was included as a control.

[00248] **FIG. 11A** shows that $CD4^{IL\text{-}10antiCD19CAR}$ cells generated from $CD4^+$ T cells isolated from three different donors (26.1, 26.2, and 26.3) killed $CD19^+$ NALM6 cells in vitro.

[00249] **FIG. 11B** shows IL-10 levels in the supernatant at day 3 of co-cultures of NALM6 cells and $CD4^{IL\text{-}10}$ T cells (control) or NALM6 cells and $CD4^{IL\text{-}10antiCD19CAR}$ cells. BLQ: below the level of quantification (31pg/mL).

[00250] **FIG. 12** shows flow cytometry plots (count versus eFluor®670) from a proliferation assay of allogeneic CD4+ T cells following co-culture with $CD4^{IL\text{-}10}$ T cells (control) and $CD4^{IL\text{-}10antiCD19CAR}$ cells isolated after a second restimulation (TF2) (see **FIG. 5C**). Top panel shows proliferation of $CD4^+$ T cells following co culture with $CD4^{IL\text{-}10antiCD19CAR}$ cells. Bottom panel shows proliferation of $CD4^+$ T cells following co-culture with $CD4^{IL\text{-}10}$ T cells (control). Flow cytometry plots show "undivided" cells and "divided" cells, where dividing cells include smaller and smaller amounts of the eFluor®670 because of dilution following each division. Suppression mediated by $CD4^{IL\text{-}10}$ T cells (control) and $CD4^{IL\text{-}10antiCD19CAR}$ cells was calculated as follows: 100-([proliferation of responders in the presence of $CD4^{IL\text{-}10}$ T cells (control) or $CD4^{IL\text{-}10antiCD19CAR}$ cells / proliferation of responders] x 100).

[00251] **FIG. 13** shows flow cytometry plots (count versus eFluor®670) from a proliferation assay of allogeneic $CD8^+$ T cells following co-culture with $CD4^{IL\text{-}10}$ T cells (control) and $CD4^{IL\text{-}10antiCD19CAR}$ cells isolated after a second restimulation (TF2) (see **FIG. 5C**). Top panel shows proliferation of $CD8^+$ T cells following co culture with $CD4^{IL\text{-}10antiCD19CAR}$ cells. Bottom panel shows proliferation of $CD8^+$ T cells following co-culture with $CD4^{IL\text{-}10}$ T cells (control). Flow cytometry plots show "undivided" cells and "divided" cells, where dividing cells include smaller and smaller amounts of the eFluor®670 because of dilution following each division. Suppression mediated by $CD4^{IL\text{-}10}$ T cells (control) and $CD4^{IL\text{-}10antiCD19CAR}$ cells was calculated as follows: 100-([proliferation of responders in the presence of $CD4^{IL\text{-}10}$ T cells (control) or $CD4^{IL\text{-}10antiCD19CAR}$ cells / proliferation of responders] x 100).

[00252] **FIG. 14** shows flow cytometry plots (count versus eFluor®670) from a proliferation assay of allogeneic PBMC following co-culture with $CD4^{IL\text{-}10}$ T cells (control) and $CD4^{IL\text{-}10antiCD19CAR}$ cells isolated after a third restimulation (TF3) (see **FIG. 5C**). Flow cytometry plots show "proliferative" cells, where dividing cells include smaller and smaller amounts of the eFluor®670 because of dilution following each division. Suppression mediated by $CD4^{IL\text{-}10}$ T cells (control) and $CD4^{IL\text{-}10antiCD19CAR}$ cells was calculated as follows: 100-([proliferation of responders in the presence of $CD4^{IL\text{-}10}$ T cells (control) or $CD4^{IL\text{-}10antiCD19CAR}$ cells / proliferation of responders] x 100).

[00253] **FIG. 15** shows a schematic of the experimental outline for assessing CD19+ tumor cell growth in vivo in Mice. At day 0, NSG mice were injected with (i) NALM6-luciferase ($1x10^5$ per mouse), (ii) NALM6-luciferase ($1x10^5$ per mouse) + PBMC ($2.5x10^6$ per mouse), (iii) NALM6-luciferase ($1x10^5$ per mouse) + $CD4^{IL\text{-}10antiCD19CAR}$ cells ($2.5x10^6$ per mouse), or (iv) NALM6-luciferase ($1x10^5$ per mouse) + PBMC ($2.5x10^6$ per mouse) + $CD4^{IL\text{-}10antiCD19CAR}$ cells ($2.5x10^6$ per mouse).

[00254] **FIG. 16A** shows bioluminescence from NALM6-luciferase cells in NSG mice at day 3, 6, 9, and 12 after injection with conditions (i) to (iv) described in **FIG. 15**.

[00255] **FIG. 16B** shows a line graph that quantifies the bioluminescence from the images in **FIG. 16A**. Total flux [p/s] over time (day post NALM6-luciferase injection) for each of the conditions (i) to (iv) described in **FIG. 15**. P-values were measured at day 12 using a Mann-Whitney test comparing conditions (ii) to (iv) to condition (i) (NALM6-luciferase alone).

[00256] **FIG. 17** shows a line graph for weight loss over time for the mice described in Fig 15 with conditions (i), (ii), and (iii). Weight loss is used as an indicator of xeno-graft-versus host disease (GvHD).

[00257] **FIGs. 18A-18C** show single donor $CD4^{IL\text{-}10antiCD19CAR}$ cells and polydonor $CD4^{IL\text{-}10antiCD19CAR}$ cells in humanized mouse model of xeno-GvHD. **FIG. 18A** shows single donor $CD4^{IL\text{-}10antiCD19CAR}$ cells tested in a humanized mouse model of xeno-GvHD induced by allogeneic PBMC in which irradiated NSG mice were injected intravenous (i.v.) with (i) allogeneic PBMC (2.5E6 cells/mouse), (ii) single donor $CD4^{IL\text{-}10antiCD19CAR}$ cells (2.5E6 cells/mouse), (iii) polydonor $CD4^{IL\text{-}10antiCD19CAR}$ cells (2.5E6 cells/mouse), (iv) PBMC+ single donor $CD4^{IL\text{-}10antiCD19CAR}$ cells, (v) or PBMC + polydonor $CD4^{IL\text{-}10antiCD19CAR}$ cells (2.5E6 cells/mouse) at Day 3. **FIG. 18B** shows $CD4^{IL\text{-}10antiCD19CAR}$ cells tested in a humanized mouse model of xeno-GvHD induced by allogeneic PBMC in which irradiated NSG mice were injected intravenous (i.v.) with allogeneic PBMC (2.5E6 cells/mouse) and/or single donor $CD4^{IL\text{-}10antiCD19CAR}$ cells at day 3. **FIG.18C** shows $CD4^{IL\text{-}10antiCD19CAR}$ cells tested in a humanized mouse model of xeno-GvHD induced by allogeneic PBMC in which irradiated NSG mice were injected intravenous (i.v.) with allogeneic PBMC (2.5E6 cells/mouse) and/or polydonor $CD4^{IL\text{-}10antiCD19CAR}$ cells (2.5E6 cells/mouse) at Day 3. PBMC: peripheral mononuclear cells; GvHD: graft vs. host disease.

[00258] **FIG. 19** illustrates an exemplary protocol for generating $CD4^{IL\text{-}10}$ cells.

[00259] **FIG. 20A** shows percentages of $CD4^{+}\Delta NGFR^{+}$ cells (mean± SD, n=10) and vector copy numbers (VCN, mean ± SD, n=10) in human $CD4^{+}$ T cells transduced with LV-IL-10/ΔNGFR (a bidirectional lentiviral vector encoding for human IL-10 and a truncated form the human NGF receptor). **FIG. 20B** shows FACS analysis of expression of CD4 and ΔNGFR in human $CD4^{+}$ T cells from two representative donors (Donor B and Donor C) transduced with LV-IL-10/ΔNGFR and purified using anti-CD271 Microbeads.

[00260] **FIG. 21** shows cytokine production profile of single donor $CD4^{IL\text{-}10}$ cells after the second (TF2) and third (TF3) restimulation. The TF2 and TF3 $CD4^{IL\text{-}10}$ cells were left unstimulated (arrows indicate spontaneous/unstimulated sample) or stimulated with immobilized CD3 (10μg/mL) and soluble CD28 mAb (1μg/mL) for 48 hours. Culture supernatants were collected and levels of IL-10, IL-4, IL-5, IFN-γ and IL-22 were determined by ELISA. All samples were tested in triplicate. Mean± SD, n=8 donors tested are presented.

[00261] **FIG. 22A** shows the percentage of $CD4^{IL\text{-}10}$ cells expressing granzyme B (GzB) after 2nd round of stimulation (TF2) analyzed by FACS. Box and whiskers of n=7 donors and single donors are presented. **FIG. 22B** shows % dead cells when $CD4^{IL\text{-}10}$ cells (10^5/well) were co-cultured with K562 and ALL-CM cells (10^5/well) at 1:1 ratio for 3 days. Box and whiskers represent data from n=4 donors and dots represent data from single donors.

[00262] **FIGs. 23A and 23B** show that single donor $CD4^{IL\text{-}10}$ cells can suppress the proliferation of allogeneic $CD4^+$ T cells. Allogeneic PBMC cells were labeled with eFluor® 670 (10^5 cells/well) and stimulated with allogeneic mature dendritic (DC) cells ($5x10^4$ cells/well) and soluble anti-CD3 mAbs in the absence or presence of $CD4^{IL\text{-}10}$ cells (10^5 cells/well) at a 1:1 Responder:Suppressor ratio. After 4 days of culture, the percentages of proliferating responder cells were determined by eFluor® 670 dilution with flow cytometry after gating on $CD4^+\Delta NGFR^-$ T cells. **FIG. 23A** show results from Donor-C, Donor-E, and Donor-F and **FIG. 23B** show results from Donor-H, Donor-I and Donor-L. Percentages of proliferation and suppression are indicated. The suppression mediated by $CD4^{IL\text{-}10}$ cells was calculated as follows: 100-([proliferation of responders in the presence of $CD4^{IL\text{-}10}$ cells/proliferation of responders alone]x 100).

[00263] **FIGs. 24A and 24B** show that single donor $CD4^{IL\text{-}10}$ cells can suppress the proliferation of allogeneic $CD8^+$ T cells. Allogeneic PBMC cells were labeled with eFluor® 670 (10^5 cells/well) and stimulated with allogeneic mature dendritic (DC) cells ($5x10^4$ cells/well) and soluble anti-CD3 mAbs in the absence or presence of $CD4^{IL\text{-}10}$ cells (10^5 cells/well) at a 1:1 Responder:Suppressor ratio. After 4 days of culture, the percentages of proliferating responder cells were determined by eFluor® 670 dilution with flow cytometry after gating on $CD4^+\Delta NGFR^-$ T cells. **FIG. 24A** show results from Donor-C, Donor-E, and Donor-F and **FIG. 24B** show results from Donor-H, Donor-I and Donor-L. Percentages of proliferation and suppression are indicated. The suppression mediated by $CD4^{IL\text{-}10}$ cells was calculated as follows: 100-([proliferation of responders in the presence of $CD4^{IL\text{-}10}$ cells/proliferation of responders alone] x 100).

[00264] **FIG. 25** shows cytokine production profile of polydonor $CD4^{IL\text{-}10}$ cells after third (TF3) restimulation, compared to mean levels (+/- SD) produced by $CD4^{IL\text{-}10}$ cells from 8 individual donors. The TF3 $CD4^{IL\text{-}10}$ cells from three donors were pooled at a 1:1:1 ratio and stimulated with immobilized CD3 (10µg/mL) and soluble CD28 mAb (1µg/mL) for 48 hours. Culture supernatants were collected and levels of IL-10, IL-4, IL-5, IFN-gamma and IL-22 were

determined by ELISA. Dots are results of polydonor $CD4^{IL\text{-}10}$ cells; gray bars represent mean± SD, n=8 single donors.

[00265] **FIG. 26A** shows the percentage of polydonor $CD4^{IL\text{-}10}$ cells expressing granzyme B (GzB) compared to mean % levels (+/- SD) of granzyme B expression by $CD4^{IL\text{-}10}$ cells of n=3 single donors used to generate the pool. Cells were analyzed by FACS after the 3rd round of stimulation (TF3). **FIG. 26B** shows % dead cells when polydonor $CD4^{IL\text{-}10}$ cells (10^5/well) were co-cultured with K562 and ALL-CM cells (10^5/well) at 1:1 ratio for 3 days. Residual leukemic cells ($CD45^+CD33^+$) were counted by FACS for each target cell. Dots are results of polydonor $CD4^{IL\text{-}10}$ and gray bars represent mean± SD of n=3 single donors used to generate the pool.

[00266] **FIGs. 27A and 27B** show that polydonor $CD4^{IL\text{-}10}$ cells can suppress the proliferation of allogeneic $CD4^+$ T cells and $CD8^+$ T cells. Allogeneic PBMC cells were labeled with eFluor® 670 (10^5 cells/well) and stimulated with allogeneic mature dendritic (DC) cells ($5x10^4$ cells/well) and soluble anti-CD3 mAbs in the absence or presence of polydonor $CD4^{IL\text{-}10}$ cells (10^5 cells/well) at a 1:1 Responder:Suppressor ratio. After 4 days of culture, the percentages of proliferating responder cells were determined by eFluor® 670 dilution with flow cytometry after gating on $CD4^+\Delta NGFR^-$ T cells and $CD8^+\Delta NGFR^-$ T cells. **FIG. 27A** shows results from polydonor $CD4^{IL\text{-}10}$ cells containing $CD4^+$ cells pooled from Donor-C, Donor-E, and Donor-F. **FIG. 27B** shows results from polydonor $CD4^{IL\text{-}10}$ cells containing $CD4^+$ cells pooled from Donor-H, Donor-I, and Donor-L. The suppression mediated by $CD4^{IL\text{-}10}$ cells was calculated as follows: 100-([proliferation of responders in the presence of $CD4^{IL\text{-}10}$ cells/proliferation of responders alone]x 100).

[00267] **FIG. 28** illustrates a protocol for testing induction of GvHD by human PBMC and/or polydonor $CD4^{IL\text{-}10}$ cells injected on day 0 post-irradiation.

[00268] **FIG. 29** shows % of NSG mice demonstrating GvHD in each day after injection of PBMC ($5x10^6$ cells/mouse), polydonor (three donors) $CD4^{IL\text{-}10}$ cells ($5x10^6$ cells/mouse), or PBMC ($5x10^6$ cells/mouse) in combination with polydonor $CD4^{IL\text{-}10}$ cells (three donors) ($5x10^6$ cells/mouse).

[00269] **FIG. 30** shows migration of $CD4^{IL\text{-}10}$ cells to spleen (left panel) and bone marrow (right panel) in NSG mice injected with PBMC ($5x10^6$ cells/mouse), polydonor (three donors) $CD4^{IL\text{-}10}$ cells ($5x10^6$ cells/mouse), or PBMC ($5x10^6$ cells/mouse) in combination with polydonor $CD4^{IL\text{-}10}$ cells (three donors) ($5x10^6$ cells/mouse). Box and whiskers on n=8 donors and single donors are presented.

[00270] **FIG. 31** illustrates a protocol for testing induction of GvHD by CD4+ T cells and polydonor or single-donor (BC-H) $CD4^{IL-10}$ cells injected on day 0 post-irradiation.

[00271] **FIG. 32** shows % of NSG mice demonstrating GvHD on each day after injection.

[00272] **FIGs. 33A-33C** shows graft-versus-leukemia (GvL) effect tested based on reduction of circulating leukemia cells and long-term leukemia free survival. Leukemia was measured as previously described (Locafaro G. et al Molecular Therapy 2017). NSG mice were sub-lethally irradiated and intravenously injected with myeloid leukemia cells (ALL-CM) ($5x10^6$) at day 0. **FIG. 33A** is an illustration of the experiment. **FIG. 33B** shows leukemia free survival rate in the animals injected with PBMC ($5x10^6$) or single donor (from donor BC-I and donor BC-H) $CD4^{IL-10}$ cells ($2.5x10^6$) at day 3. **FIG. 33C**.shows leukemia free survival rate in the animals injected with PBMC ($5x10^6$) or polydonor $CD4^{IL-10}$ cells (from donor BC-I and donor BC-H) ($2.5x10^6$) at day 3.

[00273] **FIGs. 34A-34C** show long-term leukemia free survival rate measured in NSG mice sub-lethally irradiated and intravenously injected with ALL-CM cells ($5x10^6$) at day 0. **FIG. 34A** shows an illustration of the experiment. **FIG. 34B** shows data from animals injected with mononuclear cells (PBMC) ($5x10^6$) alone or mononuclear cells (PBMC) ($5x10^6$) + single donor (from donor BC-H and donor BC-I) $CD4^{IL-10}$ cells ($2.5x10^6$) at day 3. **FIG. 34C** shows data from animals injected with mononuclear cells (PBMC) ($5x10^6$) alone or mononuclear cells (PBMC) ($5x10^6$) + polydonor $CD4^{IL-10}$ cells (BC-I/H) ($2.5x10^6$) at day 3.

[00274] **FIG. 35A-35G** show inhibition of NLPR3 inflammasome activation by $CD4^{IL-10}$ cells. **FIG. 35A** shows the effect of $CD4^{IL-10}$ cell supernatant from a single donor (#1) on the production of IL-1β by LPS activated monocytes. **FIG. 35B** shows the effect of $CD4^{IL-10}$ cell supernatant from another single donor (#2) on the production of IL-1β by LPS activated monocytes. **FIG. 35C** shows the effect of $CD4^{IL-10}$ cell supernatant from a single donor (#1) on the inhibition of LPS induced IL-1β production enhanced by NLPR3 inflammasome activator nigericine (NIG). **FIG. 35D** shows the effect of $CD4^{IL-10}$ cell supernatant from a single donor (#2) on the inhibition of LPS induced IL-1β production enhanced by NLPR3 inflammasome activator nigericine (NIG). **FIG. 35E** is a bar graph that shows the effect of $CD4^{IL-10}$ cell supernatant from a single donor and pooled cells from 2 different donors on LPS induced IL-1β production by monocytes in the presence or absence of anti-IL-10 receptor (anti-IL-10R) mAb. **FIG. 35F** shows the effect of polydonor $CD4^{IL-10}$ cell supernatants on IL-1β production by monocytes in the presence or absence of anti-IL-10 receptor (anti-IL-10R) mAb. **FIG. 35G**

shows the effects of polydonor $CD4^{IL\text{-}10}$ cell supernatants on IL-18 production induced by LPS in combination with nigericin in the presence or absence of anti-IL-10R mAb.

[00275] **FIG. 36** illustrates an experimental protocol for testing graft versus myeloid leukemia and xeno-GvHD effects. NSG-mice were intravenously injected with ALL-CM cells ($2.5x10^6$) on day 0. At day 3 the mice were divided into five groups and each group was treated with (i) none as a control , (ii) allogeneic mononuclear cells (PBMC) ; (iii) allogeneic PBMC and polydonor $CD4^{IL\text{-}10}$ cells (BC-E, BC-V, BC-T, pooled 1:1:1); (iv) allogeneic PBMC and single-donor $CD4^{IL\text{-}10}$ cells (BC-E); or (v) polydonor $CD4^{IL\text{-}10}$ cells at concentrations as indicated in **FIG. 36**.

[00276] **FIG. 37** is a bar graph depicting the cytokine secretion profiles of single-donor (BC-T, BC-V, and BC-E) and polydonor $CD4^{IL\text{-}10}$ cells (POOL: BC-E, BC-V and BC-T pooled 1:1:1).

[00277] **FIG. 38** show suppressive effects of single-donor (BC-V and BC-E) and polydonor $CD4^{IL\text{-}10}$ cells (pool of BC-V/E/T) on in vitro proliferation of allogeneic CD4+ and CD8+ T cells. Allogeneic PBMC cells were labeled with eFluor® 670 ($5x10^4$ cells/well) and stimulated with allogenic mature dendritic (DC) cells ($1x10^4$ cells/well) and soluble anti-CD3 mAbs in the absence or presence of $CD4^{IL\text{-}10}$ cells ($5x10^4$ cells/well) at a 1:1 Responder:Suppressor ratio. After 3 days of culture, the percentages of proliferating responder cells were determined by eFluor® 670 dilution with flow cytometry after gating on $CD4^+$ - $\Delta NGFR^-$ (top) or $CD8^+$ $\Delta NGFR^-$ (bottom) T cells. **FIG. 38** shows results from single donors BC-V and BC-E and pooled cells from donors BC-V, BC-E and BC-T. Percentages of proliferation and suppression are indicated. The suppression mediated by $CD4^{IL\text{-}10}$ cells was calculated as follows: 100-([proliferation of responders in the presence of $CD4^{IL\text{-}10}$ cells/proliferation of responders alone]x 100).

[00278] **FIG. 39** shows % of alive cells in a co-culture of single (BC-E and BC-V) or polydonor $CD4^{IL\text{-}10}$ cells (BC-E+BC-V+BC-T) with ALL-CM myeloid tumor cells or K562 cells. The results show selective cytotoxic effect of single-donor and polydonor $CD4^{IL\text{-}10}$ cells on ALL-CM myeloid tumor cells, but not on K562 cells which lack Class I MHC expression.

[00279] **FIG. 40** shows leukemia-free survival rate measured in NSG-mice intravenously injected with ALL-CM cells ($2.5x10^6$) on day 0. At day 3 the mice were divided into five groups and each group was treated with (i) none as a control; (ii) allogeneic mononuclear cells (PBMC); (iii) allogeneic PBMC and polydonor $CD4^{IL\text{-}10}$ cells (B BC-

V/T/ET); (iv) allogeneic PBMC and single-donor $CD4^{IL-10}$ cells (BC-E) or (v) polydonor $CD4^{IL-10}$ cells. The graph shows leukemia-free survival rate of animals in each group.

[00280] **FIG. 41** shows % of NSG mice free of GvHD on each day following injection with ALL-CM cells ($2.5x10^6$) and subsequent administration of (i) none as a control; (ii) allogeneic mononuclear cells (PBMC); (iii) allogeneic PBMC and polydonor $CD4^{IL-10}$ cells (BC-E, BC-V, BC-T); (iv) allogeneic PBMC and single-donor $CD4^{IL-10}$ cells (BC-E) or (v) polydonor $CD4^{IL-10}$ cells were administered at day 3.

[00281] **FIG. 42** shows that NSG mice dosed with 2.5E+06 of PBMC (allogeneic to the donors C, E, F and H) all succumbed to acute, and lethal xeno-GvHD at day 22. Administration of single-donor $CD4^{IL-10}$ cells (lot C) or polydonor $CD4^{IL-10}$ cells (lot CEFH) in combination with the PBMCs prevented the development of lethal xeno-GvHD in 75% (3/4 mice) and 80% (4/5 mice) of the mice, respectively. In contrast, transfer of 2.5E+06 polydonor $CD4^{IL-10}$ cells did not induce any sign of GvHD. Taken together these results indicate that polydonor $CD4^{IL-10}$ cells from 4 different donors suppress pathogenic human T cell responses as potently, or slightly more potently than single- donor $CD4^{IL-10}$ cells. PBMC: peripheral mononuclear cells; GvHD: graft vs. host disease.

[00282] **FIG. 43A** shows alignment of IL-10 protein sequences of various species, including human (SEQ ID NO: 1), *Mus musculus*, "MOUSE" (SEQ ID NO: 58); *Rattus norvegicus*, "RAT" (SEQ ID NO: 59); *Macaca mulatta*, "MACMU" (SEQ ID NO: 60); Gorilla gorilla, "GORILLA" (SEQ ID NO: 61); *Macaca fascicularis*, "CYNO" (SEQ ID NO: 62); *Papio Anubis*, "OLIVE BABOON" (SEQ ID NO: 63); *Pan paniscus*, "BONOBO" (SEQ ID NO: 64); *Pan troglodytes*, "CHIMP" (SEQ ID NO: 65); and EBVB9 (SEQ ID NO: 66).

[00283] **FIG. 43B** provides sequences of IL-10 variants generated by substituting one or more amino acids of human IL-10 by amino acids of viral IL-10 (EBVB9) at the corresponding positions. Also provided are sequences of the exemplary variants, possible huIL-10 hybrid #1 (SEQ ID NO: 67) and possible huIL-10 hybrid #2 (SEQ ID NO: 68). "*" indicates the one or more amino acid positions that are substituted. "#" indicates the preferred I105 to A105 amino acid substitution for IL-10 hybrid #2 (SEQ ID NO: 68).

[00284] **FIG. 43C** shows alignment of human IL-10 (SEQ ID NO: 1) with IL10 EBVB9 (SEQ ID NO: 66). "*"indicates the one or more amino acid positions that are substituted in IL-10 hybrid #1. "#"indicates the preferred I105 to A105 amino acid substitution for IL-10 hybrid #2.

[00285] The figures depict various embodiments of the present invention for purposes of illustration only. One skilled in the art will readily recognize from the following discussion that alternative embodiments of the structures and methods illustrated herein may be employed without departing from the principles of the invention described herein.

6. DETAILED DESCRIPTION

6.1. Definitions

[00286] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them below.

[00287] "**Graft-versus-leukemia effect**" or "**GvL**" refers to an effect that appears after allogeneic hematopoietic stem cell transplantation (HSCT) or bone marrow transplantation (BMT). T lymphocytes in the allogeneic graft eliminate malignant residual host leukemia cells.

[00288] "**Graft versus tumor effect**" or "**GvT** refers to an effect that appears after allogeneic hematopoietic stem cell transplantation (HSCT) or bone marrow transplantation (BMT). T lymphocytes in the allogeneic graft eliminate malignant residual host cancer cells, e.g., cells of myeloma and lymphoid and myeloid leukemias, lymphoma, multiple myeloma and possibly breast cancer. The term GvT is generic to GvL.

[00289] The terms "**treatment**", "**treating**", and the like are used herein in the broadest sense understood in the medical arts. In particular, the terms generally mean obtaining a desired pharmacologic and/or physiologic effect. "Treatment" as used herein covers any treatment of a disease or condition of a mammal, particularly a human, and includes: (a) preventing the disease or condition from occurring in a subject which may be predisposed to the disease or condition but has not yet been diagnosed as having it; (b) inhibiting the disease or condition (e.g., arresting its development); or (c) relieving the disease or condition (e.g., causing regression of the disease or condition, providing improvement in one or more symptoms). Improvements in any conditions can be readily assessed according to standard methods and techniques known in the art. The population of subjects treated by the method of the disease includes subjects suffering from the undesirable condition or disease, as well as subjects at risk for development of the condition or disease.

[00290] "**HLA-matched**" as used herein refers to a pair of individuals having a matching HLA allele in the HLA class I (HLA-A, HLA-B, and HLA-C) and class II (HLA-

DRB1 and HLA-DQB1) loci that allow the individuals to be immunologically compatible with each other. HLA compatibility can be determined using any of the methods available in the art, for example, as described in Tiervy, Haematologica 2016 Volume 101(6):680-687, which is incorporated by reference herein.

[00291] For a given locus, a pair of individuals have 2/2 match when each of two alleles of one individual match with the two alleles of the other individual. A pair of individuals have ½ match when only one of two alleles of one individual match with one of two alleles of the other individual. A pair of individuals have 10/10 match at the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci when all of the ten alleles (two for each of the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci) of one individual match with all ten alleles of the other individual.

[00292] In preferred embodiments, allele level typing is used for determination of HLA compatibility. Allele level typing corresponds to a unique nucleotide sequence for an HLA gene, as defined by using all digits in the first, second, third and fourth fields, e.g. A*02:01:01:01. Functionally, the third and fourth fields which characterize alleles that differ, respectively, by silent substitutions in the coding sequence and by substitutions in the non-coding sequence, are irrelevant, except when substitutions prevent the expression of HLA alleles (*e.g.*, the null allele B*15:01:01:02N). Missing a null allele will lead to a mismatch that is very likely to be recognized by alloreactive T cells and have a deleterious clinical impact. Substitutions in non-coding sequences may influence the level of expression (*e.g.,* the A24low allele A*24:02:01:02L). Such variability may also have an impact on anti-HLA allorecognition.

[00293] The term "**HLA-mismatched**" as used herein refers to a pair of individuals having a mis-matching HLA allele in the HLA class I (HLA-A, HLA-B, and HLA-C) and class II (HLA-DRB1 and HLA-DQB1) loci that make the individuals to be immunologically incompatible with each other.

[00294] The term "**partially HLA-mismatched**" as used herein refers to a pair of individuals having a mis-matching HLA allele in the HLA class I (HLA-A, HLA-B, and HLA-C) and class II (HLA-DRB1 and HLA-DQB1) loci that make the individuals to be immunologically incompatible with each other in a permissible degree. Some studies have identified permissive mismatches. Some HLA class I incompatibilities are considered to be more permissive.

[00295] "**HLA haplotype**" refers to a series of HLA loci-alleles by chromosome, one passed from the mother and one from the father. Genotypes for HLA class I (HLA-A, HLA-B, and HLA-C) and class II (HLA-DRB1 and HLA-DQB1) loci can be used to determine the HLA haplotype.

[00296] The term "**therapeutically effective amount**" is an amount that is effective to treat, and thus ameliorate a symptom of a disease.

[00297] The term "**prophylactically effective amount**" is an amount that is effect in terms of completely or partially preventing a disease, condition, or symptoms thereof.

[00298] The term "**ameliorating**" refers to any therapeutically beneficial result in the treatment of a disease state, e.g., a neurodegenerative disease state, including prophylaxis, lessening in the severity or progression, remission, or cure thereof.

[00299] The term "**$CD4^{IL\text{-}10/CAR}$**" refers to any $CD4^{IL\text{-}10}$ cell genetically modified to express a chimeric antigen receptor. In cases where $CD4^{IL\text{-}10/CAR}$ refers to a specific chimeric antigen receptor, nomenclature can include a designation of the specificity of the CAR, for example, "$CD4^{IL\text{-}10/anti\text{-}[antigen]\ CAR}$" cell. For example, $CD4^{IL\text{-}10/CAR}$ and $CD4^{IL\text{-}10/CD19\ CAR}$ can be used interchangeably.

[00300] The term "**autologous**" as used herein refers to cells derived from the same individual (e.g., patient) to whom the cells are re-introduced.

[00301] The term "**allogeneic**" as used herein refers to cells taken from two or more different individuals of the same species that are not genetically identical.

6.2. Other interpretational conventions

[00302] Ranges recited herein are understood to be shorthand for all of the values within the range, inclusive of the recited endpoints. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50.

6.3. $CD4^{IL\text{-}10/CAR}$ cells

[00303] In a first aspect, a $CD4^+$ T cell or a population of $CD4^+$ T cells are described that have been genetically modified to express a chimeric antigen receptor (CAR) from a first exogenous polynucleotide encoding the CAR and to express IL-10 from a second exogenous polynucleotide encoding IL-10 ($CD4^{IL\text{-}10/CAR}$ cells).

6.3.1. $CD4^+$ T cells and T cell donors

[00304] $CD4^+$ T cells used for generating $CD4^{IL\text{-}10/CAR}$ (autologous or allogeneic single-donor $CD4^{IL\text{-}10/CAR}$ or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$) populations can be isolated from peripheral blood, cord blood, or other blood samples from a donor, preferably a human donor, using methods available in the art. In typical embodiments, $CD4^+$ T cells are isolated from peripheral blood. In certain embodiments, $CD4^+$ T cell are isolated using leukapheresis and buffy coats. In certain embodiments, $CD4^+$ T cells are isolated from peripheral blood obtained from third parties.

[00305] In some embodiments, $CD4^+$ T cells are isolated from a prior-frozen stock of blood or a prior-frozen stock of peripheral blood mononuclear cells (PBMCs) or a prior-frozen stock of $CD4^+$ T cells. In some embodiments, $CD4^+$ T cells are isolated from peripheral blood or from PBMCs that have not previously been frozen. In some embodiments, the $CD4^+$ T cells are separately isolated from blood or PBMCs obtained from individual donors, and then pooled. In some embodiments, the $CD4^+$ T cells are isolated from blood or PBMCs that have first been pooled from a plurality of donors.

[00306] In some embodiments, the $CD4^+$ T cells are obtained from a single T cell donor. In some embodiments, the $CD4^+$ T cells are obtained from two, three, four, five, six, seven, eight, nine, or ten different T cell donors.

[00307] In some embodiments, the $CD4^+$ T cells are obtained from the patient who will be treated with the $CD4^{IL\text{-}10/CAR}$ cells.

[00308] In some embodiments, the one or more T cell donors are selected without regard to genotype. In some embodiments, the one or more T cell donors are selected based on genotype.

[00309] In certain embodiments, the one or more T cell donors are selected based on their HLA haplotypes.

[00310] In some embodiments, some or all of the at least two different T cell donors have matching HLA haplotypes. In some embodiments, some or all of the at least two different T cell donors have a mis-matched HLA haplotype.

[00311] In some embodiments, all of the $CD4^+$ T cells in the population have at least 1/10, 2/10, 3/10, 4/10, 5/10, 6/10, 7/10, 8/10, or 9/10 match at the HLA-A, HLA-B, HLA-C,

HLA-DRB1, and HLA-DQB1 loci to each other. In some embodiments, all of the $CD4^+$ T cells in the population have at least 1/8, 2/8, 3/8, 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci to each other. In some embodiments, all the $CD4^+$ T cells in the population have 2/2 match at the HLA-A locus to each other. In some embodiments, all the $CD4^+$ T cells in the population have 2/2 match at the HLA-B locus to each other. In some embodiments, all the $CD4^+$ T cells in the population have 2/2 match at the HLA-C locus to each other. In some embodiments, all the $CD4^+$ T cells in the population have at least 3/4 or 4/4 match at the HLA-DRB1 and HLA DQB1 loci with each other. In some embodiments, all the $CD4^+$ T cells in the population have an A*02 allele or A*24 allele.

[00312] In some embodiments, none of the one or more T cell donors is a host to be treated with the $CD4^{IL\text{-}10/CAR}$ cells. In some embodiments, none of the one or more T cell donors is a donor of stem cells (e.g., HSC), tissue or organ that will be used together with the $CD4^{IL\text{-}10/CAR}$ cells in the methods of treatment described herein. In some embodiments, one or more of the T cell donors is a host to be treated with the $CD4^{IL\text{-}10/CAR}$ cells.

[00313] In some embodiments, one or more of the T cell donors are HLA-mismatched or partially HLA-mismatched to the patient to be treated (host). In some embodiments, one or more of the T cell donors have less than 5/10, 6/10, 7/10, 8/10, 9/10 or 10/10 match at the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci to the patient. In some embodiments, one or more of the T cell donors have less than 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci to the patient. In some embodiments, one or more of the T cell donors have less than 2/2 match at the HLA-A, HLA-B, or HLA-C locus to the patient. In some embodiments, one or more of the T cell donors have less than 2/4, 3/4 or 4/4 match at the HLA-DRB1 and HLA-DQB1 loci to the patient.

[00314] In some embodiments, one or more of the T cell donors are HLA-mismatched or partially HLA-mismatched with the HSC donor. In some embodiments, one or more of the T cell donors have less than 5/10, 6/10, 7/10, 8/10, 9/10 or 10/10 match at the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci to the HSC donor. In some embodiments, one or more of the T cell donors have less than 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci to the HSC donor. In some embodiments, one or more of the T cell donors have less than 2/2 match at the HLA-A, HLA-B, or HLA-C locus to the HSC donor. In some embodiments, one or more of the T cell donors have less than 3/4 or 4/4 match at the HLA-DRB1 and HLA-DQB1 loci to the HSC donor.

[00315] In the preferred embodiments, none of the $CD4^+$ T cells are immortalized.

6.3.2. Exogenous polynucleotide encoding CAR

[00316] $CD4^{IL10/CAR}$ cells of the present disclosure are $CD4^+$ T cells that have been genetically modified to express comprise a first exogenous polynucleotide segment encoding a chimeric antigen receptor (CAR) from a first exogenous polynucleotide segment encoding the CAR. CARs have a modular design with four major components: an antigen- binding domain, a hinge, a transmembrane domain and an intracellular signaling domain. Each of these elements has a distinct function and, optimal molecular design of the CAR can be achieved through many variations of the constituent protein domains, as described in Rafiq et al. (*Nat. Rev. Clin. Onco.*, 17: 147–167 (2020), which is herein incorporated by reference in its entirety).

[00317] In some embodiments, the CAR encoding polynucleotide segment encodes a first-generation CAR, a second-generation CAR, or a third-generation CAR. In some embodiments, a first-generation CAR includes an antigen-binding domain, a hinge region, a transmembrane domain, and an intracellular signaling domain. In some embodiments, a second-generation CAR includes an antigen-binding domain, a hinge region, a transmembrane domain, a co-stimulatory domain, and an intracellular signaling domain. Non-limiting examples of second-generation CAR include those described in U.S. Patent Publication Nos. 2004/0043401, 2013/0287748, 2014/0227237, 2014/0099309, and 2014/0050708; WO 2012/079000; and WO 2015/157252, which are herein incorporated by reference in their entireties.

[00318] In some embodiments, a second-generation CAR is a pCAR. In some embodiments, a pCAR comprises a second generation CAR comprising: (i) (a) a signaling region; (b) a co-stimulatory signaling region; (c) a transmembrane domain; and (d) a first binding element that specifically interacts with a first epitope on a target antigen; and (ii) a chimeric co-stimulatory receptor (CCR) comprising (e) a co-stimulatory signaling region which is different from that of (b); (f) a transmembrane domain; and (g) a second binding element that specifically interacts with a second epitope on a second target antigen. Non-limiting examples of pCAR are as described in US Pat. No. 10,703,794, which is herein incorporated by reference in its entirety.

[00319] In some embodiments, a third-generation CAR comprises an antigen-binding domain, a hinge region, a transmembrane domain, a first co-stimulatory domain and a second co-stimulatory domain, and an intracellular signaling domain. Non-limiting examples of a third-

generation CAR include those described in U.S. Patent Publication Nos. 2014/0322275A1; 2019/0345217; 2019/0112380A1; and 2020/0031904A1; which are herein incorporated by reference in their entireties.

[00320] In some embodiments, the antigen binding domain comprises a single chain antibody fragment (e.g., an scFv), nanobody (e.g., camelid V_HH domains), cytokines, ligands, or a peptide (adenectins and DARPins).

6.3.2.1.Antigen-binding domain

[00321] In some embodiments, the antigen-binding domain of the CAR comprises a single chain antibody fragment. In some embodiments, the single chain antibody fragment comprises a single chain Fv (scFv).

[00322] In some embodiments, the antigen-binding domain targets an antigen associated with an autoimmune disease, inflammatory disorders, or cancer. In some embodiments, the antigen-binding domain targets an autoantigen.

[00323] In some embodiments, the antigen-binding domain targets an antigen associated with an autoimmune disease or inflammatory disorder. In some embodiments, an antigen associated with an autoimmune disease or inflammatory disorder is selected from the group consisting of but not limited to: anti-HLA-A*02, anti-HLA-A*24 or citrullinated peptides, insulin, MOG, GAD65, IA2, gliadin, and desmoglein in the context of relevant MHC molecules. In some embodiments, an antigen associated with an autoimmune disease or inflammatory disorder is selected from the group consisting of: CD19, CD20, CD22, CD27, BCMA and CD38.

[00324] In some embodiments, the antigen-binding domain targets a cancer-associated antigen. In some embodiments, a cancer-associated antigen is selected from the group consisting of: CD19, CD20, CD22, BCMA, B7-H3, CEA, BCMA, CD23, Lyml, Lym2, CLEC5A, CDH179b, FLT3, GCC, Muc, CSF2RA, GFRa4, CD32, CD33, IL1lRa, IL13Ra, NYBRI, SLea, CD200R, TGFBetaR2, CD276, TROP2, LAMP1, PTK7, DLL3, CDH1, CDH6, CDH17, CDH19, TSHR and tyrosinase.

[00325] In some embodiments, the antigen-binding domain targets a B cell antigen. In some embodiments, a B cell antigen is selected based in part on its expression during B-cell differentiation, for example, as shown in FIG. 4. Non-limiting examples of antigen-binding domains that target B cell antigens are described in WO 2020/010235, which is herein

incorporated by reference in its entirety. In some embodiments, the antigen-binding domain targets CD19. In some embodiments, the antigen-binding domain targets CD20. In some embodiments, the antigen-binding domain targets CD22. In some embodiments, the antigen-binding domain targets BCMA. In some embodiments, the antigen-binding domain targets B7-H3. In some embodiments, the antigen-binding domain targets CD27. In some embodiments, the antigen-binding domain targets CD38. In some embodiments, the antigen-binding domain targets B cell maturation antigen (BCMA). In some embodiments, the antigen-binding domain targets carcinoembryonic antigen (CEA).

[00326] In some embodiments, the single chain antibody fragment comprises a single chain Fv (scFv) that binds to CD19 (e.g., cluster of differentiation 19 protein (CD19) (e.g., OMIM Acc. No. 107265)).

[00327] In some embodiments, the scFv that targets CD19 is derived from an anti-human CD19-specific mAb clone FMC63 (Nicholson et al., *Mol. Immunol.*, 34(16-17): 1157-65 (1997), which is herein incorporated by reference in its entirety). In some embodiments, the scFv that targets CD19 is described in U.S. Patent Publication Nos. 2018/0355052 or 2020/0392200, or WO 2020/010235, each of which are herein incorporated by reference in their entireties.

[00328] In some embodiments, the anti-CD19 antigen binding domain comprises the sequence of SEQ ID NO: 11. In some embodiments, the anti-CD19 antigen-binding domain comprises a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 11. In some embodiments, the polynucleotide segment encoding an anti-CD19 antigen-binding domain comprises the SEQ ID NO: 12. In some embodiments, the polynucleotide segment encoding an anti-CD19 antigen-binding domain comprises a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 12.

[00329] In some embodiments, the anti-CD19 antigen-binding domain comprises a heavy chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 13. In some embodiments, the anti-CD19 antigen-binding domain comprises a light chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 14. In some embodiments, the anti-CD19 antigen-binding domain comprises a heavy chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to

SEQ ID NO: 13 and a light chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 14.

[00330] In some embodiments, the single chain antibody fragment comprises a single chain Fv (scFv) that binds to CD20 (e.g., cluster of differentiation 20 protein (CD20) (e.g., OMIM Acc. No. 112210)). Non-limiting examples of scFvs that target CD20 include those described in WO 2020/010235 and U.S. Patent Publication No. 2020/0392200, which are herein incorporated by reference in their entireties.

[00331] In some embodiments, the anti-CD20 antigen binding domain comprises the sequence of SEQ ID NO: 18. In some embodiments, the anti-CD20 antigen-binding domain comprises a sequence having at least 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 18. In some embodiments, the polynucleotide segment encoding an anti-CD20 antigen-binding domain comprises the SEQ ID NO: 19. In some embodiments, the polynucleotide segment encoding an anti-CD20 antigen-binding domain comprises a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 19.

[00332] In some embodiments, the anti-CD20 antigen-binding domain comprises a heavy chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 20. In some embodiments, the anti-CD20 antigen-binding domain comprises a light chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 21. In some embodiments, the anti-CD20 antigen-binding domain comprises a heavy chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 20 and a light chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 21.

[00333] In some embodiments, the single chain antibody fragment comprises a single chain Fv (scFv) that binds to CD22 (e.g., cluster of differentiation 22 protein (CD22) (e.g., OMIM Acc. No. 107266)). Non-limiting examples of scFvs that target CD22 include those described in WO 2020/010235 and U.S. Patent Publication No. 2020/0392200, which are herein incorporated by reference in their entireties.

[00334] In some embodiments, the anti-CD22 antigen binding domain comprises the sequence of SEQ ID NO: 24. In some embodiments, the anti-CD22 antigen-binding domain comprises a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 24. In some embodiments, the polynucleotide segment encoding an anti-CD22

antigen-binding domain comprises the SEQ ID NO: 25. In some embodiments, the polynucleotide segment encoding an anti-CD22 antigen-binding domain comprises a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 25.

[00335] In some embodiments, the anti-CD22 antigen-binding domain comprises a heavy chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 26. In some embodiments, the anti-CD22 antigen-binding domain comprises a light chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 27. In some embodiments, the anti-CD22 antigen-binding domain comprises a heavy chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 26 and a light chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 27.

[00336] In some embodiments, the single chain antibody fragment comprises a single chain Fv (scFv) that binds to B-cell maturation antigen (BCMA) also known as tumor necrosis factor receptor superfamily member 17 (TNFRSF17).

[00337] Non-limiting examples of scFvs that target BCMA include those described in US9765342B2 or WO 2010/104949 which are incorporated by reference in their entireties. For example, as described in WO 2010/104949, the anti-BCMA scFv can include the antigen binding domain of the A7D12.2, C11 D5.3, C12A3.2, or C13F12.1 antibody.

[00338] In some embodiments, the anti-BCMA antigen-binding domain comprises a heavy chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 50 or 52. In some embodiments, the anti-BCMA antigen-binding domain comprises a light chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 51 or 53. In some embodiments, the anti-BCMA antigen-binding domain comprises a heavy chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 50 or 52 and a light chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 51 or 53.

[00339] In some embodiments, the single chain antibody fragment comprises a single chain Fv (scFv) that binds to B7-H3, also known as CD276 (e.g., cluster of differentiation 276 protein (CD276) (e.g., OMIM Acc. No. 605715)).

[00340] Non-limiting examples of scFvs that target B7-H3 include those described in U.S. Patent Publication Nos. 2016/0053017 and 2018/0346544, which are herein incorporated by reference in their entireties. For example, as described in U.S. Patent Publication No. 2018/0346544, the anti-CD276 scFv can include the antigen binding domain of the MGA271 (CD276.MG), CD276.N1, CD276.N2, CD276.N3, CD276.N4, or CD276.N5 antibody.

[00341] In some embodiments, the anti- B7-H3 antigen binding domain comprises the sequence of SEQ ID NO: 36. In some embodiments, the anti- B7-H3 antigen-binding domain comprises a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 36. In some embodiments, the polynucleotide segment encoding an anti- B7-H3 antigen-binding domain comprises the SEQ ID NO: 37. In some embodiments, the polynucleotide segment encoding an anti- B7-H3 antigen-binding domain comprises a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 37.

[00342] In some embodiments, the anti- B7-H3 antigen-binding domain comprises a heavy chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 38. In some embodiments, the anti- B7-H3 antigen-binding domain comprises a light chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 39. In some embodiments, the anti- B7-H3 antigen-binding domain comprises a heavy chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 38 and a light chain variable domain comprising a sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 39.

[00343] In some embodiments, the single chain antibody fragment comprises a single chain Fv (scFv) that binds to CD27 (e.g., OMIM Acc. No. 186711).

[00344] In some embodiments, the single chain antibody fragment comprises a single chain Fv (scFv) that binds to CD38 (e.g., OMIM Acc. No. 107270)).

[00345] In some embodiments, the single chain antibody fragment comprises a single chain Fv (scFv) that binds to CEA (e.g., carcinoembryonic antigen protein (CEA) (e.g., OMIM Acc. No. 114890)).

[00346] In some embodiments, the single chain antibody fragment comprises a single chain Fv (scFv) that binds to BCMA (e.g., B cell maturation antigen (BCMA) (e.g., OMIM Acc. No. 109545)).

6.3.2.2.Hinge region

[00347] The hinge region connects the extracellular antigen-binding domain to the intracellular signaling domains (e.g., one or more co-stimulatory domains and the intracellular signaling domains) through the transmembrane domain. The hinge provides sufficient flexibility to overcome steric hindrance and adequate length to facilitate access and binding of the antigen-binding domain to the target antigen. In some embodiments, differences in the length and composition of the hinge can affect antigen binding and signaling through the CAR. For example, spacer sequences in the hinge region, or a spacer sequences added to the hinge region, facilitate access and binding of the antigen-binding domain to the target antigen. In some embodiments, the hinge region influences cytokine production.

[00348] In some embodiments, the hinge region is selected from a human CD8 hinge region, a human CD28 hinge region, a IgG1 hinge region, or a IgG4 hinge region. In some embodiments, the hinge region is derived from a human CD8. In some embodiments, the hinge region derived from human CD8 comprises the sequence of SEQ ID NO: 28. In some embodiments, the hinge region derived from human CD8 comprises a sequence having at least at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 28.

6.3.2.3. Transmembrane domain

[00349] The transmembrane region anchors the CAR in the $CD4^{IL10/CAR}$ T cell. In some embodiments, the transmembrane domain influences stability and function of the CAR.

[00350] In some embodiments, the transmembrane domain is selected from a group consisting of: TNFRSF 19 transmembrane domain, a CD3zeta transmembrane domain, a CD8α transmembrane domain, a CD4 transmembrane domain, a CD28 transmembrane domain, or an B7-family inducible costimulatory (ICOS) transmembrane domain. In some embodiments, the transmembrane domain is derived from a TNFRSF 19. In some embodiments, the transmembrane domain derived from TNFRSF 19 comprises a sequence of SEQ ID NO: 29. In some embodiments, the transmembrane domain derived from TNFRSF 19 comprises a sequence having at least at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 29.

6.3.2.4. Intracellular signaling domain and co-stimulatory domains

[00351] The intracellular signaling domain activates the $CD4^{IL10/CAR}$ T cell. In some embodiments, the intracellular signaling domain participates in T cell function, metabolism and persistence.

[00352] In some embodiments, the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM). In some embodiments, the intracellular signaling domain comprises two or more immunoreceptor tyrosine-based activation motifs (ITAM). In some embodiments, the immunoreceptor tyrosine-based activation motif (ITAM) is derived from CD3zeta. In some embodiments, the intracellular signaling domain derived from CD3zeta comprises a sequence of SEQ ID NO: 30 In some embodiments, the intracellular signaling domain derived from CD3zeta comprises a sequence having at least at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 30.

[00353] In some embodiments, the intracellular signaling domain further comprises one or more co-stimulatory domains. In such cases, the one or more co-stimulatory domains enhance T cell activation , function, metabolism, and persistence as compared to $CD4^+$ T cells comprising a CAR without one or more co-stimulatory domains. $CD4^+$ (e.g., $CD4^{IL10/CAR}$) T cells with CARs containing co- stimulatory domains in addition to activation domains produce IL-2 and can proliferate upon repeated antigen exposure.

[00354] In some embodiments, co-stimulatory domains derived from different sources (e.g., 4-1BB and CD28) induce different functional and metabolic profiles in $CD4^+$ (e.g., $CD4^{IL10/CAR}$) T cells. For example, $CD4^+$ (e.g., $CD4^{IL10/CAR}$) T cells having a CAR comprising a co-stimulatory domain derived from CD28 experience enhanced differentiation into effector memory T cells. In another example, $CD4^+$ (e.g., $CD4^{IL10/CAR}$) T cells having a CAR comprising a co-stimulatory domain derived from 4-1BB experience enhanced differentiation into central memory T cells.

[00355] In some embodiments, the one or more co-stimulatory domains is 4-1BB, CD28, OX40, ICOS, CD27, MYD88-CD40, and KIR2DS2.

[00356] In some embodiments, the one or more co-stimulatory domains is derived from 4-1BB. In some embodiments, the co-stimulatory domain derived from 4-1BB comprises a sequence of SEQ ID NO: 31. In some embodiments, the co-stimulatory domain derived from 4-

1BB comprises a sequence having at least at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 31.

[00357] In some embodiments, the one or more co-stimulatory domains is derived from CD28. In some embodiments, the one or more co-stimulatory domains comprises a CD28 co-stimulatory domain. In some embodiments, the co-stimulatory domain derived from CD28 comprises the sequence of SEQ ID NO: 32. In some embodiments, the co-stimulatory domain derived from CD28 comprises a sequence having at least at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 32.

[00358] In some embodiments, a CAR comprises two co-stimulatory domains. In some embodiments, a CAR includes a first co-stimulatory domain comprising a co-stimulatory domain derived from 4-1BB and a second co-stimulatory domain comprising a co-stimulatory domain derived from a CD28 co-stimulatory domain.

6.3.2.5.Chimeric antigen receptors (CARs)

[00359] In some embodiments, the CAR encoding polynucleotide segment encodes a CAR comprising an antigen-binding domain that targets an antigen associated with an autoimmune disease, inflammatory disorders, or cancer.

[00360] In some embodiments, the CAR encoding polynucleotide segment encodes a CAR comprising an antigen-binding domain that targets an antigen associated with an autoimmune disease or inflammatory disorder. In some embodiments, an antigen associated with an autoimmune disease or inflammatory disorder is selected from the group consisting of but not limited to: anti-HLA-A*02, anti-HLA-A*24 or citrullinated peptides, insulin, MOG, GAD65, IA2, gliadin, and desmoglein in the context of relevant MHC molecules.

[00361] In some embodiments, the CAR encoding polynucleotide segment encodes a CAR comprising an antigen-binding domain that targets a cancer-associated antigen. In some embodiments, a cancer-associated antigen is selected from the group consisting of: CD19, CD20, CD22, B7-H3, CEA, BCMA, CD23, Lyml, Lym2, CLEC5A, CDH179b, FLT3, GCC, Muc, CSF2RA, GFRa4, CD32, CD33, IL1lRa, IL13Ra, NYBRI, SLea, CD200R, TGFBetaR2, CD276, TROP2, LAMP1, PTK7, DLL3, CDH1, CDH6, CDH17, CDH19, TSHR and tyrosinase.

[00362] In some embodiments, the CAR encoding polynucleotide segment encodes a CAR comprising an antigen-binding domain that targets a B cell antigen. In some embodiments,

a B cell antigen is selected based in part on its expression during B-cell differentiation, for example, as shown in FIG. 4. Non-limiting examples of antigen-binding domains that target B cell antigens are described in WO 2020/010235, which is herein incorporated by reference in its entirety. In some embodiments, the antigen-binding domain targets CD19. In some embodiments, the antigen-binding domain targets CD20. In some embodiments, the antigen-binding domain targets CD22. In some embodiments, the antigen-binding domain targets B7-H3. In some embodiments, the antigen-binding domain targets CD27. In some embodiments, the antigen-binding domain targets CD38. In some embodiments, the antigen-binding domain targets B cell maturation antigen (BCMA). In some embodiments, the antigen-binding domain targets carcinoembryonic antigen (CEA).

[00363] In some embodiments, the CAR encoding polynucleotide segment encodes a CAR comprising an anti-CD19 antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a second-generation CAR comprising an anti-CD19 antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a third-generation CAR comprising an anti-CD19 antigen-binding domain.

[00364] In some embodiments, the CAR encoding polynucleotide segment encodes an anti-CD19 CAR including: an anti-CD19 antigen-binding domain, a hinge region, a transmembrane domain, a co-stimulatory domain, and an intracellular signaling domain. In some embodiments, the CAR encoding polynucleotide segment encodes an anti-CD19 antigen-binding domain; a human CD8 hinge region; a TNFRSF 19 transmembrane region; a 4-1BB co stimulatory domain; and a CD3zeta chain intracellular signaling domain. In some embodiments, the anti-CD19 CAR comprises the sequence of SEQ ID NO: 9. In some embodiments, the anti-CD19 CAR comprises a sequence having at least at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 9. In some embodiments, the first exogenous polynucleotide segment comprises the sequence of SEQ ID NO: 10. In some embodiments, the first exogenous polynucleotide segment comprises a sequence having at least at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 10.

[00365] Non-limiting examples of CARs comprising an anti-CD19 antigen-binding domain include: U.S. Patent Publication No. 2020/0392200, WO 2020/010235 or WO 2012/079000, which are herein incorporated by reference in their entireties.

[00366] In some embodiments, the CAR encoding polynucleotide segment encodes a CAR comprising an anti-CD20 antigen-binding domain. In some embodiments, the CAR

encoding polynucleotide segment encodes a second-generation CAR comprising an anti-CD20 antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a third-generation CAR comprising an anti-CD20 antigen-binding domain.

[00367] In some embodiments, the CAR encoding polynucleotide segment encodes an anti-CD20 CAR including: an anti-CD20 antigen-binding domain, a transmembrane domain, a co-stimulatory domain, and an intracellular signaling domain. In some embodiments, the CAR encoding polynucleotide segment encodes an anti-CD20 antigen-binding domain; a human CD8 transmembrane region; a 4-1BB co stimulatory domain; and a CD3zeta chain intracellular signaling domain. In some embodiments, the anti-CD20 CAR comprises the sequence of SEQ ID NO: 16. In some embodiments, the anti-CD20 CAR comprises a sequence having at least at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 16. In some embodiments, the first exogenous polynucleotide segment comprises the sequence of SEQ ID NO: 17. In some embodiments, the first exogenous polynucleotide segment comprises a sequence having at least at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 17.

[00368] Non-limiting examples of CARs comprising an anti-CD20 antigen-binding domain include: U.S. Patent Publication No. 2020/0392200 or WO 2020/010235, both of which are herein incorporated by reference in their entireties.

[00369] In some embodiments, the CAR encoding polynucleotide segment encodes a CAR comprising an anti-CD22 antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a second-generation CAR comprising an anti-CD22 antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a third-generation CAR comprising an anti-CD22 antigen-binding domain.

[00370] . In some embodiments, the anti-CD22 CAR comprises the sequence of SEQ ID NO: 22. In some embodiments, the anti-CD22CAR comprises a sequence having at least at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 22. In some embodiments, the first exogenous polynucleotide segment comprises the sequence of SEQ ID NO: 23. In some embodiments, the first exogenous polynucleotide segment comprises a sequence having at least at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 23.

[00371] Non-limiting examples of CARs that include an anti-CD22 CAR include those described in WO 2020/010235 and U.S. Patent Publication No. 2020/0392200, which are herein incorporated by reference in their entireties.

[00372] Non-limiting examples of CARs that include an anti-B7-H3 CAR include those described in U.S. Patent Publication Nos. 2016/0053017, 2017/0369585, and 2018/0346544, which are herein incorporated by reference in their entireties.

[00373] In some embodiments, the CAR encoding polynucleotide segment encodes a CAR comprising an anti- B7-H3 antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a second-generation CAR comprising an anti- B7-H3 antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a third-generation CAR comprising an anti- B7-H3 antigen-binding domain.

[00374] . In some embodiments, the anti-B7-H3 CAR comprises the sequence of SEQ ID NO: 34. In some embodiments, the anti- B7-H3 CAR comprises a sequence having at least at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 34. In some embodiments, the first exogenous polynucleotide segment comprises the sequence of SEQ ID NO: 35. In some embodiments, the first exogenous polynucleotide segment comprises a sequence having at least at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 35.

[00375] In some embodiments, the CAR encoding polynucleotide segment encodes a CAR comprising an anti-CD27 antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a second-generation CAR comprising an anti-CD27 antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a third-generation CAR comprising an anti-CD27 antigen-binding domain.

[00376] In some embodiments, the CAR encoding polynucleotide segment encodes a CAR comprising an anti-CD38 antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a second-generation CAR comprising an anti-CD38 antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a third-generation CAR comprising an anti-CD38antigen-binding domain.

[00377] In some embodiments, the CAR encoding polynucleotide segment encodes a CAR comprising an anti-CEA antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a second-generation CAR comprising an anti-CEA

antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a third-generation CAR comprising an anti-CEA antigen-binding domain.

[00378] In some embodiments, the CAR encoding polynucleotide segment encodes a CAR comprising an anti-BCMA antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a second-generation CAR comprising an anti-BCMA antigen-binding domain. In some embodiments, the CAR encoding polynucleotide segment encodes a third-generation CAR comprising an anti-BCMA antigen-binding domain. In some embodiments, the anti-BCMA CAR comprises the sequence of SEQ ID NOs: 41-49 and 54. In some embodiments, the anti-BCMA CAR comprises a sequence having at least at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NOs: 41-49 and 54.

[00379] The first exogenous polynucleotide further comprises one or more regulatory elements (e.g., any of the regulatory elements described herein or known in the art) operably linked to a coding sequence of the CAR. In some embodiments, the regulatory element is a promoter.

[00380] In some embodiments, the regulatory element comprises a promoter capable of directing expression of the CAR in $CD4^+$ T cells. In some embodiments, the promoter drives constitutive expression of the CAR in $CD4^+$ T cells. In some embodiments, the promoter drives expression of the CAR in activated $CD4^+$ T cells.

[00381] In some embodiments, the first exogenous polynucleotide segment further comprises a segment encoding a selection marker (e.g., any of the selection markers described herein or known in the art) that permits selection of successfully transduced $CD4^+$ T cells.

[00382] In some embodiments, the first exogenous polynucleotide segment is delivered into $CD4^+$ T cells using a vector. In some embodiments, the vector is a plasmid vector. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a retroviral vector (e.g., a gammaretroviral vector). In some embodiments, the vector is a lentiviral vector.

[00383] In some embodiments, the first exogenous polynucleotide segment is delivered into $CD4^+$ T cells using a lentiviral vector and the first exogenous polynucleotide segment comprises lentiviral vector sequences. In certain embodiments, a lentiviral vector disclosed in Mátrai *et al.*, *Molecular Therapy* 18(3):477–490 (2010) ("Mátrai"), incorporated by reference herein, is used. In some embodiments, the first exogenous polynucleotide segment encoding a chimeric antigen receptor (CAR); and the second exogenous polynucleotide segment encoding

interleukin-10 (IL-10) are present in a single lentiviral vector. In such cases, the first exogenous polynucleotide and second exogenous polynucleotide segment are operably linked to a first promoter. For example, the lentiviral vector includes a human PGK promoter, the first exogenous polynucleotide operably linked to the second exogenous polynucleotide segment via an internal ribosome entry site (IRES) or a self-cleaving peptide (e.g., a 2A self-cleaving peptide).

[00384] In some embodiments, the first exogenous polynucleotide segment is integrated in the T cell nuclear genome. In some embodiments, the first exogenous polynucleotide segment is not integrated in the nuclear genome. In some embodiments, the first exogenous segment polynucleotide exists in the T cell cytoplasm.

6.3.3. Exogenous polynucleotide encoding IL-10

[00385] The single donor $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells of the present disclosure have been further genetically modified to comprise a second exogenous polynucleotide segment encoding IL-10. The second exogenous polynucleotide segment comprises an IL-10-encoding polynucleotide segment operably linked to one or more regulatory elements (e.g., any of the one or more regulatory elements described herein or known in the art).

[00386] The IL-10-encoding polynucleotide segment can encode IL-10 of a human, bonobo or rhesus. In some embodiments, the IL-10-encoding polynucleotide segment encodes human IL-10 having the sequence of SEQ ID NO: 1. In some embodiments, the IL-10-encoding polynucleotide segment encodes a variant of human IL-10 having at least 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 1. In some embodiments, the IL-10-encoding polynucleotide segment has the nucleotide sequence of SEQ ID NO: 2. In some embodiments, the IL-10-encoding polynucleotide segment has at least 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 2.

[00387] In some embodiments, the exogenous polynucleotide encodes viral-IL-10. In various embodiments, the exogenous polypeptide encodes IL-10 from HCMV, GMCMV, RhCMV, BaCMV, MOCMV, SMCMV, EBV, Bonobo-HV, BaLCV, OvHV-2, EHV-2, CyHV-3, AngHV-1, ORFV, BPSV, PCPV, LSDV, SPV, GPV, or CNPV. In some embodiments, the exogenous polypeptide encodes viral IL-10 from EBV or ORFV.

[00388] In some embodiments, the viral-IL-10 comprises the sequence of SEQ ID NO: 6. In some embodiments, the viral IL-10 comprises a sequence having at least 80%, 85%, 90%,

95%, 97%, 98%, 98% or 99% sequence identity to SEQ ID NO: 6 or 18. In some embodiments, the exogenous polypeptide coding a viral IL-10 comprises the sequence of SEQ ID NO: 7. In some embodiments, the exogenous polypeptide coding a viral IL-10 comprises a sequence having at least 80%, 85%, 90%, 95%, 97%, 98%, 98% or 99% sequence identity to SEQ ID NO: 7.

[00389] In some embodiments, the IL-10 is a protein comprising human IL-10 with one, two, three, four, five, six, seven, eight, nine or ten amino acid modifications. In some embodiments, the one, two, three, four, five, six, seven, eight, nine or ten amino acid modifications are substituted with amino acids of viral IL-10 at corresponding amino acid positions.

[00390] In some embodiments, the IL-10-encoding polynucleotide segment encodes a variant of human IL-10 having one, two, three, four, five, six, seven, eight, nine, ten or more amino acid substitutions compared to human IL-10 (e.g., SEQ ID NO: 1). In some embodiments, the one, two, three, four, five, six, seven, eight, nine or ten amino acid substitution are substitution(s) with amino acid(s) of viral IL-10 at corresponding amino acid position(s). In some embodiments, the IL-10-encoding polynucleotide segment encodes a variant of human IL-10 having one, two, three, four, five, six, seven, eight, nine, ten or more amino acid insertion, deletion or modification compared to human IL-10 (e.g., SEQ ID NO: 1). In some embodiments, the variant of human IL-10 has the sequence of SEQ ID NO: 56 or 57.

[00391] In some embodiments, the IL-10-encoding polynucleotide segment encodes IL-10 of a *Mus musculus*, "MOUSE" (SEQ ID NO: 58); *Rattus norvegicus*, "RAT" (SEQ ID NO: 59); *Macaca mulatta*, "MACMU" (SEQ ID NO: 60); *Gorilla gorilla*, "GORILLA" (SEQ ID NO: 61); *Macaca fascicularis*, "CYNO" (SEQ ID NO: 62); *Papio Anubis*, "OLIVE BABOON" (SEQ ID NO: 63); *Pan paniscus*, "BONOBO" (SEQ ID NO: 64); *Pan troglodytes*, "CHIMP" (SEQ ID NO: 65); and EBVB9 (SEQ ID NO: 66). In some embodiments, the IL-10-encoding polynucleotide segment encodes a protein having at least 90%, 95%, 98%, or 99% sequence identity to IL-10 of a *Mus musculus*, "MOUSE" (SEQ ID NO: 58); *Rattus norvegicus*, "RAT" (SEQ ID NO: 59); *Macaca mulatta*, "MACMU" (SEQ ID NO: 60); *Gorilla gorilla*, "GORILLA" (SEQ ID NO: 61); *Macaca fascicularis*, "CYNO" (SEQ ID NO: 62); *Papio Anubis*, "OLIVE BABOON" (SEQ ID NO: 63); *Pan paniscus*, "BONOBO" (SEQ ID NO: 64); *Pan troglodytes*, "CHIMP" (SEQ ID NO: 65); and EBVB9 (SEQ ID NO: 66).

[00392] In some embodiments, the IL-10-encoding polynucleotide segment encodes a variant of human IL-10 having one, two, three, four, five, six, seven, eight, nine, ten or more amino acid substitutions, insertions, and/or deletions compared to human IL-10 (e.g., SEQ ID NO: 1). In some embodiments, the modifications are substitutions, insertions, and/or deletions with amino acids of *Mus musculus*, "MOUSE" (SEQ ID NO: 58); *Rattus norvegicus*, "RAT" (SEQ ID NO: 59); *Macaca mulatta*, "MACMU" (SEQ ID NO: 60); *Gorilla gorilla*, "GORILLA" (SEQ ID NO: 61); *Macaca fascicularis*, "CYNO" (SEQ ID NO: 62); *Papio Anubis*, "OLIVE BABOON" (SEQ ID NO: 63); *Pan paniscus*, "BONOBO" (SEQ ID NO: 64); *Pan troglodytes*, "CHIMP" (SEQ ID NO: 65); and EBVB9 (SEQ ID NO: 66), at the corresponding positions. In some embodiments, the variant of human IL-10 has the sequence of SEQ ID NO: 67 or SEQ ID NO: 68.

[00393] In some embodiments, the IL-10-encoding polynucleotide segment encodes a variant of human IL-10 having reduced immunostimulatory activity compared to human IL-10. In some embodiments, the variant of human IL-10 includes I105A substitution. In some embodiments, a variant of human IL-10 is made using the method described in A Single Amino Acid Determines the Immunostimulatory Activity of Interleukin 10, J Exp Med, 191, 2, 2000, p.213-223.

[00394] The second exogenous polynucleotide segment further comprises one or more regulatory element operably linked to a coding sequence of IL-10 where the one or more regulatory element directs expression of the encoded IL-10 in transduced $CD4^+$ T cells.

[00395] In some embodiments, the regulatory element(s) comprise a promoter capable of directing expression of IL-10 in $CD4^+$ T cells. In some embodiments, the promoter drives constitutive expression of IL-10 in $CD4^+$ T cells. In some embodiments, the promoter drives expression of IL-10 in activated $CD4^+$ T cells.

[00396] In some embodiments, the second exogenous polynucleotide segment further comprises a segment encoding a selection marker (e.g., any of the selection markers described herein or known in the art) that permits selection of successfully transduced $CD4^+$ T cells.

[00397] In typical embodiments, the exogenous polynucleotide is delivered into CD4+ T cells using a vector. In some embodiments, the vector is a plasmid vector. In some embodiments, the vector is a viral vector.

[00398] In some embodiments, the first exogenous polynucleotide segment encoding CAR and the second exogenous polynucleotide segment encoding IL-10 are in the same vector. In some embodiments, the first exogenous polynucleotide segment encoding CAR and the second exogenous polynucleotide segment encoding IL-10 are in the same viral vector. In some embodiments, the first exogenous polynucleotide segment encoding CAR and the second exogenous polynucleotide segment encoding IL-10 are in the same lentiviral vector.

[00399] In certain embodiments, the exogenous polynucleotide is delivered into CD4+ T cells using a lentiviral vector and the exogenous polynucleotide comprises lentiviral vector sequences. In certain embodiments, a lentiviral vector disclosed in Mátrai *et al.*, *Molecular Therapy* 18(3):477–490 (2010) ("Mátrai"), incorporated by reference herein, is used.

[00400] In some embodiments, the second exogenous polynucleotide segment is integrated in the T cell nuclear genome. In some embodiments, the second exogenous polynucleotide segment is not integrated in the nuclear genome. In some embodiments, the second exogenous polynucleotide segment exists in the T cell cytoplasm.

[00401] In particular embodiments, the second exogenous polynucleotide segment has the sequence of SEQ ID NO: 5. In some embodiments, the exogenous polynucleotide has at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 5.

6.3.4. Regulatory elements

[00402] In some embodiments, the first and/or second exogenous polynucleotide segments further comprise a regulatory element operably linked to a coding sequence of a CAR or IL-10, respectively. In some embodiments, the regulatory element comprises a promoter sequence, an enhancer sequence, a non-coding sequence, or any combination thereof.

[00403] In some embodiments, the first exogenous polynucleotide segment further comprises a regulatory element operably linked to a coding sequence of the CAR where the regulatory elements direct expression of the encoded CAR in transduced $CD4^+$ T cells. In some embodiments, an inducible promoter is used to induce expression of the CAR when therapeutically appropriate.

[00404] In some embodiments, the second exogenous polynucleotide segment further comprises a regulatory element operably linked to a coding sequence of IL-10 where the regulatory elements direct expression of the encoded IL-10 in transduced $CD4^+$ T cells. In some

embodiments, an inducible promoter is used to induce expression of IL-10 when therapeutically appropriate. In some embodiments, the IL-10 promoter is used.

[00405] In some embodiments, the promoter is a tissue-specific promoter. In some embodiments, the promoter is a lineage-specific promoter. In some embodiments, the promoter is promoter that can be used to drive ubiquitous expression of the CAR or IL-10.

[00406] In some embodiments, the promoter is a native human promoter. In some embodiments, the promoter is a human elongation factor (EF)1α promoter. In some embodiments, the promoter is a human phosphoglycerate kinase promoter (PGK). In some embodiments, the promoter is a human ubiquitin C promoter (UBI-C).

[00407] In some embodiments, the promoter is a synthetic promoter. In certain embodiments, the promoter is a minimal CMV core promoter. In particular embodiments, the promoter is an inducible or constitutive bidirectional promoter. In specific embodiments, the synthetic bidirectional promoter disclosed in Amendola et al., *Nature Biotechnology*, 23(1):108-116 (2005) is used. This promoter can mediate coordinated transcription of two mRNAs in a ubiquitous or a tissue-specific manner. In certain embodiments, the bidirectional promoter induces expression of CAR and a selection marker or IL-10 and a selection marker.

[00408] In some embodiments, the promoter is a native human promoter. In some embodiments, the promoter is a human elongation factor (EF)1α promoter. In some embodiments, the promoter is a human phosphoglycerate kinase promoter (PGK). In some embodiments, the promoter is a human ubiquitin C promoter (UBI-C).

[00409] In some embodiments, the first and/or second exogenous polynucleotide segment further comprises one or more non-coding sequences 3' to the coding sequence. Non-limiting examples of non-coding sequences 3' to the coding sequence include 3'untranslated region (UTR), a poly(A) signal and a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE).

[00410] In some embodiments, an exogenous polynucleotide comprises more than one coding sequence. For example, an exogenous polynucleotide includes the first exogenous polynucleotide segment and the second exogenous polynucleotide segment on a single contiguous polynucleotide sequence (e.g., a single polynucleotide construct). In some embodiments, the one or more coding sequences include a sequence encoding a CAR and a sequence encoding a selection marker. In some embodiments, the one or more coding sequences

include a sequence encoding IL-10 and a sequence encoding a selection marker. In some embodiments, the one or more coding sequences include a sequence encoding a CAR, a sequence encoding IL-10, and one or more sequences encoding a selection marker. In some embodiments, the exogenous polynucleotide is free of a selection marker.

[00411] In some embodiments, the multiple coding sequences are separated by one or more internal ribosome entry site (IRES).

[00412] In some embodiments, the multiple coding sequences are separated by one or more self-cleaving peptides. In some embodiments, the self-cleaving peptides can be 2A self-cleaving peptides. Non-limiting examples of self-cleaving peptides include 2A peptides (18-22 amino acids), including a peptide from foot-and-mouth disease virus (F2A), porcine teschovirus-1 (P2A), Thosea asigna virus (T2A), or equine rhinitis A virus (E2A). In some embodiments, the first exogenous polynucleotide, the second exogenous polynucleotide, or both, comprise a sequence encoding a Furin P2A peptide. In some embodiments, the first exogenous polynucleotide, the second exogenous polynucleotide, or both, comprise a sequence encoding a T2A peptide. In some embodiments, the first exogenous polynucleotide, the second exogenous polynucleotide, or both, comprise a sequence encoding an E2A peptide.

[00413] In some embodiments, the coding sequence further comprises a self-cleaving peptide between the coding sequence of the heavy chain and the coding sequence of the light chain. In some embodiments, the self-cleaving peptide is selected from the group consisting of F2A, P2A, T2A and E2A.

6.3.5. Selection markers

[00414] In some embodiments, the first exogenous polynucleotide segment, the second exogenous nucleotide segment, or both, further comprise a polynucleotide segment encoding a selection marker that permits selection of successfully transduced $CD4^+$ T cells. In some embodiments, the first exogenous polynucleotide segment, the second exogenous nucleotide segment, or both, are free of a selection marker.

[00415] In some embodiments, an exogenous polynucleotide comprises the first exogenous polynucleotide segment and the second exogenous polynucleotide segment are on a single contiguous polynucleotide sequence (e.g., a single polynucleotide construct). In such cases, the exogenous polynucleotide includes one or more selection makers that permits selection of successfully transduced $CD4^+$ T cells.

[00416] In some embodiments, the selection marker is ΔNGFR. In certain embodiments, the selection marker is a polypeptide having the sequence of SEQ ID NO: 3. In certain embodiments, the selection marker is a polypeptide having at least 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 3. In particular embodiments, the nucleotide sequence encoding the ΔNGFR selection marker has the sequence of SEQ ID NO: 4. In some embodiments, the nucleotide sequence encoding the ΔNGFR selection marker has at least 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 4.

[00417] In some embodiments, expression of the selection marker correlates with expression of IL-10 from the exogenous polynucleotide. In some embodiments, expression of the selection marker linearly correlates with expression of IL-10 from the exogenous polynucleotide. Accordingly, in some embodiments, expression of the selection marker is measured to infer expression of IL-10 from the exogenous polynucleotide.

[00418] In some embodiments, the selection marker is a truncated form of EGFR polypeptide. In some embodiments, the selection marker is a truncated form of the human EGFR polypeptide, optionally huEGFR disclosed in Wang et al. "A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells", *Blood*, v. 118, n. 5 (2011), incorporated by reference in its entirety herein.

[00419] In some embodiments, the exogenous polynucleotide further comprises a sequence encoding an antibiotic resistance gene. In some embodiments, the exogenous polynucleotide comprises a sequence encoding an ampicillin resistance gene.

6.3.6. Gene expression of $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$

[00420] $CD4^{IL\text{-}10/CAR}$ cells express IL-10. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells constitutively express IL-10. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells express IL-10 when activated.

[00421] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells constitutively express at least 100 pg of IL-10 per 10^6 of the $CD4^+$ T cells/mL of culture. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells constitutively express at least 200pg, 500pg, 1ng, 5ng, 10ng, or 50ng of IL-10 per 10^6 of the $CD4^+$ T cells/mL of culture.

[00422] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express at least 1ng or 2ng IL-10 per

10^6 of the $CD4^+$ T cells/mL of culture after activation with a combination of anti-CD3 and anti-CD28 antibodies, or anti-CD3 antibody and anti-CD28 antibody coated beads. In some embodiments, polydonor $CD4^{IL\text{-}10}$ T cells express at least 5ng, 10ng, 100ng, 200ng, or 500ng IL-10 per 10^6 of the $CD4^+$ T cells/mL of culture after activation with anti-CD3 and anti-CD28 antibodies or CD3 antibody and CD28 antibody coated beads.

[00423] In various embodiments, the amount of IL-10 production is determined 12 hours, 24 hours, or 48 hours after activation using various methods for protein detection and measurement, such as ELISA, real-time polymerase chain reaction (PCR), spectroscopic procedures, colorimetry, amino acid analysis, radiolabeling, Edman degradation, HPLC, western blotting, etc. In preferred embodiments, the amount of IL-10 production is determined by ELISA 48 hours after activation with anti-CD3 and anti-CD28 antibodies.

[00424] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express IL-10 at a level at least 5-fold higher than unmodified $CD4^+$ T cells. In some embodiments, polydonor $CD4^{IL\text{-}10}$ T cells express IL-10 at a level at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 30, 40, or 50-fold higher than unmodified $CD4^+$ T cells.

[00425] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express at least 100 pg IL-5 per 10^6 of the $CD4^+$ T cells/mL. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express at least 100 pg, 200 pg, 500pg, 1ng, 5ng, 10ng, or 50ng IL-5 per 10^6 of the $CD4^+$ T cells/mL.

[00426] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express at least 1ng IL-5 per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express at least 2ng, 5ng, 10ng, 100ng, 200ng, or 500ng IL-5 per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies.

[00427] In various embodiments, the amount of IL-5 production is determined 12 hours, 24 hours, or 48 hours after activation using various methods for protein detection and measurement, such as ELISA, real-time polymerase chain reaction (PCR), spectroscopic procedures, colorimetry, amino acid analysis, radiolabeling, Edman degradation, HPLC, western

blotting, etc. In preferred embodiments, the amount of IL-5 production is determined by ELISA 48 hours after activation with anti-CD3 and anti-CD28 antibodies.

[00428] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express at least 100 pg IFN-γ per 10^6 of the $CD4^+$ T cells/mL. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express at least 100 pg, 200 pg, 500pg, 1ng, 5ng, 10ng, or 50ng IFN-γ per 10^6 of the $CD4^+$ T cells/mL.

[00429] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express at least 1ng I IFN-γ per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express at least 2ng, 5ng, 10ng, 100ng, 200ng, or 500ng IFN-γ per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies.

[00430] In various embodiments, the amount of IFN-γ production is determined 12 hours, 24 hours, or 48 hours after activation using various methods for protein detection and measurement, such as ELISA, real-time polymerase chain reaction (PCR), spectroscopic procedures, colorimetry, amino acid analysis, radiolabeling, Edman degradation, HPLC, western blotting, etc. In preferred embodiments, the amount of IFN-γ production is determined by ELISA 48 hours after activation with anti-CD3 and anti-CD28 antibodies.

[00431] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express at least 25 pg IL-4 per 10^6 of the $CD4^+$ T cells/mL. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express at least 25 pg, 50 pg, 75 pg, 100 pg, 200 pg, 500pg, 1ng, 5ng, 10ng, or 50ng IL-4 per 10^6 of the $CD4^+$ T cells/mL.

[00432] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express at least 100 pg IL-4 per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express at least 100 pg, 200 pg, 300 pg, 400 pg, 500 pg, 600 pg, 700 pg, 800 pg, 900 pg, 1000 pg, 2ng, 5ng, 10ng, 100ng, 200ng, or 500ng IL-4 per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies.

[00433] In various embodiments, the amount of IL-4 production is determined 12 hours, 24 hours, or 48 hours after activation using various methods for protein detection and measurement, such as ELISA, real-time polymerase chain reaction (PCR), spectroscopic procedures, colorimetry, amino acid analysis, radiolabeling, Edman degradation, HPLC, western blotting, etc. In preferred embodiments, the amount of IL-4 production is determined by ELISA 48 hours after activation with anti-CD3 and anti-CD28 antibodies.

[00434] In some embodiments, the expression one or more of IL-10, IL-4, IFN-γ, IL-22, and IL-5 is stable after one or more re-stimulations.

[00435] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells further express a selection marker. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells express a protein typically expressed in Tr1 cells. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells express a marker protein characteristic of Tr1 cells.

[00436] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells express CD49b. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells express LAG-3. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells express TGF-β. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells express IFN-γ. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells express GzB. In some embodiments, polydonor $CD4^{IL\text{-}10}$ cells release GzB when activated with myeloid antigen-presenting cells. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells express perforin. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells release perforin when activated with myeloid antigen-presenting cells. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells express CD18. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells express CD2. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells express CD226. In some embodiments, polydonor $CD4^{IL\text{-}10}$ cells express IL-22. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells express IL-10.

[00437] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) or polydonor $CD4^{IL\text{-}10/CAR}$ cells exhibit at least one function of Tr1 cells. In various embodiments, the function is secretion of IL-10, secretion of TGF-β, and by the specific killing of myeloid antigen-presenting cells through the release of Granzyme B (GzB) and perforin.

6.3.7. Additional properties of $CD4^{IL\text{-}10/CAR}$ cells

[00438] In some embodiments, a $CD4^{IL\text{-}10/CAR}$ cell comprises an anti-CD19 CAR. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cell is capable of in vitro cytotoxicity to a $CD19^+$ target cell. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cell is capable of in vivo cytotoxicity to a $CD19^+$ target cell. In some embodiments, the $CD19^+$ target cell is an autoantibody-producing B cell. In some embodiments, the $CD19^+$ target cell is a $CD19^+$ cancer cell. In some embodiments, the cytotoxicity to a $CD19^+$ target cell is maintained after one or more restimulations (see, e.g., production methods described in FIGs. 5A-5C).

[00439] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cell is capable of in vitro cytotoxicity to a myeloid target cell. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cell is capable of in vivo cytotoxicity to a myeloid target cell. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cell is capable of cytotoxicity to a $CD19^+$ target cell and a myeloid target cell. In some embodiments, the myeloid target cell expressing one or more of Class I MHC, CD13, CD54 and CD112. In some embodiments, the cytotoxicity to a myeloid target cell is maintained after one or more restimulations (see, e.g., production methods described in FIGs. 5A-5C).

[00440] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cell is capable of suppressing allogeneic $CD4^+$ T cell proliferation. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cell is capable of suppressing allogeneic $CD4^+$ T cell proliferation by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%.

[00441] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cell is capable of suppressing allogeneic $CD8^+$ T cell proliferation. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cell is capable of suppressing allogeneic $CD8^+$ T cell proliferation by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%.

[00442] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cell is capable of suppressing allogeneic $CD4^+$ T cell proliferation and allogenic $CD8^+$ T cell proliferation. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cell is capable of suppressing allogeneic $CD4^+$ T cell proliferation by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% and suppressing allogeneic $CD8^+$ T cell proliferation by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%.

[00443] In some embodiments, suppression mediated by $CD4^{IL\text{-}10/CAR}$ cells is calculated as follows: 100-([proliferation of responders in the presence of $CD4^{IL\text{-}10}$ T cells (control) and $CD4^{IL\text{-}10/CAR}$ T cells / proliferation of responders] x 100).

[00444] In some embodiments, the suppressive property is stable after one or more restimulations.

6.3.8. Product by process

[00445] In some embodiments $CD4^{IL\text{-}10/CAR}$ T cells (autologous or allogeneic single-donor or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells) are obtained by modifying $CD4^+$ T cells with an exogenous polynucleotide segment encoding a CAR and an exogenous polynucleotide segment encoding IL-10. In some embodiments, modifying $CD4^+$ T cells with a first exogenous polynucleotide segment encoding a CAR and a second exogenous polynucleotide segment encoding IL-10 comprises: (i) a first transduction with the polynucleotide segment encoding IL-10 followed by a second transduction with the exogenous polynucleotide segment encoding the CAR; (ii) a first transduction with the polynucleotide segment encoding the CAR followed by a second transduction with the exogenous polynucleotide segment encoding IL-10; (iii) simultaneous transduction (i.e., co-transduction) of the polynucleotide segment encoding IL-10 and the exogenous polynucleotide segment encoding the CAR; or (iv) transduction of a single polynucleotide construct comprising both the polynucleotide segment encoding IL-10 and the polynucleotide segment encoding the CAR. In some embodiments, the polynucleotide encoding IL-10 further comprises a coding sequence of a marker protein (e.g., deltaNGFR).

[00446] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells) are obtained by modifying $CD4^+$ T cells with (i) a first polynucleotide construct comprising a polynucleotide segment encoding a CAR and second polynucleotide construct comprising a polynucleotide segment encoding IL-10 or (ii) a single polynucleotide construct comprising a polynucleotide segment encoding a CAR and a polynucleotide segment encoding IL-10.

[00447] In some embodiments, the first exogenous polynucleotide segment encoding a CAR, the second exogenous polynucleotide segment encoding IL-10, or a polynucleotide construct comprising the first and second exogenous polynucleotide segments is introduced to $CD4^+$ T cells by a viral vector or a plasmid vector. In some embodiments, $CD4^+$ T cells are transduced with a first viral vector (e.g., any of the viral vectors described herein) containing the

polynucleotide segment encoding IL-10 followed by a second transduction with a second viral vector (e.g., any of the viral vectors described herein) containing the polynucleotide segment encoding a CAR. In another embodiment, $CD4^+$ T cells are co-transduced with a first viral vector (e.g., any of the viral vectors described herein) containing the first exogenous polynucleotide segment and a second viral vector containing the second exogenous polynucleotide segment. In yet another embodiment, $CD4^+$ T cells are transduced with a viral vector (e.g., any of the viral vectors described herein) containing both the first and second exogenous polynucleotide segments.

[00448] In some embodiments, polydonor $CD4^{IL\text{-}10/CAR}$ are generated by (i) pooling primary $CD4^+$ T cells obtained from at least two different T cell donors; and (ii) modifying the pooled $CD4^+$ T cells according to the methods described herein. In some embodiments, polydonor $CD4^{IL\text{-}10/CAR}$ T cells are generated by (i) obtaining primary $CD4^+$ T cells from at least two different T cell donors; (ii) separately modifying each donor's $CD4^+$ T cells according to the methods described herein, and then (iii) pooling the genetically modified $CD4^+$ T cells.

[00449] In some embodiments, $CD4^{IL\text{-}10/CAR}$ T cells have been cultured in the presence of proteins capable of activating $CD4^+$ T cells. In some embodiments, $CD4^{IL\text{-}10/CAR}$ T cells have been cultured in the presence of anti-CD3 antibody and anti-CD28 antibody, or anti-CD3 antibody and anti-CD28 antibody coated beads. In some embodiments, $CD4^{IL\text{-}10/CAR}$ T cells have been cultured in the presence of anti-CD3 antibodies, anti-CD28 antibodies, and IL-2, or anti-CD3 antibody and anti-CD28 antibody coated beads and IL-2. In some embodiments, $CD4^{IL\text{-}10/CAR}$ T cells have been cultured in the presence of a polymeric nanomatrix reagent to activate and expand human T cells via CD3 and CD28. In some embodiments, $CD4^{IL\text{-}10/CAR}$ T cells have been cultured in the presence of other T-cell specific immune cell culture media, activators, and supplements.

[00450] In some embodiments, $CD4^{IL\text{-}10/CAR}$ (single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ T cells) are in a frozen stock (e.g., frozen suspension). In some embodiments, $CD4^{IL\text{-}10/CAR}$ (single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ T cells) are in a liquid suspension.

6.4. Population of $CD4^{IL10/CAR}$ T cells

[00451] In another aspect, the disclosure features a population of $CD4^{IL10/CAR}$ T cells as described herein above. In some embodiments, the population further comprises $CD4^+$ T cells (e.g., primary $CD4^+$ T cells).

[00452] In some embodiments, the population of $CD4^+$ T cells comprise $CD4^+$ T cells obtained from an autologous or allogeneic single T cell donor (single-donor $CD4^{IL\text{-}10/CAR}$ cells). In some embodiments, the primary $CD4^+$ T cells are from a single T cell donor. In some embodiments, the primary $CD4^+$ T cells are from the same individual (i.e., the same patient). In some embodiments, the population of $CD4^+$ T cells comprise $CD4^+$ T cells obtained from at least two different T cell donors (polydonor $CD4^{IL\text{-}10/CAR}$ cells). In some embodiments, the primary $CD4^+$ T cells are from the donor of the allo-HSCT.

[00453] In some embodiments, the $CD4^+$ T cells were obtained from two, three, four, five, six, seven, eight, nine, or ten different T cell donors and pooled. Polydonor $CD4^{IL\text{-}10}$ T cells and methods of making and using the same are described in PCT/US2021/039464, which is herein incorporated by reference in its entirety. Methods and/or compositions described therein can be adopted to generate polydonor $CD4^{IL\text{-}10/CAR}$ cells.

[00454] In some embodiments, the primary $CD4^+$ T cells are from donors selected from donors after analyzing a donors' genetic information. In some embodiments, genetic information of a donor (i.e., the donor of the primary $CD4^+$ T cells) is not analyzed. In some embodiments, the primary $CD4^+$ T cells are from donors selected based on their HLA haplotypes. In some embodiments, the $CD4^+$ T cells in the population collectively have six, seven, eight, nine, ten, eleven, twelve, or more different HLA haplotypes. In some embodiments, all the $CD4^+$ T cells in the population have at least 1/10, 2/10, 3/10, 4/10, 5/10, 6/10, 7/10, 8/10, or 9/10 match at the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci to each other. In some embodiments, all the $CD4^+$ T cells in the population have at least 1/8, 2/8, 3/8, 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci to each other.

[00455] In some embodiments, all the $CD4^+$ T cells in the population have 2/2 match at the HLA-A locus to each other. In some embodiments, all the $CD4^+$ T cell in the population have 2/2 match at the HLA-B locus to each other. In some embodiments, all the $CD4^+$ T cell in the population have 2/2 match at the HLA-C locus to each other. In some embodiments, all the $CD4^+$ T cells in the population have at least 3/4 or 4/4 match at the HLA-DRB1 and HLA-DQB1 loci with each other. In some embodiments, all the $CD4^+$ T cells in the population have an A*02 or an A*24 allele. In some embodiments, all the CD4+ T cells are A*02 or A*24 negative.

[00456] In some embodiments, all of the $CD4^+$ T cells in the population have less than 5/10, 6/10, 7/10, 8/10, or 9/10 match at the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-

DQB1 loci to each other. In some embodiments, all of the $CD4^+$ T cells in the population have less than 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci to each other. In some embodiments, all the $CD4^+$ T cells in the population have less than 2/2 match at the HLA-A locus to each other. In some embodiments, all the $CD4^+$ T cells in the population have less than 2/2 match at the HLA-B locus to each other. In some embodiments, all the $CD4^+$ T cells in the population have less than 2/2 match at the HLA-C locus to each other. In some embodiments, all the $CD4^+$ T cells in the population have less than 3/4 or 4/4 match at the HLA-DRB1 and HLA DQB1 loci with each other.

[00457] In some embodiments, at least 30% (e.g., at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%) of the $CD4^+$ T cells within the population express the CAR and IL-10 as inferred by the expression of ΔNGFR.

[00458] In some embodiments, the $CD4^+$ T cells are in a frozen suspension. In some embodiments, the $CD4^+$ T cells are in a liquid suspension. In some embodiments, the liquid suspension has previously been frozen.

6.5. Pharmaceutical compositions

[00459] In another aspect, pharmaceutical compositions are provided. In some embodiments, the pharmaceutical composition comprises any of the single donor $CD4^{IL\text{-}10/CAR}$ T cells provided herein, any of the polydonor $CD4^{IL\text{-}10/CAR}$ T cells provided herein, or any of the populations of polydonor $CD4^{IL\text{-}10/CAR}$ T cells provided herein, and a pharmaceutically acceptable carrier or diluent.

[00460] The pharmaceutical composition can be formulated for administration by any route of administration appropriate for human or veterinary medicine. In typical embodiments, the composition is formulated for intravenous (IV) administration. In some embodiments, the composition is formulated for intravenous (IV) infusion. In embodiments formulated for IV administration, the pharmaceutical composition will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability.

[00461] In some embodiments, the pharmaceutically acceptable carrier or diluent is saline, lactated Ringer's solution, or other physiologically compatible solution. In various embodiments, the pharmaceutical composition solution comprises 2-20%, preferably 5 %, human serum albumin.

[00462] In some embodiments, unit dosage forms of the pharmaceutical composition are provided that are adapted for administration of the pharmaceutical composition by systemic administration, in particular, for intravenous administration.

[00463] In some embodiments, the unit dosage form contains 10^4 to 10^{11} $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells, 10^4 to 10^{10} $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells, 10^4 to 10^9 $CD4^{IL\text{-}10/CAR}$ T cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells, 10^5 to 10^{10} $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells, 10^5 to 10^9 $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells, 10^5 to 10^8 $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or 10^5 to 10^7 $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$ cells.

[00464] In typical embodiments, the pharmaceutical composition in the unit dosage form is in liquid form.

6.6. Methods of making single donor $CD4^{IL10/CAR}$ cells or polydonor $CD4^{IL10/CAR}$ cells

[00465] In another aspect, the present disclosure provides a method of making $CD4^{IL10/CAR}$ cells or polydonor $CD4^{IL10/CAR}$ cells.

[00466] In some embodiments, the methods provided herein include modifying the $CD4^+$ T cells or pooled $CD4^+$ T cells by introducing an exogenous polynucleotide segment encoding a CAR and an exogenous polynucleotide segment encoding IL-10. In some embodiments, modifying the $CD4^+$ T cells or pooled $CD4^+$ T cells with a exogenous polynucleotide segment encoding a CAR and a exogenous polynucleotide segment encoding IL-10 comprises:(i) a first transduction with the polynucleotide segment encoding IL-10 followed by a second transduction with the exogenous polynucleotide segment encoding the CAR; (ii) a first transduction with the polynucleotide segment encoding the CAR followed by a second transduction with the exogenous polynucleotide segment encoding IL-10; (iii) simultaneous transduction (i.e., co-transduction) of the polynucleotide segment encoding IL-10 and the exogenous polynucleotide segment encoding the CAR; or (iv) transduction of a single polynucleotide construct comprising both the polynucleotide segment encoding IL-10 and the polynucleotide segment encoding the CAR.

[00467] In some embodiments, the single polynucleotide construct comprising the polynucleotide segment encoding IL-10 and the polynucleotide segment encoding the CAR is a bidirectional vector in which IL-10 is under one promoter (e.g., PGK or EF1a) and the CAR is

under a second promoter (for example CMV). In such cases, if the construct includes a polynucleotide sequence encoding a selection marker, the polynucleotide sequence can be downstream of a either the IL-10 or the CAR.

[00468] In some embodiments, the single polynucleotide construct comprising the polynucleotide segment encoding IL-10 and the polynucleotide segment encoding the CAR comprises from 5' to 3' the sequence encoding IL-10 and the sequence encoding the CAR. In such embodiments, the sequence encoding IL-10 and the sequence encoding the CAR are operably linked to a single promoter (e.g., constitutive or inducible). In such embodiments, a sequence encoding an internal ribosome entry site (IRES) or a sequence encoding a 2A-peptide is located between the sequence encoding IL-10 and the sequence encoding the CAR.

[00469] In some embodiments, the single polynucleotide construct comprising the polynucleotide segment encoding IL-10 and the polynucleotide segment encoding the CAR comprises from 5' to 3' the sequence encoding the CAR and the sequence encoding IL-10. In such embodiments, the sequence encoding the CAR and the sequence encoding IL-10 are operably linked to a single promoter (e.g., constitutive or inducible). In such embodiments, a sequence encoding an internal ribosome entry site (IRES) or a sequence encoding a 2A-peptide is located between the sequence encoding the CAR and the sequence encoding IL-10.

[00470] In some embodiments, the method comprises the steps of: (a) pooling primary $CD4^+$ T cells obtained from one or more T cell donors; and (b) modifying the pooled $CD4^+$ T cells by introducing a first exogenous polynucleotide segment encoding a CAR and a second exogenous polynucleotide segment encoding IL-10 according to the methods provided herein. In other embodiments, the method comprises the steps of: (a) obtaining primary $CD4^+$ T cells from one or more T cell donors; (b) separately modifying each donor's $CD4^+$ T cells by introducing a first exogenous polynucleotide segment encoding a CAR and a second exogenous polynucleotide segment encoding IL-10 (e.g., according to the methods provided herein); and then pooling the genetically modified $CD4^+$ T cells, thereby obtaining the $CD4^{IL\text{-}10/CAR}$ cells. Various methods known in the art can be used to introduce a first exogenous polynucleotide segment encoding a CAR, a second exogenous polynucleotide segment encoding IL-10, or both, to primary $CD4^+$ T cells.

[00471] In some embodiments, the method further comprises the step of incubating the primary $CD4^+$ T cells or genetically-modified $CD4^+$ T cells in the presence of an anti-CD3 antibody and anti-CD28 antibody, or anti-CD3 antibody and anti-CD28 antibody coated beads.

In some embodiments, the method further comprises the step of incubating the primary $CD4^+$ T cells or genetically-modified $CD4^+$ T cells in the presence of anti-CD3 antibody, anti-CD28 antibody and IL-2 or anti-CD3 antibody and anti-CD28 antibody coated beads and IL-2. In some embodiments, the method further comprises the step of incubating the primary $CD4^+$ T cells or genetically-modified $CD4^+$ T cells in the presence of a mixture of feeder cells. In some embodiments, the method further comprises the step of incubating the primary $CD4^+$ T cells or genetically-modified $CD4^+$ T cells in the presence of nanopreparations of anti-CD3 antibody and anti-CD28 antibody. In some embodiments, the incubation is done in the presence of a polymeric nanomatrix reagent to activate and expand human T cells via CD3 and CD28. In some embodiments, $CD4^{IL\text{-}10/CAR}$ T cells have been cultured in the presence of other T-cell specific immune cell culture media, activators, and supplements.

[00472] In some embodiments, the incubation step is performed before introducing a first exogenous polynucleotide segment encoding a CAR and a second exogenous polynucleotide segment encoding IL-10 according to the methods provided herein. In some embodiments, the incubation step is performed after (a) pooling primary $CD4^+$ T cells obtained from one or more different T cell donors; but before (b) modifying the pooled $CD4^+$ T cells by introducing a first exogenous polynucleotide segment encoding a CAR, and a second exogenous polynucleotide segment encoding IL-10 according to the methods provided herein. In some embodiments, the incubation step is performed after (a) obtaining primary $CD4^+$ T cells from one or more different T cell donors; but before (b) separately modifying each donor's $CD4^+$ T cells by introducing a first exogenous polynucleotide segment encoding a CAR and a second exogenous polynucleotide segment encoding IL-10 according to the methods provided herein.

[00473] In some embodiments, the incubation step is performed after step (b). In other words, in some embodiments, the incubation step is performed after (b) modifying the pooled $CD4^+$ T cells by introducing a first exogenous polynucleotide segment encoding a CAR and a second exogenous polynucleotide segment encoding IL-10 according to the methods provided herein. In some embodiments, the incubation step is performed after (b) separately modifying each donor's $CD4^+$ T cells by introducing a first exogenous polynucleotide segment encoding a CAR and a second exogenous polynucleotide segment encoding IL-10 according to the methods provided herein, but before pooling the genetically modified $CD4^+$ T cells, thereby obtaining the genetically-modified $CD4^+$ T cells. In some embodiments, the incubation step is performed after pooling the genetically modified $CD4^+$ T cells, thereby obtaining the $CD4^{IL\text{-}10/CAR}$ cells.

[00474] In some embodiments, the incubation step is performed more than once. In some embodiments, the incubation step is performed both before and after genetic modification of $CD4^+$ T cells.

[00475] In some embodiments, the first exogenous polynucleotide segment, the second exogenous polynucleotide segment, or both, is introduced into the primary $CD4^+$ T cells using a viral vector. In some embodiments, the first and second exogenous polynucleotide segments are located on the same viral vector. In some embodiments, the viral vector is a lentiviral vector.

[00476] In some embodiments, the first exogenous polynucleotide comprises a segment encoding a CAR having the sequence of SEQ ID NOs: 9, 16, 22, 34, 41-49, and 54 and the second exogenous polynucleotide segment encoding IL-10 having the sequence of SEQ ID NO: 1. In some embodiments, the first exogenous polynucleotide comprises a segment encoding a CAR having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NOs: 9, 16, 22, 34, 41-49, and 54 and the second exogenous polynucleotide comprises a segment encoding IL-10 having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 1.

[00477] In some embodiments, the CAR-encoding polynucleotide segment has the sequence of SEQ ID NOs: 10, 17, 23, 35, or 55 and the IL-10-encoding polynucleotide segment has the sequence of SEQ ID NO: 2. In some embodiments, the CAR-encoding polynucleotide segment has at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NOs: 10, 17, 22, 35, or 55and the IL-10-encoding polynucleotide segment has at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 2. In some embodiments, the first exogenous polynucleotide segment, the second exogenous polynucleotide segment, or both, further comprise a segment encoding a marker permitting selection of successfully transduced $CD4^+$ T cells. In some embodiments, the encoded selection marker is ΔNGFR. In certain embodiments, the encoded selection marker has the sequence of SEQ ID NO: 4. In some embodiments, the encoded selection marker is a truncated form of human EGFR polypeptide.

[00478] In some embodiments, the method further comprises the step of isolating the genetically-modified $CD4^+$ T cells expressing one or more selection markers, thereby generating an enriched population of genetically-modified $CD4^{IL\text{-}10/CAR}$ cells.

[00479] In some embodiments, at least 40% (e.g., at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%) of the genetically-modified $CD4^+$ T cells in the enriched population express a selection marker, wherein the selection marker is a

proxy for IL-10 expression. In some embodiments, at least 75% (e.g., at least 80%, at least 90%, at least 95%, or at least 98%) of the genetically-modified $CD4^+$ T cells in the enriched population express a selection marker, wherein the selection marker is a proxy for IL-10 expression. In some embodiments, at least 75% of the genetically-modified $CD4^+$ T cells in the enriched population express a selection marker. In some embodiments, at least 95% of the genetically-modified $CD4^+$ T cells in the enriched population express a selection marker. In some embodiments, at least 96, 97, 98, or 99% of the genetically-modified $CD4^+$ T cells in the enriched population express a selection marker.

[00480] In some embodiments, at least 75% (e.g., at least 80%, at least 90%, at least 95%, or at least 98%) of the genetically-modified $CD4^+$ T cells in the enriched population express IL-10. In some embodiments, at least 75% of the genetically-modified $CD4^+$ T cells in the enriched population express IL-10. In some embodiments, at least 95% of the genetically-modified CD4+ T cells in the enriched population express IL-10. In some embodiments, at least 96, 97, 98, or 99% of the genetically-modified CD4+ T cells in the enriched population express IL-10.

[00481] In some embodiments, at least 30 % (e.g., at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%) of the genetically-modified $CD4^+$ T cells in the enriched population express a CAR. In some embodiments, at least 50% of the genetically-modified $CD4^+$ T cells in the enriched population express a CAR. In some embodiments, at least 75% of the genetically-modified $CD4^+$ T cells in the enriched population express a CAR. In some embodiments, at least 95% of the genetically-modified $CD4^+$ T cells in the enriched population express a CAR. In some embodiments, at least 96, 97, 98, or 99% of the genetically-modified $CD4^+$ T cells in the enriched population express a CAR.

[00482] In some embodiments, at least 30 % (e.g., at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%) of the genetically-modified $CD4^+$ T cells in the enriched population express IL-10 and a CAR. In some embodiments, at least 50% of the genetically-modified $CD4^+$ T cells in the enriched population express IL-10 and a CAR. In some embodiments, at least 75% of the genetically-modified $CD4^+$ T cells in the enriched population express IL-10 and a CAR. In some embodiments, at least 95% of the genetically-modified $CD4^+$ T cells in the enriched population express IL-10 and a CAR. In some embodiments, at least 96,

97, 98, or 99% of the genetically-modified $CD4^+$ T cells in the enriched population express IL-10 and a CAR.

[00483] In some embodiments, the method further comprises the step of incubating the enriched population of the genetically-modified $CD4^+$ T cells. In some embodiments, the incubation is performed in the presence of anti-CD3 antibody and anti-CD28 antibody, or anti-CD3 antibody and anti-CD28 antibody coated beads. In some embodiments, the incubation is performed further in presence of IL-2. In some embodiments, the incubation is performed in the presence of feeder cells. In some embodiments, the incubation is performed in the presence of nanopreparations of anti-CD3 antibody and anti-CD28 antibody. In some embodiments, the incubation is performed in the presence of a polymeric nanomatrix reagent to activate and expand human T cells via CD3 and CD28. In some embodiments, $CD4^{IL\text{-}10/CAR}$ T cells have been cultured in the presence of other T-cell specific immune cell culture media, activators, and supplements.

[00484] In some embodiments, the method further comprises the step of freezing the genetically-modified $CD4^+$ T cells.

[00485] In some embodiments, the primary $CD4^+$ T cells are from donors selected based on their HLA haplotypes. In some embodiments, the method further comprises the step of selecting T cell donors by analyzing their genetic information. In some embodiments, the method comprises the step of analyzing genetic information or HLA haplotype of potential T cell donors.

[00486] In some embodiments, the primary $CD4^+$ T cells are from donors having at least a partial HLA match with a host to be treated with the primary $CD4^+$ T cells or a modification thereof. In some embodiments, the primary $CD4^+$ T cells are from donors having at least a partial HLA match with a stem cell (HSC), tissue or organ donor. In some embodiments, the $CD4^+$ T cells are from donors HLA matched with a stem cell (HSC), tissue, or organ donor. In some embodiments, the primary $CD4^+$ T cells are obtained from third party donors who are not biologically related with a host. In some embodiments, the primary $CD4^+$ T cells are obtained from third party donors who are not biologically related with a stem cell, tissue or organ donor.

[00487] In some embodiments, in step (a), the primary $CD4^+$ T cells are obtained from two, three, four, five, six, seven, eight, nine, or ten different T cell donors. In some embodiments, the at least two T cell donors have at least 1/10, 2/10, 3/10, 4/10, 5/10, 6/10, 7/10, 8/10, or 9/10 match at the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci to each

other. In some embodiments, the at least two T cell donors have at least 1/8, 2/8, 3/8, 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci to each other. In some embodiments, the at least two T cell donors have 2/2 match at the HLA-A locus to each other. In some embodiments, the at least two T cell donors have 2/2 match at the HLA-B locus to each other. In some embodiments, the at least two T cell donors have 2/2 match at the HLA-C locus to each other. In some embodiments, the at least two T cell donors have at least 3/4 or 4/4 match at the HLA-DRB1 and HLA-DQB1 loci to each other. In some embodiments, each of the at least two T cell donors has an A*02 or A*24 allele. In some embodiments, the T-cell donor is HLA-A*02 negative. In some embodiments, each of the at least two T cell donors is HLA-A*02 or HLA-A*24 negative.

[00488] In some embodiments, the at least two T cell donors have less than 5/10, 6/10, 7/10, 8/10, or 9/10 match at the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci to each other. In some embodiments, the at least two T cell donors have less than 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci to each other. In some embodiments, the at least two T cell donors have less than 2/2 match at the HLA-A locus to each other. In some embodiments, the at least two T cell donors have less than 2/2 match at the HLA-B locus to each other. In some embodiments, the at least two T cell donors have less than 2/2 match at the HLA-C locus to each other. In some embodiments, the at least two T cell donors have less than 3/4 or 4/4 match at the HLA-DRB1 and HLA-DQB1 loci to each other.

[00489] In some embodiments, in step (a), the primary $CD4^+$T cells are obtained from one or more frozen stocks. In some embodiments, in step (a), the primary $CD4^+$ T cells are obtained from unfrozen peripheral blood mononuclear cells of the at least two different T cell donors. In some embodiments, the method further comprises the step of isolating $CD4^+$ T cells from the peripheral blood mononuclear cells. In some embodiments, in step (a), the primary $CD4^+$ T cells are obtained from a liquid suspension. In some embodiments, the liquid suspension is obtained from a previously frozen stock.

[00490] In some embodiments, the method does not comprise the step of anergizing the $CD4^+$ T cells in the presence of peripheral blood mononuclear cells (PBMCs) from a host. In some embodiments, the method does not comprise the step of anergizing the $CD4^+$ T cells in the presence of recombinant IL-10 protein, wherein the recombinant IL-10 protein is not expressed from the $CD4^+$ T cells. In some embodiments, the method does not comprise the step of anergizing the $CD4^+$ T cells in the presence of DC10 cells from a host.

[00491] In some embodiments, the methods provided herein include modifying the $CD4^+$ T cells or pooled $CD4^+$ T cells by introducing an exogenous polynucleotide segment encoding a CAR and an exogenous polynucleotide segment encoding IL-10 after one or more of the pooling, purification, restimulation, and expansion steps.

6.7. Methods of using $CD4^{IL\text{-}10/CAR}$ cells or polydonor $CD4^{IL\text{-}10/CAR}$

[00492] In another aspect, the present disclosure provides a method of treating a patient, comprising the step of administering any of the $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous single-donor or allogeneic single-donor) provided herein, any of the allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ T cells provided herein, any of the populations of polydonor $CD4^{IL\text{-}10/CAR}$ cells provided herein, or any of the pharmaceutical composition provided herein to a patient in need thereof.

[00493] In some embodiments, the method further comprises the preceding step of thawing a frozen suspension of $CD4^{IL\text{-}10/CAR}$ cells.

[00494] In some embodiments, the autologous single-donor or allogeneic single-donor $CD4^{IL\text{-}10/CAR}$ cells or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition prevents or reduces severity of pathogenic T cell response in the patient.

[00495] In some embodiments, the polydonor $CD4^{IL\text{-}10}$ cells or the pharmaceutical composition prevents or reduces the severity of an inflammatory or autoimmune response.

[00496] In some embodiments, the polydonor $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition prevents or reduces severity of pathogenic T cell response in the patient. In some embodiments, the polydonor $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition reduces inflammation. In some embodiments, the polydonor $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition enhances tissue repair. In some embodiments, the polydonor $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition enhances immunological tolerance to self and non-pathogenic antigens and maintain immune homeostasis. In some embodiments, the polydonor $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition downregulates pathogenic T-cell responses associated with organ transplantation, GvHD and various autoimmune and inflammatory diseases. In some embodiments, the polydonor $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition treats autoimmune disease. In some embodiments, the polydonor $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition reduces hyperactivity of NLPR3 inflammasome or reduces symptoms associated with hyperactivity of NLPR3 inflammasome. In some embodiments, the polydonor $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition induces death of tumor cells or reduces tumor growth. In some embodiments, the polydonor $CD4^{IL\text{-}10/CAR}$ cells or the

pharmaceutical composition increases disease free survival (e.g., absence of minimal residual disease). In some embodiments, the polydonor $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition induces wound healing or tissue repair.

[00497] In some embodiments, the polydonor $CD4^{IL\text{-}10}$ cells or the pharmaceutical composition are administered at an amount effective to prevent or reduce severity of pathogenic T cell response in the patient. In some embodiments, the polydonor $CD4^{IL\text{-}10}$ cells or the pharmaceutical composition are administered at an amount effective to reduce inflammation. In some embodiments, the polydonor $CD4^{IL\text{-}10}$ cells or the pharmaceutical composition are administered at an amount effective to enhance tissue repair. In some embodiments, the polydonor $CD4^{IL\text{-}10}$ cells or the pharmaceutical composition are administered at an amount effective to enhance immunological tolerance to self and non-pathogenic antigens and maintain immune homeostasis. In some embodiments, the polydonor $CD4^{IL\text{-}10}$ cells or the pharmaceutical composition are administered at an amount effective to downregulate pathogenic T-cell responses associated with organ transplantation, GvHD and various autoimmune or inflammatory diseases. In some embodiments, the polydonor $CD4^{IL\text{-}10}$ cells or the pharmaceutical composition are administered at an amount effective to treat autoimmune disease. In some embodiments, the polydonor $CD4^{IL\text{-}10}$ cells or the pharmaceutical composition are administered at an amount effective to reduce hyperactivity of NLPR3 inflammasome or reduces symptoms associated with hyperactivity of NLPR3 inflammasome. In some embodiments, the polydonor $CD4^{IL\text{-}10}$ cells or the pharmaceutical composition are administered at an amount effective to induce death of tumor cells or reduces tumor growth. In some embodiments, the polydonor $CD4^{IL\text{-}10}$ cells or the pharmaceutical composition are administered at an amount effective to increase disease free survival (e.g., absence of minimal residual disease).

[00498] In some embodiments, the single donor $CD4^{IL\text{-}10/CAR}$ cells (e.g., autologous or allogeneic) or the pharmaceutical composition prevents or reduces severity of pathogenic T cell response in the patient. In some embodiments, the single donor $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition reduces inflammation. In some embodiments, the single donor $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition enhances tissue repair. In some embodiments, the single donor $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition enhances immunological tolerance to self and non-pathogenic antigens and maintain immune homeostasis. In some embodiments, the single donor $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition downregulates pathogenic T-cell responses associated with organ transplantation, GvHD and

various autoimmune and inflammatory diseases. In some embodiments, the single donor $CD4^{IL-10/CAR}$ cells or the pharmaceutical composition treats autoimmune disease. In some embodiments, the single donor $CD4^{IL-10/CAR}$ cells or the pharmaceutical composition reduces hyperactivity of NLPR3 inflammasome or reduces symptoms associated with hyperactivity of NLPR3 inflammasome. In some embodiments, the single donor $CD4^{IL-10/CAR}$ cells or the pharmaceutical composition induces death of tumor cells or reduces tumor growth. In some embodiments, the single donor $CD4^{IL-10/CAR}$ cells or the pharmaceutical composition increases disease free survival (e.g., absence of minimal residual disease). In some embodiments, the single donor $CD4^{IL-10/CAR}$ cells or the pharmaceutical composition induces wound healing or tissue repair.

[00499] In some embodiments, the treatment method further comprises monitoring $CD4^{IL-10/CAR}$ cells in a patient after administration. In some embodiments, the method comprises the step of detecting a selection marker in a biological sample obtained from the patient, thereby detecting presence or absence of $CD4^{IL-10/CAR}$ cells or polydonor $CD4^{IL-10/CAR}$ cells. In some embodiments, the method comprises the step of detecting a selection marker in a patient, and using detection of the selection marker to track and/or monitor $CD4^{IL-10/CAR}$ cells following administration to a patient. In some embodiments, the selection marker is detected at multiple time points to trace changes in presence of $CD4^{IL-10/CAR}$ cells in a patient. In some embodiments, the biological sample is a biopsy or blood sample from the patient.

[00500] The $CD4^{IL-10/CAR}$ cells (autologous single-donor, allogeneic single-donor or allogeneic polydonor $CD4^{IL-10/CAR}$ cells) are administered in a therapeutically effective amount. The amount can be determined based on the body weight and other clinical factors. In some embodiments, 10^3 to 10^{11} cells/kg are administered. In some embodiments, 10^3 to 10^{10} cells/kg are administered. In some embodiments, 10^3 to 10^9 cells/kg are administered. In some embodiments, 10^3 to 10^8 cells/kg are administered. In some embodiments, 10^3 to 10^7 cells/kg are administered. In some embodiments, 10^3 to 10^6 cells/kg are administered. In some embodiments, 10^3 to 10^5 cells/kg are administered. In some embodiments, 10^3 to 10^4 cells/kg are administered.

[00501] In various embodiments, $CD4^{IL-10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL-10/CAR}$ cells) are administered on a therapeutically effective schedule. In some embodiments, $CD4^{IL-10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL-10/CAR}$ cells) are administered once. In some embodiments, $CD4^{IL-10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or

allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells) are administered every day, every 3 days, every 7 days, every 14 days, every 21 days, or every month.

[00502] The $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells) can be administered according to different administration routes, such as systemically, subcutaneously, or intraperitoneally. In some embodiments, the cells are administered within a saline or physiological solution which may contain 2-20%, preferably 5 % human serum albumin.

6.7.1. Methods of reducing or preventing GvHD

[00503] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein is used to treat a patient before a hematopoietic stem cell (HSC) transplant (HSCT), concurrently with an HSCT, or following an HSCT.

[00504] In various embodiments, the HSCT is a matched related HSCT, or a matched unrelated HSCT. In various embodiments, the HSCT is a haploidentical HSCT, a mismatched related HSCT, or a mismatched unrelated HSCT.

[00505] In some embodiments, the patient has a hematological malignancy which requires treatment with allo-HSCT. In some embodiments, the hematological malignancy is mediated by aberrant myeloid cells. In some embodiments, the malignancy or hematological cancer is a myeloid leukemia. In some embodiments, the malignancy or hematological cancer is a $CD19^+$, $CD20^+$, $CD22^+$, $BCMA^+$, or $B7\text{-}H3^+$ hematological cancer. In some embodiments, the $CD19^+$, $CD20^+$, $CD22^+$, or $B7\text{-}H3^+$ hematological cancer is selected from chronic lymphocytic leukemia, acute lymphoblastic leukemia (ALL), and non-Hodgkin's lymphomas.

[00506] In some embodiments, T cell donors are selected based on genetic information of a patient to be treated with $CD4^{IL\text{-}10/CAR}$ (autologous single-donor or allogeneic single-donor) or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells and HSC, and/or genetic information of the HSC donor. In some embodiments, T cell donors are selected based on HLA haplotype of a patient to be treated with polydonor $CD4^{IL\text{-}10}$ cells and HSC, and/or HLA haplotype of the HSC donor. In some embodiments, the method further comprises the step, prior to administering $CD4^{IL\text{-}10}$ cells, of analyzing genetic information or HLA haplotype of T cell donors. In some embodiments, the method further comprises the step of analyzing genetic information or HLA haplotype of a host.

In some embodiments, the method further comprises the step of analyzing genetic information or HLA haplotype of an HSC donor.

[00507] In some embodiments, T cell donors, a host and an HSC donor are not biologically related. In some embodiments, T cell donors, a host and an HSC donor have different HLA haplotypes. In some embodiments, T cell donors, a host and an HSC donor have at least partial mismatch in HLA haplotype. In some embodiments, T cell donors are selected when they have HLA haplotype with an HLA match over a threshold value.

[00508] In some embodiments, the HSC donor is partially HLA mismatched to the patient. In some embodiments, the HSC donor has less than 5/10, 6/10, 7/10, 8/10, 9/10 or 10/10 match at the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci to the patient. In some embodiments, the HSC donor has less than 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci to the patient. In some embodiments, the HSC donor has less than 2/2 match at the HLA-A, HLA-B, or HLA-C locus to the patient. In some embodiments, the HSC donor has less than 3/4 or 4/4 match at the HLA-DRB1 and HLA-DQB1 loci to the patient.

[00509] In some embodiments, one or more of the T cell donors are HLA-mismatched or partially HLA-mismatched to the patient. In some embodiments, one or more of the T cell donors have less than 5/10, 6/10, 7/10, 8/10, 9/10 or 10/10 match at the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci to the patient. In some embodiments, one or more of the T cell donors have less than 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci to the patient. In some embodiments, one or more of the T cell donors have less than 2/2 match at the HLA-A, HLA-B, or HLA-C locus to the patient. In some embodiments, one or more of the T cell donors have less than 2/4, 3/4 or 4/4 match at the HLA-DRB1 and HLA-DQB1 loci to the patient.

[00510] In some embodiments, one or more of the T cell donors are HLA-mismatched or partially HLA-mismatched with the HSC donor. In some embodiments, one or more of the T cell donors have less than 5/10, 6/10, 7/10, 8/10, 9/10 or 10/10 match at the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci to the HSC donor. In some embodiments, one or more of the T cell donors have less than 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci to the HSC donor. In some embodiments, one or more of the T cell donors have less than 2/2 match at the HLA-A, HLA-B, or HLA-C locus to the HSC donor.

In some embodiments, one or more of the T cell donors have less than 3/4 or 4/4 match at the HLA-DRB1 and HLA-DQB1 loci to the HSC donor.

[00511] In some embodiments, when administered to a patient, $CD4^{IL\text{-}10/CAR}$ cells (single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein prevents or reduces severity of GvHD by the transplanted hematopoietic stem cells.

[00512] In some embodiments, when administered to a patient, $CD4^{IL\text{-}10/CAR}$ cells (single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein prevents or reduces severity of pathological T cell response by the transplanted hematopoietic cells. In specific embodiments, the $CD4^{IL\text{-}10/CAR}$ or polydonor $CD4^{IL\text{-}10/CAR}$ cells prevents or reduces GvHD.

[00513] In some embodiments, the polydonor $CD4^{IL\text{-}10}$ cells or the pharmaceutical composition prevents or reduces severity of tissue damage induced by the pathogenic T cells or the inflammation.

6.7.2. Methods of treating cancer

[00514] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are used for treatment of cancer. In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are used for treating a malignancy.

[00515] In preferred embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells) directly mediate anti-tumor effects and in particular embodiments, an anti-leukemic effect.

[00516] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein and allogeneic are administered prior or after hematopoietic stem cell transplantation (HSCT), peripheral blood stem cells (PBSC), cord blood (CB), or bone marrow (BM) transplantation.

[00517] In some embodiments, the neoplastic cells express CD19. In some embodiments, the neoplastic cells express CD20. In some embodiments, the neoplastic cells express CD22. In some embodiments the neoplastic cells express BCMA. In some embodiments, the neoplastic cells express B7-H3. In some embodiments, the neoplastic cells express CD13. In some embodiments, the neoplastic cells express HLA-class I. In some embodiments, the neoplastic cells express CD54. In some embodiments, the neoplastic cells express CD13, HLA-class I and CD54. In some embodiments, the neoplastic cells express CD112. In some embodiments, the neoplastic cells express CD58. In some embodiments, the neoplastic cells express CD155. In some embodiments, the tumor expresses CD112, CD58, or CD155. In various embodiments, the tumor is a solid or hematological tumor. In some embodiments, the tumor is a solid tumor. In some embodiments, the solid tumor expresses B7-H3. In some embodiment, the solid tumor is selected from the group consisting of: breast cancer, brain cancer, lung cancer, liver cancer, stomach cancer, spleen cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, uterine cancer, skin cancer, head cancer, neck cancer, sarcomas, neuroblastomas and ovarian cancer.

[00518] In some embodiments, the patient has a cancer selected from the group consisting of: Adrenal Cancer, Anal Cancer, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Brain/CNS Tumors In Adults, Brain/CNS Tumors In Children, Breast Cancer, Breast Cancer In Men, Cancer of Unknown Primary, Castleman Disease, Cervical Cancer, Colon/Rectum Cancer, Endometrial Cancer, Esophagus Cancer, Ewing Family Of Tumors, Eye Cancer, Gallbladder Cancer, Gastrointestinal Carcinoid Tumors, Gastrointestinal Stromal Tumor (GIST), Gestational Trophoblastic Disease, Hodgkin Disease, Kaposi Sarcoma, Kidney Cancer, Laryngeal and Hypopharyngeal Cancer, Leukemia, Acute Lymphocytic (ALL), Acute Myeloid (AML, including myeloid sarcoma and leukemia cutis), Chronic Lymphocytic (CLL), Chronic Myeloid (CML) Leukemia, Chronic Myelomonocytic (CMML), Leukemia in Children, Liver Cancer, Lung Cancer, Lung Cancer with Non-Small Cell, Lung Cancer with Small Cell, Lung Carcinoid Tumor, Lymphoma, Lymphoma of the Skin, Malignant Mesothelioma, Multiple Myeloma, Myelodysplastic Syndrome, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Neuroblastoma, Non-Hodgkin Lymphoma, Non-Hodgkin Lymphoma In Children, Oral Cavity and Oropharyngeal Cancer, Osteosarcoma, Ovarian Cancer, Pancreatic Cancer, Penile Cancer, Pituitary Tumors, Prostate Cancer, Retinoblastoma, Rhabdomyosarcoma, Salivary Gland Cancer, Sarcoma - Adult Soft Tissue Cancer, Skin Cancer, Skin Cancer - Basal and Squamous Cell, Skin Cancer - Melanoma, Skin Cancer - Merkel Cell, Small Intestine Cancer, Stomach Cancer,

Testicular Cancer, Thymus Cancer, Thyroid Cancer, Uterine Sarcoma, Vaginal Cancer, Vulvar Cancer, Waldenstrom Macroglobulinemia, and Wilms Tumor.

[00519] In some embodiments, the cancer is a myeloid tumor. In particular embodiments, the cancer is AML or CML. In some embodiments, the cancer is a myeloid tumor. In some embodiments, the cancer is ALL.

[00520] In some embodiments, the method is used to treat a hematological cancer affecting blood, bone marrow, and lymph nodes. In various embodiments, the hematological cancer is a lymphoma (e.g., Hodgkin's Lymphoma), lymphocytic leukemias, myeloma. In various embodiments, the hematological cancer is acute or chronic myelogenous (myeloid) leukemia (AML, CML), or a myelodysplastic syndrome.

[00521] In some embodiments, the malignancy or hematological cancer is a $CD19^+$, $CD20^+$, $CD22^+$, $BCMA^+$, or $B7\text{-}H3^+$ hematological cancer. In some embodiments, the $CD19^+$, $CD20^+$, $CD22^+$, $BCMA^+$, or $B7\text{-}H3^+$ hematological cancer is chronic lymphocytic leukemia, acute lymphoblastic leukemia (ALL), and non-Hodgkin's lymphomas.

[00522] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells) are used for treating a patient with a hematological cancer where the method includes administering a therapeutically effective amount of the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein to the patient.

[00523] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are used for treating a patient with a $CD19^+$ hematological cancer where the method includes administering a therapeutically effective amount of $CD4^{IL\text{-}10/anti\text{-}CD19\ CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/anti\text{-}CD19\ CAR}$), populations of single-donor or polydonor $CD4^{IL\text{-}10/anti\text{-}CD19\ CAR}$ cells, or any of the pharmaceutical compositions provided herein to the patient.

[00524] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are used for treating a patient with a $CD20^+$ hematological cancer where the method includes administering a therapeutically effective amount of $CD4^{IL\text{-}10/anti\text{-}CD20\ CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/anti\text{-}CD20\ CAR}$), populations of single-donor or polydonor $CD4^{IL\text{-}10/anti\text{-}CD20\ CAR}$ cells, or any of the pharmaceutical compositions provided herein to the patient.

[00525] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are used for treating a patient with a $CD22^+$ hematological cancer where the method includes administering a therapeutically effective amount of $CD4^{IL\text{-}10/anti\text{-}CD22\ CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/anti\text{-}CD22\ CAR}$), populations of single-donor or polydonor $CD4^{IL\text{-}10/anti\text{-}CD22\ CAR}$ cells, or any of the pharmaceutical compositions provided herein to the patient.

[00526] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are used for treating a patient with a $B7\text{-}H3^+$ cancer (e.g., a solid cancer) where the method includes administering a therapeutically effective amount of $CD4^{IL\text{-}10/anti\text{-}B7\text{-}H3\ CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/anti\text{-}B7\text{-}H3\ CAR}$), populations of single-donor or polydonor $CD4^{IL\text{-}10/anti\text{-}B7\text{-}H3\ CAR}$ cells, or any of the pharmaceutical compositions provided herein to the patient.

[00527] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells are used for treating a patient with a $BCMA^+$ cancer (e.g., a solid cancer) where the method includes administering a therapeutically effective amount of $CD4^{IL\text{-}10/anti\text{-}BCMA\ CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/anti\text{-}BCMA\ CAR}$), populations of single-donor or polydonor $CD4^{IL\text{-}10/anti\text{-}BCMA\ CAR}$ cells, or any of the pharmaceutical compositions provided herein to the patient.

[00528] In some embodiments, the cancer is refractory or resistant to a therapeutic intervention.

[00529] In some embodiments, the methods provided herein are used for preventing relapse of a $CD19^+$, $CD20^+$, $CD22^+$, $BCMA^+$, or $B7\text{-}H3^+$ hematological and solid cancers in a patient, comprising: administering to a patient, identified as having a $CD19^+$, $CD20^+$, $CD22^+$ $BCMA^+$, or $B7\text{-}H3^+$ hematological or solid cancers or at risk of having a relapse of a $CD19^+$, $CD20^+$, $CD22^+$, $BCMA^+$, or $B7\text{-}H3^+$ hematological or solid cancers, a therapeutically effective amount of the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are sufficient to induce an anti-cancer effect.

[00530] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells) are used in combination with a therapeutic intervention. The combination may be simultaneous or performed at different times. Preferably the therapeutic intervention is selected from the group consisting of: chemotherapy, radiotherapy, allo-HSCT, immune suppression, blood transfusion, bone marrow transplant, growth factors, biologicals.

[00531] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells) are used in treating a patient with a malignancy, where the method includes administering an allo-HSCT graft to the patient, and administering a therapeutically effective amount of the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein to the patient.

[00532] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells) are used to treat cancer patients receiving allogeneic HSCT (allo-HSCT) in order to prevent GvHD and to induce long term tolerance (in addition to direct anti-tumor effects). The amount of $CD4^{IL\text{-}10/CAR}$ cells is sufficient to suppress or prevent graft versus host disease (GvHD) without suppressing graft versus leukemia (GvL) or graft versus tumor (GvT) efficacy of the allo-HSCT (mediated by donor T-cells present in stem cell preparation).

[00533] Also provided herein are methods for treating a patient with minimal residual disease, comprising: administering to a patient, identified as having minimal residual disease or at risk of having minimal residual disease, a therapeutically effective amount the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein sufficient to induce an anti-cancer effect.

[00534] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein induce cell death of tumor infiltrating tumor growth promoting myeloid lineage cells (e.g., monocytes, macrophages, neutrophils).

[00535] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein induce cell death of B cells (e.g., $CD19^+$ B cells, $CD20^+$ B cells, $CD22^+$ B cell, $BCMA^+$, $B7\text{-}H3^+$ B cells, or $B7\text{-}H3^+$ solid tumor cells).

6.7.3. Methods of treating inflammatory or autoimmune disease

[00536] In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are administered to treat inflammatory or autoimmune disease. In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are administered to treat a disease or disorder involving hyperactivity of NLPR3 inflammasome.

[00537] The NOD-like receptor family (NLR) protein NLRP3 is an intracellular signaling molecule that senses danger signals from pathogenic, environmental or endogenous source. Following activation, NLPR3 interacts with caspase-1, forming a complex termed the inflammasome. This results in the activation of caspase-1, which cleaves the pro-inflammatory cytokines IL-1β and IL-18 to their active forms and mediates a type of inflammatory cell death known as pyroptosis.

[00538] In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are administered to treat an inflammatory disease selected from Muckle-Wells syndrome (MWS), familial cold auto- inflammatory syndrome (FCAS) and neonatal onset multi-system inflammatory disease (NOMID). In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are administered to treat a chronic disease selected from metabolic syndrome, type 2 diabetes, atherosclerosis, Alzheimer, Parkinson, ALS, non-alcoholic steatohepatitis, osteoarthritis, silicosis, asbestosis, gout, and lung fibrosis. In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are administered to treat Crohn's disease, Ulcerative colitis, Multiple sclerosis and systemic lupus erythromytosis or inflammatory eye diseases such as diabetic retinopathy, acute glaucoma and age related macular degeneration.

[00539] In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are administered to treat a disease associated with NLRP3. The disease can be selected from the group consisting of: CAPS, NASH, Alzheimer, Parkinson, cardiovascular disease, osteoarthritis, gout, pseudogout, nephrocalcitosis, type II diabetes, Sjogren syndrome, sickle cell disease (SCD), AMD, infections, cerebral malaria, asbestosis, contact hypersensitivity, sunburn, silicosis, cystic fibrosis, inflammatory bowel disease, ALS, myelodysplastic syndrome, and uveitis.

[00540] In some embodiments, the disease is a brain disorder selected from Parkinson, Alzheimer, age-related cognitive impairment, frontotemporal dementia, traumatic brain injury, intracerebral hemorrhage, sepsis-associated encephalopathy, cerebral ischemia, subarachnoid hemorrhage, epilepsy, acrylamide poisoning, opioid-induced neuroinflammation, chronic

migraine, perioperative neurocognitive disorder, poststroke cognitive impairment, post-cardiac arrest cognitive impairment, social isolation-induced cognitive impairment, anxiety and post-traumatic stress disorder.

[00541] In some embodiments, the disease is a lung disorder selected from asthma, IR lung injury, ARDS/COPD, particulate matter-induced lung injury, radiation pneumonitis, pulmonary hypertension, sarcoidosis, cystic fibrosis, and allergic rhinitis.

[00542] In some embodiments, the disease is a heart disorder selected from atherosclerosis, heart failure, hypertension, myocardial infarction, atrial fibrillation, cardiac injury induced by metabolic dysfunction, heart failure, and endothelial dysfunction.

[00543] In some embodiments, the disease is a gastrointestinal disease, such as colitis. In some embodiments, the disease is a liver disorder selected from acute liver failure, circadian regulation of immunity, NASH, cognitive dysfunction in diabetes, IR liver injury, idiosyncratic drug-induced liver injury and liver fibrosis. In some embodiments, the disease is a pancreas or kidney disorder selected from diabetic encephalopathy, diabetes-associated atherosclerosis, insulin resistance, islet transplantation rejection, chronic crystal nephropathy, renal fibrosis, I/R kidney injury, obesity-associated renal disease, and renal hypertension. In some embodiments, the disease is a skin or eye disorder selected from psoriasis and retinal neovascularization. In some embodiments, the disease is a reproductive disorder such as preterm birth. In some embodiments, the disease is an immune disorder selected from primary dysmenorrhea, innate immunity, innate to adaptive immunity, systemic lupus erythematosus-lupus nephritis, and multiple sclerosis. In some embodiments, the disease is an inheritable disorder selected from Muckle-Wells syndrome, rheumatoid arthritis, sickle cell disease and VCP-associated disease. In some embodiments, the disease is a pain disorder selected from multiple sclerosis-associated neuropathic pain, chronic prostatitis/chronic pelvic pain, cancer-induced bone pain, and hyperalgesia. In some embodiments, the disease is cancer, such as human squamous cell carcinoma of head and neck cancer. In some embodiments, the disease is an infective disorder, such as bacterial, viral or parasitic infection.

[00544] In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are used in combination with a currently available treatments for NLRP3 related diseases, such as a biologic agent that target IL-1. The biologic agent includes the recombinant IL-1 receptor antagonist Anakinra, the neutralizing IL-1β antibody Canakinumab and the soluble decoy IL-1 receptor Rilonacept.

[00545] In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are administered to treat a disease selected from Type 2 diabetes, metabolic syndrome, cardiovascular diseases, SLE, MS, CD, Ulcerative colitis (UC), osteoarthritis, Nonalcoholic steatohepatitis (Nash), Parkinson, ALS, lung fibrosis, silicosis, asbestosis, diabetic retinopathy, and age-related macular degeneration.

[00546] In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are administered to treat inflammation. The inflammation can be related to coronary artery disease (CAD), Type 2 diabetes, neurodegenerative diseases, or inflammatory bowel disease, but is not limited thereto.

[00547] In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are administered to treat a disease or disorder involving increased IL-1β production by activated monocytes, macrophages or dendritic cells. In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are administered to treat a disease or disorder involving increased IL-18 production by activated monocytes, macrophages or dendritic cells. In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are administered to treat a disease or disorder involving increased mature caspase 1 production by activated monocytes, macrophages or dendritic cells.

[00548] In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are administered to reduce IL-1β production by activated monocytes, macrophages or dendritic cells. In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are administered to reduce IL-18 production by activated monocytes, macrophages or dendritic cells. In some embodiments, single donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells are administered to reduce mature caspase 1 production by activated monocytes, macrophages or dendritic cells.

6.7.4. Methods of treating other disorders

[00549] In some embodiments, $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells) are administered to a patient in need of immune tolerization. In some embodiments, a method provided herein includes administering a therapeutically effective amount the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein, to a patient in need of immune tolerization.

[00550] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are administered to a patient to treat autoimmune disease.

[00551] In some embodiments, the autoimmune disease is selected from the group consisting of: autoimmune uveitis, psoriasis, vitiligo, alopecis areata, psoriatic arthritis, inflammatory bowel disease, Hashimoto's thyroiditis, autoimmune vasculitis, ulcerative colitis, bullous diseases, scleroderma, celiac disease, graves disease, systemic sclerosis, myasthenia gravis, anti-NMDA encephalitis, pemphigoid diseases (vulgaris and foliaceus), epidermolysis bullosa acquisita, thrombotic thrombocytopenic purpura, ididopathic thrombocytic purpora, autoantibody induced vascular inflammation, autoantibody induced carditis, rheumatoid arthritis, autoantibody induced rheumatoid arthritis, neuromyelitis optica spectrum disorders, systemic lupus erythematosus (SLE), multiple sclerosis (MS), sjögren's syndrome, autoimmune myopathies, type I diabetes, addison disease, pernicious anemia, autoimmune hepatitis, primary biliary cholangitis (PBC), autoimmune pancreatitis, goodpasture's disease, primary membranous nephropathy, ovarian insufficiency, autoimmune orchitis, dry eye disease, and idiopathic interstitial pneumonias. In some embodiments, the autoimmune disease is Crohn's disease, ulcerative colitis, celiac disease, type-1 diabetes, lupus, psoriasis, psoriatic arthritis, or rheumatoid arthritis. In some embodiments, the patient has an allergic or atopic disease. The allergic or atopic disease can be selected from the group consisting of: asthma, atopic dermatitis, and rhinitis. In some embodiments, the patient has a food allergy.

[00552] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are administered to prevent or reduce severity of pathogenic T cell response to cell and organ transplantation other than HSCT. In some embodiments, the method comprises the step of organ transplantation to the patient, either prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein. In certain embodiments, the organ is a kidney, a heart, a lung, a liver, or pancreatic islet cells. In preferred embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical

compositions provided herein prevents or reduces severity of host rejection of the organ transplantation.

[00553] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are administered to prevent or reduce immune response associated with gene therapy, *e.g.*, administration of recombinant Adenovirus, Adeno-Associated Virus (AAV), Herpes simplex virus (HSV), Retrovirus, Lentivirus, a non-integration Lentivirus, Alphavirus, Flavivirus, Rhabdovirus, Measles virus, Newcastle disease Virus, Poxvirus, or Picornavirus. In these embodiments, the method further comprises the step of administering a recombinant Adenovirus, Adeno-Associated Virus (AAV), Herpes simplex virus (HSV), Retrovirus, Lentivirus, a non-integrating lentivirus, Alphavirus, Flavivirus, Rhabdovirus, Measles virus, Newcastle disease Virus, Poxvirus, or Picornavirus to the patient, either prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition.

[00554] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are administered to prevent or reduce immune response against a recombinant viral vector other than AAV. In these embodiments, the method further comprises the step of administering a recombinant viral vector other than AAV to the patient, either prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition. Non-limiting examples of viral vectors other than AAV include: Herpes simplex virus (HSV), Retrovirus, Lentivirus, Alphavirus, Flavivirus, Rhabdovirus, Measles virus, Newcastle disease Virus, Poxvirus, or Picornavirus.

[00555] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are administered to prevent or reduce immune response associated with transplantation of iPS-derived tissues or cells. The iPS-derived tissues and cells include, but are not limited to cardiomyocytes, hepatocytes, epithelial cells, cartilage, bone and muscle cells, neurons.

[00556] In some embodiments, the method further comprises the step of administering an immunogenic therapeutic protein to the patient, either prior to or subsequent to administration

of the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition reduces immune responses against the immunogenic therapeutic protein. In some embodiments, the immunogenic therapeutic protein is selected from a therapeutic antibody, a factor VIII replacement, a cytokine, and a cytokine mutein.

[00557] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are administered to treat inflammation. The inflammation can be related to coronary artery disease (CAD), Type 2 diabetes, neurodegenerative diseases, non-alcohol steatohepatitis(NASH) , or inflammatory bowel disease, but is not limited thereto.

[00558] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are administered to treat a disease or disorder involving hyperactivity of NLPR3 inflammasome. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are administered to treat a disease or disorder involving increased IL-1β production by activated monocytes, macrophages or dendritic cells. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are administered to treat a disease or disorder involving increased IL-18 production by activated monocytes, macrophages or dendritic cells. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are administered to treat a disease or disorder involving increased mature caspase 1 production by activated monocytes, macrophages or dendritic cells.

[00559] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided

herein are administered to reduce IL-1β production by activated monocytes, macrophages or dendritic cells. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are administered to reduce IL-18 production by activated monocytes, macrophages or dendritic cells. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are administered to reduce mature caspase 1 production by activated monocytes, macrophages or dendritic cells.

[00560] In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (s autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are administered to reduce patient hyperactive immune response to viral infection. In some embodiments, the virus is SARS-coV-2. In some embodiments, the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein are administered to reduce hyperactive immune responses to bacterial infections, such as toxic shock, cytokine storm, a therapeutic antibody, a factor VIII replacement, a cytokine, and a cytokine mutein..

[00561] In another aspect, the present disclosure provides a method for treating or inhibiting autoimmune disease, allergic disease, or inflammatory disease in a patient, comprising the step of administering to a patient, identified as having autoimmune disease, allergic disease, or inflammatory disease, a therapeutically effective amount of the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein sufficient to treat or inhibit the autoimmune disease, allergic disease, or inflammatory disease.

[00562] In another aspect, the present disclosure provides a method for reducing transplant rejection in a patient transplanted with hematopoietic stem cells, bone marrow cells, or a solid organ, comprising the step of administering to a patient, identified as having rejection of transplanted hematopoietic stem cells, bone marrow cells, or a solid organ, a therapeutically

effective amount of the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein sufficient to reduce transplant rejection.

[00563] In another aspect, the present disclosure provides a method for treating graft-versus-host disease (GvHD) in a patient, comprising: administering to a patient, identified as having graft-versus-host disease (GvHD) or at risk of having graft-versus-host disease (GvHD), a therapeutically effective amount of the $CD4^{IL\text{-}10/CAR}$ cells (autologous single-donor, allogeneic single-donor, or allogeneic polydonor $CD4^{IL\text{-}10/CAR}$ cells), the population of single-donor or polydonor $CD4^{IL\text{-}10/CAR}$ cells, or any of the pharmaceutical compositions provided herein sufficient to suppress or prevent GvHD. In some embodiments, the GvHD is acute GvHD. In some embodiments, the GvHD is chronic GvHD.

[00564] In another aspect, the present disclosure features a method for treating tissue or organ damage (e.g., wound healing) in a patient, comprising: administering to a patient, identified as having tissue or organ damage or at risk of having tissue or organ damage, a therapeutically effective amount of any of the $CD4^{IL\text{-}10/CAR}$ cells provided herein, any of the populations of $CD4^{IL\text{-}10/CAR}$ cells provided herein, or the pharmaceutical composition provided herein sufficient to induce repair of tissue or organ damage.

6.8. Polynucleotides and vectors

[00565] This disclosure also features one or more polynucleotide constructs comprising (a) a first polynucleotide segment encoding a chimeric antigen receptor (CAR) (e.g., any of the CARs provided herein); and (b) a second polynucleotide segment encoding interleukin-10 (IL-10) and (c) a third polynucleotides segment encoding the truncated form of the NGFR. In some embodiments, the first polynucleotide segment and the second and third polynucleotide are in the same construct. In some embodiments, the first polynucleotide is in the first polynucleotide construct, and the second and third polynucleotide segment is in the second polynucleotide construct. In some embodiments, the third polynucleotide segment is optional.

[00566] In some embodiments, the first polynucleotide segment comprises a regulatory element (e.g., any of the exemplary regulatory elements (e.g., any of the promoters) described herein) operably linked to the coding sequence of the CAR. In some embodiments, the regulatory element drives expression of the CAR.

[00567] In some embodiments, the second polynucleotide segment comprises a regulatory element (e.g., any of the exemplary regulatory elements (e.g., any of the promoters) described herein) operably linked to a coding sequence of the IL-10. In some embodiments, the regulatory element drives constitutive expression of the IL-10.

[00568] In some embodiments, the third polynucleotide segment comprises a regulatory element (e.g., any of the exemplary regulatory elements (e.g., any of the promoters) described herein) operably linked to a coding sequence of the selection marker (e.g., ΔNGFR). In some embodiments, the regulatory element drives constitutive expression of the ΔNGFR.

[00569] In some embodiments, the polynucleotide construct includes an internal ribosome entry site (IRES) or a self-cleaving peptide between the first polynucleotide segment and the second polynucleotide segment. In some embodiments, the polynucleotide construct includes an internal ribosome entry site (IRES) or a self-cleaving peptide between the second polynucleotide segment and the first polynucleotide segment. In some embodiments, the self-cleaving peptide is selected from the group consisting of F2A, P2A, T2A and E2A.

[00570] In some embodiments, the polynucleotide construct includes from 5' to 3': a promoter operably linked to the first polynucleotide segment, a self-cleaving peptide or IRES, and a second polynucleotide segment. In some embodiments, the polynucleotide construct includes from 5' to 3': a promoter operably linked to the first polynucleotide segment, a self-cleaving peptide or IRES, and a second polynucleotide segment, and a second promoter operably linked to the third polynucleotide segment.

[00571] In some embodiments, the polynucleotide construct includes from 5' to 3': a promoter operably linked to the second polynucleotide segment, a self-cleaving peptide or IRES, and a first polynucleotide segment.

[00572] In some embodiments, the polynucleotide construct includes a first promoter operably linked to the first polynucleotide segment and a second promoter operably linked to the second polynucleotide segment. In some embodiments, the polynucleotide construct includes a first promoter operably linked to the first polynucleotide segment, a second promoter operably linked to the second polynucleotide segment, and a third promoter operably linked to the third polynucleotide segment.

[00573] In some embodiments, the polynucleotide construct encodes an antigen-binding domain that targets an antigen associated with an autoimmune disease, inflammatory disorder, or

cancer. In some embodiments, the antigen is selected from the group consisting of: CD19, CD20, CD22, CD27, BCMA, CD38, HLA*A2, HLA*A24 or citrullinated peptides, insulin, MOG, GAD65, IA2, gliadin, and desmoglein in the context of relevant MHC molecules.

[00574] In some embodiments, the polynucleotide construct includes a sequence encoding an antigen-binding domain that targets a cancer-associated antigen. In some embodiments, a cancer-associated antigen is selected from the group consisting of: CD19, CD20, CD22, CD23, CD27, CD38, CEA, BCMA, Lyml, Lym2, CLEC5A, CDH179b, FLT3, GCC, Muc, CSF2RA, GFRa4, CD32, CD33, IL1lRa, IL13Ra, NYBRI, SLea, CD200R, TGFBetaR2, CEA, CD276, TROP2, LAMP1, PTK7, DLL3, CDH1, CDH6, CDH17, CDH19, TSHR, B7-H3 and tyrosinase.

[00575] In some embodiments, the first polynucleotide segment comprises the sequence of SEQ ID NOs: 10, 17, 23, 35, or 55.

[00576] In some embodiments, the second polynucleotide segment comprises the sequence of SEQ ID NO: 2.

[00577] In some embodiments, the polynucleotide construct further comprises one or more selection markers (e.g., any of the selection markers provided herein). In some embodiments, the first polynucleotide segment, second polynucleotide segment, or both, further comprising a sequence encoding a selection marker.

[00578] In some embodiments, the polynucleotide construct includes a ΔNGFR as a selection marker.

[00579] In some embodiments, the polynucleotide construct includes a ΔNGFR comprising the sequence of SEQ ID NO: 4.

[00580] In some embodiments, the polynucleotide construct includes a truncated form of EGFR as a selection marker.

[00581] In some embodiments, the one or more polynucleotide constructs are one or more vectors. In some embodiments, the vector is a viral vector. Non-limiting examples of viral vectors include: lentivirus, retrovirus, gammaretroviruses, adeno-associated virus, adenovirus, helper-dependent adenovirus, sendai virus, or a baculovirus. In some embodiments, the polynucleotide construct is a lentiviral vector.

[00582] In some embodiments, the first polynucleotide is in the first lentiviral vector, and the second polynucleotide segment is in the second lentiviral vector.

[00583] In some embodiments, the lentiviral vector is capable of integrating into the T cell nuclear genome. In some embodiments, the lentiviral vector is not capable of integrating into T cell nuclear genome. In some embodiments, an integration-deficient lentiviral vector is used. For example, in some embodiments, an integration-deficient or other lentiviral vector disclosed in Mátrai is used. In some embodiments, an integrase-defective lentivirus is used. For example, an integrase-defective lentivirus containing an inactivating mutation in the integrase (D64V) can be used as described in Mátrai *et al.*, *Hepatology* 53:1696-1707 (2011), which is incorporated by reference herein, is used.

6.9. EXAMPLES

[00584] The following examples are provided by way of illustration not limitation.

6.9.1. Summary of experimental observations

[00585] Overall, the data in the experiments described below demonstrated that human CD4+T cells can be transduced with two different lentiviral vectors: 1) a LVV encoding a synthetic CAR comprising a human B cell surface antigen binding domain, a CD8α hinge, and a CD8α transmembrane domain, a CD28 costimulatory domain, and a CD3zeta activation domain and 2) a bidirectional vector encoding the human IL-10 gene, plus a truncated form of the NGFR gene. The resulting transduced $CD4^+$ T cells expressed both the CD19 CAR (as measured by CD19 expression) and IL-10/NGFR as measured by NGFR expression. No differences in transduction efficiencies were found, when the double transduced $CD4^+$ T cells (designated generically as $CD4^{IL\text{-}10/CAR}$ cells or as $CD4^{IL\text{-}10antiCD19CAR}$ cells when expressing the anti-CD19 CAR described in FIGs. 2A-2B) were generated following sequential or simultaneous transduction with the two different lentiviral vectors.

[00586] Following activation through CD3 and CD28, the $CD4^{IL\text{-}10/CAR}$ cells displayed cytokine production profiles similar to those of $CD4^{IL\text{-}10}$ cells and those described for Tr1 cells. For example, the $CD4^{IL\text{-}10/CAR}$ cells produced high levels of IL-10, and no or low levels of IL-4, IFN-γ, and IL-5. Notably, this cytokine production profile was stable after prolonged culture periods.

[00587] Interestingly, co-culture of $CD19^+$ target cells and $CD4^{IL\text{-}10antiCD19CAR}$ cells resulted in specific activation of the $CD4^{IL\text{-}10anti\text{-}CD19\ CAR}$ cells and induction of IL-10 production.

[00588] $CD4^{IL\text{-}10antiCD19CAR}$ cells suppressed PBMC proliferation, allogeneic $CD4^+$ T cell proliferation, and allogeneic $CD8^+$ T cell proliferation *in vitro*, as also seen with $CD4^{IL\text{-}10}$ cells.

[00589] $CD4^{IL\text{-}10antiCD19CAR}$ cells specifically killed $CD19^+$ target cells *in vitro*, but like $CD4^{IL\text{-}10}$ cells, they maintained their ability to kill myeloid target cells, which depend on the expression of one or more of Class I MHC, CD13, CD54 and CD112. In addition, the $CD4^{IL\text{-}10anti\text{-}CD19\ CAR}$ T cells strongly inhibited $CD19^+$ target cell growth vivo. Importantly, single donor and polydonor $CD4^{IL\text{-}10antiCD19\ CAR}$ cells, similarly as described for single donor and polydonor $CD4^{IL\text{-}10}$ cells, did not induce xeno-GvHD, whereas PBMC's induced severe xeno-GvHD. Finally, the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells synergized with the PBMC in their antitumor effect, indicating that they do not interfere with protective GvL responses, as described for $CD4^{IL\text{-}10}$ cells (Locafaro et al. Mol Ther. 2017;25(10):2254-2269). In addition, the single donor and polydonor $CD4^{IL\text{-}10antiCD19\ CAR}$ cells protected mice from xeno-GvHD in a humanized mouse model of GvHD induced by allogeneic PBMC (see **FIGs. 18A-18C**).

[00590] This data showed that specific interactions between the $CD4^{IL\text{-}10anti\text{-}CD19\ CAR}$ and CD19 expressed on the target cells induce activation of these cells resulting in specific killing of $CD19^+$ target cells and IL-10 production.

[00591] Taken together, the data showed $CD4^{IL\text{-}10/CAR}$ T cells have unique clinical utility for the treatment of all B-cell and T-cell mediated auto immune diseases because they will not only eliminate autoantibody producing B cells but also simultaneously suppress pathogenic autoimmune $CD4^+$ T cell and $CD8^+$ T cell responses through the production of IL-10. The data also showed that single donor or polydonor $CD4^{IL\text{-}10antiCD19\ CAR}$ cells have unique clinical utility for the treatment/elimination of $CD19^+$ tumor cells and prevention of tumor relapses, particularly in patients undergoing allogeneic HSCT, as the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells did not induce GvHD by themselves and did not interfere with the protective anti-tumor effects (GvL effects) of the PBMC.

[00592] Importantly, even at high concentrations, $CD4^{IL\text{-}10antiCD19\ CAR}$ cells (either autologous or allogeneic) did not induce GvHD by themselves. These results demonstrate that $CD4^{IL\text{-}10/CAR}$ cells can be used for treatment of i) leukemias and other malignancies expressing the target antigen, ii) leukemias and other malignancies in patients undergoing allogeneic HSCT or BM transplantation to reduce GvHD while preserving GvL or GvT therapeutic effects of the HSCT, iii) auto immune diseases through the removal of auto immune B-cells, and down regulation of pathogenic T cell responses and the NLPR3 inflammasome, iv) inflammatory

diseases through down regulation of pathogenic T cell responses and the NLPR3 inflammasome, v) cell and organ transplant rejection, vi) solid tumors expressing tumor associated antigens including but not limited to B7-H3 and vii) immune-mediated diseases through tissue repair and wound healing.

6.9.2. Example 1: Generation of $CD4^{IL\text{-}10/anti\text{-}CD19\ CAR}$ cells

6.9.2.1. Vector production for IL-10 / ΔNGFR and anti-CD19 CAR

[00593] A second generation CD19 CAR was used for the production of $CD4^{IL\text{-}10anti\text{-}CD19\ CAR}$ cells. The CD19 CAR included a scFv from a fully-human anti-CD19 monoclonal antibody (SEQ ID NO: 12), a CD8α hinge, and a CD8α transmembrane domain, a CD28 costimulatory domain, and a CD3ζ (CD3 zeta) activation domain (FIG. 2A). The CD19 CAR as shown in FIG. 2A was inserted into a lentiviral vector (LVV) (see FIG. 2B). See FIG. 1 for illustration of exemplary CARs targeting CD19, CD20, CD22, BCMA or B7H3. The lentiviral vector was generated by ligating the coding sequence of the anti-CD19 scFv (FMC63) into the lentiviral backbone including the human CD8α hinge region, and CD8α trans membrane region, the CD28 co stimulatory domain and the CD3ζ intracellular signaling domain. Surface expression of the anti-CD19 CAR was monitored with an anti-CD19-CAR detection agent (Miltenyi).

[00594] To introduce a polynucleotide encoding IL-10 into the $CD4^+$ T cells to produce the $CD4^{IL\text{-}10anti\text{-}CD19\ CAR}$ cells a lentiviral vector (LVV) containing coding sequences of both the human IL-10 (SEQ ID NO: 2) and a truncated form of the NGFR (also known as CD271) (ΔNGFR) (SEQ ID NO: 4) (FIG. 4) was used. The LVV containing IL-10 and ΔNGFR coding sequence is further described in WO2016/146542, which is hereby incorporated by reference in its entirety.

[00595] The lentiviral vector was generated by ligating the coding sequence of human IL-10 from 549 bp fragment of pH15C (ATCC 68192)) into plasmid pLVIL-10. The presence of the bidirectional promoter (human PGK promoter plus minimal core element of the CMV promoter in the opposite direction) allowed co-expression of the two transgenes. The plasmid further contained a coding sequence of an antibiotic resistance gene (e.g., ampicillin or kanamycin). See FIG. 3A and FIG. 3B for exemplary illustration of the IL-10 construct.

[00596] The lentiviral vectors were produced by Ca_3PO_4 transient four-plasmid co-transfection into 293T cells and concentrated by ultracentrifugation: 1 μM sodium butyrate was added to the cultures for vector collection. Titer was estimated on 293T cells by limiting

dilution, and vector particles are measured by HIV-1 Gag p24 antigen immune capture (NEN Life Science Products; Waltham, MA). Vector infectivity was calculated as the ratio between titer and particle. For concentrated vectors, titers ranged from $1x10^8$ to $6x10^9$ transducing units/mL, and infectivity from $5x10^4$ to $5x10^5$ transducing units/ng.

6.9.2.2. Production of $CD4^{IL\text{-}10/CAR}$ cells

[00597] As shown in FIGs. 5A-5C, three different methods were used to generate $CD4^{IL\text{-}10antiCD19\ CAR}$ cells. For each of the different methods, $CD4^+$ T cells were purified from healthy human donors. The purified human $CD4^+$ T cells were activated with anti-CD3/anti-CD28 beads (Miltenyi) (3:1 cells:beads ratio), in complete culture medium (X-vivo supplemented with 5% human AB serum and rhIL-2 (50U/mL).

[00598] FIG. 5A shows that the activated $CD4^+$ T cells were transduced with a lentiviral vector encoding an anti-CD19 CAR ($LVV^{CD19\text{-}CAR}$) 48 hours after activation and transduced 24 hours later (i.e., 72 hours after activation) with a bidirectional lentiviral vector encoding human IL-10 and a truncated form of the human NGF receptor ($LVV^{IL\text{-}10\text{-}NGFR}$). Both LVVs were used at a multiplicity of infection (MOI) of 20.

[00599] FIG. 5B shows that activated $CD4^+$ T cells were transduced with the $LVV^{IL\text{-}10\text{-}NGFR}$ 48 hours after activation and transduced 24 hours later (i.e., 27 hours after activation) with $LVV^{CD19\text{-}CAR}$.

[00600] FIG. 5C shows the activated $CD4^+$ T cells were transduced simultaneously with $LVV^{IL\text{-}10\text{-}NGFR}$ and $LVV^{CD19\text{-}CAR}$ 48 hours after activation. Controls $CD4^{IL10}$ cells were generated by transducing activated $CD4^+$ T cells 48 hours after activation with $LVV^{IL\text{-}10\text{-}NGFR}$ only. For each of FIGs. 5A-5C, the transductions were conducted in the presence of polybrene (8 μg/mL). Additionally, for each of FIGs. 5A-5C,10-28 days after transduction, transduced cells (i.e., $\Delta NGFR^+$ cells) were purified using anti-CD271 microbeads (Miltenyi) and anti-CD19 CAR microbeads (Miltenyi). Purified cells were re-stimulated every 14 days as previously described (Andolfi et al. Mol. Ther. 20(9):1778-1790 (2012) and Locafaro et al. Mol. Ther. 25(10): 2254-2269 (2017)). After the second (TF2) and third (TF3) re-stimulation the resulting cells ($CD4^{IL\text{-}10antiCD19\ CAR}$) were characterized in vitro and in vivo. Culture medium was refreshed every 2-3 days, as needed through the entire culture period.

[00601] $CD4^{IL\text{-}10antiCD19\ CAR}$ cells were analyzed at TF3 (i.e., after the third re-stimulation) by staining with an anti-CD271 mAb (Biolegend) and an anti-CD19 CAR detection

reagent (Miltenyi) and analyzed by flow cytometry. Staining indicated that identity and the purity of the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells determine as co-expression of NGFR and the anti-CD19 CAR for the three different transduction methods was 88.6% (FIG. 6A from method described in FIG. 5A), 64.1% (FIG. 6B from method described in FIG. 5B) and 52.6% (FIG. 6C from method described in FIG. 5C), respectively. As expected, $CD4^{IL\text{-}10}$ cells generated respectively from the same donors (donor 26.1 and 26.2) using the method described in FIG. 5C and used as controls did not express the CD19 CAR (FIG. 7).

[00602] As shown in FIG. 7, $CD4^{IL\text{-}10antiCD19\ CAR}$ cells expressed both IL-10 (as measured by the expression of NGFR) and the anti-CD19 CAR. In this experiment, $CD4^+$ T cells from two different donors (26.1 and 26.2) were transduced simultaneously with $LVV^{IL\text{-}10\text{-}NGFR}$ and $LVV^{anti\text{-}CD19\ CAR}$ 48 hours after bead activation (method 5C). To generate the $CD4^{IL\text{-}10}$ T cells (control), $CD4^+$ T cells from donors 26.1 and 26.2 were transduced solely with $LVV^{IL\text{-}10\text{-}NGFR}$ 48 hours after bead activation. For this analysis, $CD4^{IL\text{-}10}$ cells (control) and $CD4^{IL\text{-}10antiCD19\ CAR}$ cells were analyzed following a second round of restimulation (i.e., TF2) with feeder cells. Both $CD4^{IL\text{-}10}$ cells (control) and $CD4^{IL\text{-}10anti\text{-}CD19\ CAR}$ T cells were stained with anti-CD271 mAb (Biolegends) and a CD19-CAR detection reagent (Miltenyi) and analyzed by flow cytometry. As shown in FIG. 7, the majority of the cells transduced with $LVV^{IL\text{-}10\text{-}NGFR}$ and $LVV^{CD19\text{-}CAR}$ expressed CD271 and the CD19-CAR (FIG. 7, right panels) whereas the majority of the cells that were only transduced with $LVV^{IL\text{-}10\text{-}NGFR}$ expressed CD271, but not the CD19-CAR (FIG. 7, left panels).

[00603] These results indicate that $CD4^{IL\text{-}10antiCD19\ CAR}$ cells can be successfully generated independent of timing and sequence of transduction with the two different LVVs. In addition, this suggest that $CD4^{IL\text{-}10/CAR}$ can be generated using a single LVV encoding both the IL-10 and the CAR.

6.9.2.3. $CD4^{IL\text{-}10antiCD19\ CAR}$ cells have a cytokine production profile which is comparable to that of naturally derived Tr1 cells and $CD4^{IL\text{-}10}$ cells

[00604] $CD4^{IL\text{-}10antiCD19\ CAR}$ cells from 3 different healthy donors (26.1, 26.2 and 26.3) were generated by simultaneous transduction with (i) LVV containing anti-CD19-CAR and (ii) LVV containing IL-10 and ΔNGFR according to the methods described in FIG. 5C. In particular, to generate the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells, $CD4^+$ T cells from the three different donors (26.1, 26.2, and 26.3) were transduced simultaneously with $LVV^{IL\text{-}10\text{-}NGFR}$ and $LVV^{CD19\text{-}CAR}$ 48 hours after bead activation. To generate the $CD4^{IL\text{-}10}$ T cells (control), $CD4^+$ T cells from the

three different donors (26.1, 26.2, and 26.3) were transduced with $LVV^{IL\text{-}10\text{-}NGFR}$ 48 hours after bead activation.

[00605] For this analysis, $2x10^5$ cells (in 200 µL) for each $CD4^{IL\text{-}10}$ cells (control) and $CD4^{IL\text{-}10/CAR}$ T cells were restimulated as previously described in Andolfi et al. Mol. Ther. 20(9): 1778-1790 (2012) and Locafaro et al. Mol. Ther. 25(10): 2254-2269 (2017). At day 14 after the second round (TF2) of restimulation, cells were stimulated with immobilized anti-CD3 and soluble anti-CD28 mAbs for 48 hours. Culture supernatants were collected and levels of IL-10, IL-4, IL-5, and IFN-γ were determined by ELISA.

[00606] As shown in FIGs. 8A-8D, although some individual differences in cytokine production levels were observed, the $CD4^{IL\text{-}10/CAR}$ cells, like $CD4^{IL\text{-}10}$ cells and Tr1 cells (not shown), produced high levels of IL-10, low levels of IL-4, and variable levels of IFN-γ, and IL-5.

[00607] Similar results were obtained with $CD4^{IL\text{-}10antiCD19\ CAR}$ cells derived from donor 24.2, which were collected at TF 3 (FIG. 9). To generate the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells for this experiment, $CD4^+$ T cells from donor 24.2 were transduced simultaneously with $LVV^{IL\text{-}10\text{-}NGFR}$ and $LVV^{CD19\text{-}CAR}$ 48 hours after bead activation (see FIG. 5C). To generate the $CD4^{IL\text{-}10}$ T cells (control), $CD4^+$ T cells from donor 24.2 were transduced solely with $LVV^{IL\text{-}10\text{-}NGFR}$ 48 hours after bead activation. For this analysis, $2x10^5$ cells (in 200 µL) for each $CD4^{IL\text{-}10}$ T cells (control) and $CD4^{IL\text{-}10/CAR}$ T cells were restimulated as previously described in Andolfi et al. Mol. Ther. 20(9): 1778-1790 (2012) and Locafaro et al. Mol. Ther. 25(10): 2254-2269 (2017). Briefly, at day 14 after the third round (TF3) of restimulation, cells were stimulated with immobilized anti-CD3 and soluble anti-CD28 mAbs for 48 hours. Culture supernatants were collected, and levels of IL-10, IL-4, IL-5, and IFN-γ were determined by ELISA.

[00608] As shown in FIG. 9, even after prolonged culture these cells still produce high levels of IL-10, low levels of IL-4, and variable levels of IL-5 and IFN-γ.

[00609] Collectively, the data in FIGs. 8A-8D and FIG. 9 indicate that simultaneous transduction of purified $CD4^{IL\text{-}10}$ cells with $LVV^{IL\text{-}10\text{-}NGFR}$ and $LVV^{CD19\text{-}CAR}$ is feasible and results in the generation of $CD4^{IL\text{-}10\text{-}CAR}$ T cells with the same unique and characteristic cytokine production profile as $CD4^{IL\text{-}10}$ T cells and Tr1 cells. In addition, no differences in the cytokine production profiles were observed when $CD4^{IL\text{-}10\text{-}CAR}$ T were harvested at TF2 or TF3, indicating that these cytokine production profiles are stable over prolonged periods of time.

6.9.3. Example 2: Cytotoxicity of $CD4^{IL\text{-}10antiCD19\ CAR}$ cells and activation following engagement with $CD19^+$ target cells

[00610] It was known that $CD4^{IL\text{-}10}$ cells are able to kill myeloid tumor cells such as ALL-CM under defined conditions (Andolfi et al. Mol Ther. 2012;20(9):1778-1790). This experiment was designed to assess whether co-expression of an anti-CD19 CAR produces cells ($CD4^{IL\text{-}10antiCD19\ CAR}$) that can kill $CD19^+$ cells when co-cultured with a cell line expressing CD19 (e.g., NALM6).

[00611] In particular, $CD4^{IL\text{-}10}$ cells (control) and $CD4^{IL\text{-}10antiCD19\ CAR}$ cells, both generated from $CD4^+$ T cells isolated from donor 24.2 were collected following a third restimulation (TF3) (see FIG. 5C) and were co-cultured with K562, NALM6 ($CD19^+$), or ALL-CM target cells (10^5 cells/well) at a 1:1 ratio for three days. Following the three day co-culture, flow cytometry was used to determine the presence of residual target cells in each co-culture.

[00612] The data showed that $CD4^{IL\text{-}10antiCD19\ CAR}$ cells, through engagement of the CD19-CAR with $CD19^+$ target cells, kill $CD19^+$ NALM6 cells. In contrast, $CD4^{IL\text{-}10}$ cells used as controls were ineffective and did not kill $CD19^+$ NALM6 cells (FIG. 10). Furthermore, $CD4^{IL\text{-}10antiCD19\ CAR}$ cells, like $CD4^{IL\text{-}10}$ cells, killed the myeloid tumor cell line ALL CM. Both the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells and the $CD4^{IL\text{-}10}$ cells had no, or very low cytotoxic effect on K562 cells, which are highly susceptible to non-specific cytotoxicity and to killing by NK cells (FIG. 10).

[00613] These results indicate that engagement of the CD19-CAR expressed on the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells with CD19 expressed on the NALM6 lymphoma cells triggers the cytotoxic machinery of the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells, resulting in selective and efficient killing of $CD19^+$ target cells. In addition, $CD4^{IL\text{-}10antiCD19\ CAR}$ cells, like $CD4^{IL\text{-}10}$ cells, maintain the ability to kill myeloid target cells that express one or more of CD13, Class I MHC antigens, CD54 and CD112 as previously described (Andolfi et al. Mol Ther. 2012;20(9):1778-1790).

[00614] In addition to inducing selective cytotoxic activity, engagement of the $CD4^{IL\text{-}10antiCD19\ CAR}$ with $CD19^+$ NALM6 target cells resulted in selective activation and IL-10 production by the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells.

[00615] For these experiments, $CD4^{IL\text{-}10}$ cells (control) and $CD4^{IL\text{-}10antiCD19\ CAR}$ cells were generated from $CD4^+$ T cells isolated from three different donors (26.1, 26.2, and 26.3). Prior to assessing IL-10 production, selective killing of $CD19^+$ cells was confirmed for $CD4^{IL\text{-}10antiCD19\ CAR}$ cells generated from each of the three different donors.

[00616] In particular, $CD4^{IL\text{-}10}$ cells (control) and $CD4^{IL\text{-}10antiCD19\ CAR}$ cells generated from $CD4^+$ T cells isolated from three different donors (26.1, 26.2, and 26.3) were collected following a second restimulation (TF2) (see FIG. 5C) and were co-cultured with K562 cells, NALM6 ($CD19^+$) cells, or ALL-CM target cells (10^5 cells/well) at a 1:1 ratio for three days. Following the three day co-culture, flow cytometry was used to determine the presence of residual leukemic target cells in each co-culture. The K562 cell line, which is highly susceptible to non-specific cytotoxicity and to killing by NK cells, served as a control. The data are shown in FIG. 11A.

[00617] As shown in FIG. 11A, $CD4^{IL\text{-}10antiCD19\ CAR}$ cells generated from 3 different donors (26.1, 26.2 and 26.3) efficiently killed $CD19^+$ NALM6 cells. $CD4^{IL\text{-}10antiCD19\ CAR}$ cells maintained their capacity to kill the myeloid tumor cell line ALL-CM, whereas they were not, or were only weakly cytotoxic for K562 cells, which do not express CD19, but are known to be sensitive to non-specific cytotoxicity and killing by NK cells (FIG. 11A). $CD4^{IL\text{-}10antiCD19\ CAR}$ also killed ALL-CM, a myeloid leukemia cell line. Significant amounts of IL-10 were measured in the supernatants of the cytotoxicity assays after only 3 days of co-culture with NALM6 cells (FIG. 11B). In contrast, no IL-10 (limit of quantification: 31 pg/mL) could be detected in co-cultures of NALM6 cells with the $CD4^{IL\text{-}10}$ cells from the same donor (FIG. 11B).

[00618] Taken together, the data showed that $CD4^{IL\text{-}10antiCD19\ CAR}$ cells are selectively activated by engagement of the CD19-CAR with CD19 expressed on relevant target cells. This activation led to (i) cytotoxic/cytolytic activity of the cells resulting in killing of the $CD19^+$ target cells and (ii) induction of IL-10 production, which is required for the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells immune regulatory and suppressor functions. Furthermore, the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells maintain the same cytotoxic capabilities towards myeloid tumor target cells that was previously observed with $CD4^{IL\text{-}10}$ cells. Lastly, the failure of $CD4^{IL\text{-}10}$ cells to kill $CD19^+$ NALM6 cells (FIG. 11B), demonstrated that the cytotoxic activities of the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells was due to anti-CD19 CAR-engagement with $CD19^+$ target cells.

6.9.4. Example 3: $CD4^{IL\text{-}10antiCD19\ CAR}$ cells suppress the proliferative responses of both allogeneic $CD4^+$ and $CD8^+$ T cells

[00619] As $CD4^{IL\text{-}10antiCD19\ CAR}$ cells and $CD4^{IL\text{-}10}$ cells harvested at TF2 or TF3 each produce high levels of IL-10, both were tested for their ability to suppress the proliferation of allogeneic $CD4^+$ and $CD8^+$ T cells taken from healthy donors.

[00620] To assess proliferation of allogeneic $CD4^+$ T cells, allogeneic PBMC cells were labeled with eFluor® 670 ($1x10^5$ cells per well) and stimulated with mature allogeneic dendritic cell (DCs) ($5x10^4$ cells per well) and soluble anti-CD3 mAbs in the presence or absence of $CD4^{IL\text{-}10}$ cells (control) or $CD4^{IL\text{-}10antiCD19\ CAR}$ cells ($1x10^5$ cells per well) isolated after a second restimulation (see FIG. 5C) at a 1:1 Responder:Suppressor ratio. After 4 days of culture, the percentage of $CD4^+$ proliferating responder T cells was determined by eFluor®670 dilution with flow cytometry. For flow cytometry, cells were gated on $CD4^+\Delta NGFR^-$ T cells.

[00621] To assess proliferation of allogeneic $CD8^+$ T cells, allogeneic PBMC cells were labeled with eFluor® 670 ($1x10^5$ cells per well) and stimulated with allogeneic mature dendritic cell (DCs) ($5x10^4$ cells per well) and soluble anti-CD3 mAbs in the presence or absence of $CD4^{IL\text{-}10}$ cells (control) or $CD4^{IL\text{-}10antiCD19\ CAR}$ cells ($1x10^5$ cells per well) isolated after a second restimulation (see FIG. 5C) at a 1:1 Responder:Suppressor ratio. After 4 days of culture, the percentage of $CD8^+$ proliferating responder T cells was determined by eFluor®670 dilution with flow cytometry. For flow cytometry, cells were gated on $CD8^+\Delta NGFR^-$ T cells.

[00622] To assess proliferation of allogeneic PBMC, allogeneic PBMC cells were labeled with eFluor® 670 ($1x10^5$ cells per well) and stimulated with allogeneic mature dendritic cell (DCs) ($5x10^4$ cells per well) and soluble anti-CD3 mAbs in the presence or absence of $CD4^{IL\text{-}10}$ (control) or $CD4^{IL\text{-}10antiCD19CAR}$ cells ($1x10^5$ cells per well) isolated after a second restimulation (see FIG. 5C) at a 1:1 Responder:Suppressor ratio. After 4 days of culture, the percentage of $CD8^+$ proliferating responder T cells was determined by eFluor®670 dilution with flow cytometry. For flow cytometry, cells were gated on $CD3^+\Delta NGFR^-$ T cells.

[00623] As shown in FIG. 12, $CD4^{IL\text{-}10antiCD19\ CAR}$ cells derived from 3 different healthy donors 26.1, 26.2, and 26.3 harvested at TF2, suppress the proliferation of allogeneic $CD4^+$ T cells by 78%, 48%, and 74%, respectively. They also suppress the proliferation of allogeneic $CD8^+$ T cells by 77%, 41%, and 42%, respectively (FIG. 13). Comparable results were obtained with $CD4^{IL\text{-}10}$ cells generated from the same 3 donors (i.e., 26.1, 26.2, and 26.3), which inhibited the proliferation of allogeneic $CD4^+$ T cells by 83%, 81%, and 61%, respectively, and $CD8^+$ T cells by 77%, 41%, and 68%, respectively (FIG. 12 and FIG. 13). Finally, $CD4^{IL\text{-}10antiCD19\ CAR}$ cells and $CD4^{IL\text{-}10}$ cells were also effective in suppressing the proliferation of allogeneic T cells when harvested at TF3. In particular, the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells, like $CD4^{IL\text{-}10}$ cells, suppressed the proliferation of the PBMC by 69% and 60%, respectively (FIG. 14).

[00624] Taken together, these results showed that $CD4^{IL\text{-}10/CAR}$ cells have immunosuppressive effects on both allogeneic $CD4^+$ T cells and allogeneic $CD8^+$ T cells, and the immunosuppressive effects are comparable to those of $CD4^{IL\text{-}10}$ cells; co-expression of the CAR did not abrogate or alter the immunosuppressive effects of $CD4^{IL\text{-}10}$ cells. The results also showed that the immune suppressive function is stable after a prolonged culture period (i.e., in $CD4^{IL\text{-}10antiCD19\ CAR}$ T cells isolated after TF3).

6.9.5. Example 4: Treatment or prevention of GvHD and cancer using $CD4^{IL\text{-}10antiCD19\ CAR}$ cells

Effects of $CD4^{IL\text{-}10antiCD19\ CAR}$ cells in vivo.

[00625] A population of $CD4^{IL\text{-}10antiCD19\ CAR}$ cells were tested in a humanized xeno-GvHD disease and tumor transplant NSG mouse model for their effect on $CD19^+$ tumor growth, GvL effects, and induction of GvHD.

[00626] According to their body weight, NSG mice were sub-lethally irradiated with 175-200 Gr. At day 0, NSG mice were injected with (i) NALM6-luciferase ($1x10^5$ per mouse), (ii) NALM6-luciferase ($1x10^5$ per mouse) + PBMC ($2.5x10^6$ per mouse), (iii) NALM6-luciferase ($1x10^5$ per mouse) + $CD4^{IL\text{-}10antiCD19\ CAR}$ cells ($2.5x10^6$ per mouse), or (iv) NALM6-luciferase ($1x10^5$ per mouse) + PBMC ($2.5x10^6$ per mouse) + $CD4^{IL\text{-}10antiCD19\ CAR}$ cells ($2.5x10^6$ per mouse) (see FIG. 15).

[00627] Effects on leukemia cell growth were determined by a standard bioluminescence measurements (see FIGs. 16A-16B). In addition, the mice were monitored for development of GvHD by measuring weight at days 0, 9, 12, and 15, after administration of PBMC and/or the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells (see FIG. 17).

[00628] As shown in FIGs. 16A-16B, although the PBMC inhibited NALM6 cell growth (FIGs. 16A-16B), modeling the graft-versus-leukemia effect of an allo-HSCT in human patients, they induced xeno-GvHD in the mice (FIG. 17), analogous to the GvHD seen in human allo-HSCT therapy. In contrast, the $CD4^{IL\text{-}10antiCD19\ CAR}$ cells inhibited NALM6 cell growth (FIGs. 16A-16B) and did not cause any signs of xeno-GvHD (FIG. 17) as determined by weight loss. Treatment with PBMC + $CD4^{IL\text{-}10/CAR}$ T cells had greatest anti-leukemia effect (FIG. 16A-16B) and greatest anti-tumor effect (FIG. 17).

[00629] These data indicate that $CD4^{IL\text{-}10antiCD19\ CAR}$ T cells are strongly cytotoxic and selectively kill $CD19^+$ target cells in vivo. In addition, they do not interfere with the protective

GvL effects of the PBMC; indeed, they work synergistically. In contrast to the PBMC, treatment with the $CD4^{IL\text{-}10antiCD19\ CAR}$ T cells alone, or in conjunction with PBMC, did not induce xeno-GvHD.

6.9.6. Example 5: $CD4^{IL\text{-}10antiCD19\ CAR}$ cells suppressive activity in vivo and effectively prevent severe xeno-GvHD

[00630] An additional study described in FIG. 18A was used to assess whether single donor or polydonor $CD4^{IL\text{-}10antiCD19\ CAR}$ cells induced GvHD and whether they protected from GvHD induced by PBMC. In particular, humanized NSG mice were sub-lethally irradiated and GvHD was induced by allogeneic PBMC. Mice were treated according to FIG. 18A. On day 3 mice were dosed intravenously with (i) PBMC (2.5E6 cells/mouse), (ii) single donor (BC26.3) $CD4^{IL\text{-}10antiCD19\ CAR}$ cells (2.5E6 cells/mouse), (iii) polydonor (BC-26.1, BC26.2, and BC26.3) $CD4^{IL\text{-}10antiCD19\ CAR}$ cells (2.5E6 cells/mouse), (iv) PBMC (2.5E6 cells/mouse) and single donor (BC26.3) $CD4^{IL\text{-}10antiCD19\ CAR}$ cells (2.5E6 cells/mouse), or (v) PBMC (2.5E6 cells/mouse) and polydonor (BC-26.1, BC26.2, and BC26.3) $CD4^{IL\text{-}10antiCD19\ CAR}$ cells (2.5e6 cells/mouse) (see FIG. 18A). GvHD was determined using a composite score of weight loss, fur appearance, skin appearance, hunch, and activity (Bondanza et al. 2006). Mice with a combined score ≥ 6 and / or a weight loss $\geq 20\%$ were euthanized for humane reasons. The donor of the PBMC was unrelated to the donors used to generate the single donor $CD4^{IL\text{-}10anti\text{-}CD19CAR}$ and polydonor $CD4^{IL\text{-}10antiCD19\ CAR}$.

[00631] As shown in FIG. 18B and FIG. 18C, the injection of 2.5E6 PBMC cells induced fulminant and rapidly lethal xeno-GvHD by day 16, whereas mice that received 2.5E6 single donor $CD4^{IL\text{-}10antiCD19\ CAR}$ cells (FIG. 18B) or 2.5E6 polydonor $CD4^{IL\text{-}10antiCD19\ CAR}$ cells (FIG. 18C) did not develop any signs of xeno-GvHD. Furthermore, when mice received both 2.5E6 PBMC and 2.5E6 single donor $CD4^{IL\text{-}10antiCD19\ CAR}$ cells (FIG. 18B) or 2.5E6 PBMC and 2.5E6 polydonor $CD4^{IL\text{-}10antiCD19\ CAR}$ cells (FIG. 18C), the co-administration of PBMC and single donor $CD4^{IL\text{-}10antiCD19\ CAR}$ cells or polydonor $CD4^{IL\text{-}10antiCD19\ CAR}$ protected the mice against PBMC-induced xeno-GvHD.

[00632] These results indicate that single donor $CD4^{IL\text{-}10antiCD19\ CAR}$ cells and polydonor $CD4^{IL\text{-}10antiCD19\ CAR}$ do not induce xeno-GvHD in vivo. In addition, single donor $CD4^{IL\text{-}10antiCD19\ CAR}$ cells and polydonor $CD4^{IL\text{-}10antiCD19\ CAR}$ effectively prevent severe xeno-GvHD induced by PBMC, demonstrating that they have suppressive activity in vivo.

Other experiments

[00633] The mice in the treatment groups are monitored for additional periods to determine effects of the $CD4^{IL\text{-}10/\ CAR}$ cells on long term survival.

[00634] The amount and localization of the $CD4^{IL\text{-}10/\ CAR}$ cells are also monitored in peripheral blood and tissues after administration. Specifically, presence of $CD4^{IL\text{-}10/\ CAR}$ cells are monitored in peripheral blood and at sites of inflammation: lymph nodes, spleen, gut, and bone marrow. The mice in the treatment group(s) are monitored for an additional 3 weeks to determine long-term survival.

[00635] The results demonstrate reduction and prevention of xeno-GvHD persists.

6.9.7. Example 6: Treatment of chronic inflammatory and autoimmune diseases using $CD4^{IL\text{-}10/\ CAR}$ cells

[00636] Activation of the NLPR3 inflammasome has been implicated in many chronic inflammatory and autoimmune diseases. The NLPR3 inflammasome can be activated by "danger signals" which lead to caspase1-mediated production of the pro-inflammatory cytokines IL-1β and IL-18 by monocytes/macrophages. A series of in vitro experiments are performed to investigate the effects of $CD4^{IL\text{-}10/anti\text{-}CD19\ CAR}$ cells on the NLPR3 inflammasome and IL-1 β/IL-18 production by human monocytes.

[00637] First, human PBMC are isolated from peripheral blood by standard density centrifugation on Ficoll/Paque (Sigma-Aldrich). Monocytes are isolated from the human PBMC by negative selection using monocyte isolation kit II (Miltenyi) according to the manufacturer's instructions. Negative selection is preferred because positive selection or adherence can lead to undesired activation of the cells. Isolated monocytes are plated at $5x10^4$ cells/200 µl in the presence of various dilutions of supernatants from $CD4^{IL\text{-}10/anti\text{-}CD19\ CAR}$ cells /200 µl per well in 96-well microtiter plates in culture medium containing 3% toxin free human AB serum.

[00638] Table 1 summarizes treatment conditions applied to different sets of monocytes, each set including 6 wells of cells. Monocytes are activated by LPS in combination with the NLPR3 inflammasome activator nigericin. The caspase 1 inhibitor Z-YVADfmk and the specific NLPR3 inhibitor MC950 are added as indicated as controls.

Table 1

Group #	Monocytes	CD4 cells/supernatant	YVADfmk*	LPS** plus nigericin	Other
Medium control					

Incubation time: 1 hour; followed by activation by LPS for 4 hours					
1	Monocytes	No	No	No	
2	Monocytes	No	No	LPS + Nig	
3	Monocytes	No	Z-YVADfmk	LPS + Nig	
4	Monocytes	No	MCC950	LPS + Nig	
Culture of monocytes in the presence of supernatants* obtained from single donor or polydonor $CD4^{IL-10/CAR}$ cell- or from $CD4^{GFP}$ cell-culture**					
5	Monocytes	50% supernatant of single donor $CD4^{IL-10/CAR}$ cells	No	LPS + Nig	
6	Monocytes	25% supernatant of single donor $CD4^{IL-10/CAR}$ cells	No	LPS + Nig	
7	Monocytes	12.5%supernatant of single donor $CD4^{IL-10/CAR}$ cells	No	LPS + Nig	
8	Monocytes	50% supernatant of single donor $CD4^{IL-10/CAR}$ cells	No	LPS + Nig	Anti-IL-10 receptor antibody
9	Monocytes	50% supernatant of poly donor $CD4^{IL-10/CAR}$ cells	No	LPS + Nig	
10	Monocytes	25% supernatant of poly donor $CD4^{IL-10/CAR}$ cells	No	LPS + Nig	
11	Monocytes	12.5%supernatant of poly donor $CD4^{IL-10/CAR}$ cells	No	LPS + Nig	
12	Monocytes	50% supernatant of poly donor $CD4^{IL-10/CAR}$ cells	No	LPS + Nig	Anti-IL-10 receptor antibody
13	Monocytes	50% supernatant of $CD4^{GFP}$ cells	No	LPS + Nig	

* Z-YVADfmk is an inhibitor specific to caspase 1. 20 µM Z-YVADfmk (Biovision, Enzo Life Sciences, or Axxora Life Sciences) dissolved in DMSO is used.
** LPS (Sigma-Aldrich) 100ng/mL plus nigericin (Nig, Invivogen) 10 µM added during the last 30min of LPS incubation, MCC950 (Invivogen) 10 µM.
*** Supernatants of $CD4^{IL-10}$ and CD4 GFP or NGFR cultures are obtained by incubating $CD4^{IL-10}$ or CD4 GFP cells at $1X10^6$/mL for 3days and collecting the supernatants. IL-10 production levels are measured by IL-10 specific ELISA. Anti-IL-10R antibody (Biolegend) 30µg/mL.

[00639] After treatments outlined in Table 1, supernatants are collected from 6 wells for each group and IL-1 β/IL-18 production is measured by ELISA specific for mature IL-1β or IL-18 (Biolegend). Cells collected from selected groups are analyzed by Western Blot to determine levels of activated caspase 1.

[00640] Data from the experiments show that autologous single-donor, allogeneic single-donor, and allogeneic polydonor $CD4^{IL\text{-}10/anti\text{-}CD19\ CAR}$ cells down-regulate IL-1β and IL-18 production by activated monocytes. They further show that autologous single-donor, allogeneic single-donor, and allogeneic polydonor $CD4^{IL\text{-}10/anti\text{-}CD19\ CAR}$ cells down-regulate mature caspase-1 production in activated monocytes. Additionally, autologous single-donor, allogeneic single-donor, and allogeneic polydonor $CD4^{IL\text{-}10/anti\text{-}CD19\ CAR}$ and IL-10 produced by the polydonor $CD4^{IL\text{-}10}$ down-regulate inflammasome.

[00641] Similar experiments are performed with human macrophages or dendritic cells instead of monocytes. Results from the experiments demonstrate that $CD4^{IL\text{-}10/anti\text{-}CD19\ CAR}$ cells further down-regulate IL-1β, IL-18, and mature caspase-1 production from activated macrophages and dendritic cells.

[00642] These data indicate that autologous single-donor, allogeneic single-donor, and allogeneic polydonor $CD4^{IL\text{-}10/anti\text{-}CD19\ CAR}$ cells can be used to treat diseases or disorders involving hyperactivation of NLPR3 inflammasome. In particular, autologous single-donor, allogeneic single-donor, and allogeneic polydonor $CD4^{IL\text{-}10/anti\text{-}CD19\ CAR}$ cells can be used to treat chronic inflammatory and autoimmune diseases. The NLPR3 inflammasome can be activated by exogenous or endogenous "danger signals", such as Pathogen Associated Molecular Patterns (PAMPs), silica, asbestos, Danger Associated Molecular Patterns (DAMPs) like products from damaged mitochondria, necrotic and stressed cells, and uremic acid crystals.

6.9.8. Example 7: Generation of polydonor $CD4^{IL\text{-}10}$ cells

Vector production

[00643] Polydonor $CD4^{IL\text{-}10}$ cells were produced by transduction with a lentiviral vector containing coding sequences of both the human IL-10 and a truncated form of the NGFR (ΔNGFR) (FIGs. 3A-3B), as described in WO2016/146542, incorporated by reference in its entirety herein. The sequence of the vector is provided as SEQ ID NO:5. In short, the lentiviral vector was generating by ligating the coding sequence of human IL-10 from 549 bp fragment of pH15C (ATCC 68192)) into plasmid #1074.1071.hPGK.GFP.WPRE.mhCMV.dNGFR.SV40PA. The presence of the bidirectional promoter (human PGK promoter plus minimal core element of the CMV promoter in the opposite direction) allows co-expression of the two transgenes. The plasmid further contains a coding sequence of an antibiotic resistance gene (e.g., ampicillin or kanamycin).

[00644] The lentiviral vectors were produced by Ca_3PO_4 transient four-plasmid co-transfection into 293T cells and concentrated by ultracentrifugation: 1 μM sodium butyrate was added to the cultures for vector collection. Titer was estimated on 293T cells by limiting dilution, and vector particles were measured by HIV-1 Gag p24 antigen immune capture (NEN Life Science Products; Waltham, MA). Vector infectivity was calculated as the ratio between titer and particle. For concentrated vectors, titers ranged from $5x10^8$ to $6x10^9$ transducing units/mL, and infectivity from $5xl0^4$ to $5x10^5$ transducing units/ng.

Production of $CD4^{IL-10}$ cells

[00645] FIG. 19 is a schematic representation of the production process of $CD4^{IL-10}$ cells. $CD4^+$ T cells from healthy donors were purified. Human $CD4^+$ T cells were activated with soluble anti-CD3, soluble anti-CD28 mAbs, and rhIL-2 (50 U/mL) for 48 hours before transduction with a bidirectional lentiviral vector encoding for human IL-10 and a truncated form the human NGF receptor (LV-IL-10/ΔNGFR) at multiplicity of infection (MOI) of 20.

[00646] After 11 days, transduced cells were analyzed by FACS for the expression of ΔNGFR, and the vector copy number (VCN) was quantified by digital droplet PCR (ddPCR).

[00647] The mean transduction efficiency of $CD4^+$ T cells from 10 different donors was 45 ± 17% with VCN of 2.7 ± 0.6%. FIG. 20A shows percentages of $CD4^+\Delta NGFR^+$ cells (mean± SD, n=10 left bar) and vector copy numbers (VCN, mean ± SD, n=10 right bar) in human $CD4^+$ T cells transduced with LV-IL-10/ΔNGFR (a bidirectional lentiviral vector encoding for human IL-10 and a truncated form the human NGF receptor). The frequency of $CD4^+\Delta NGFR^+$ cells and the vector copy numbers were quantified by digital droplet PCR (ddPCR) in $CD4^{IL-10}$ cells.

[00648] $\Delta NGFR^+$ T cells were purified using anti-CD271 mAb-coated microbeads and resulted in > 95% pure $CD4^{IL-10}$ cells populations. After purification, cells were stained with markers for CD4 and ΔNGFR and analyzed by FACS. The data showed purity resulting from the purification step was over 98%. FIG. 20B shows FACS data from two representative donors (Donor B and Donor C) out of 10 donors tested. The purified $CD4^{IL-10}$ cells were restimulated 3 times at 14 day intervals and their *in vitro* and *in vivo* functions were tested after the second (TF2) and or third restimulation (TF3) functions.

[00649] Resting $CD4^{IL-10}$ cells produced IL-10 constitutively. Upon activation, the level of IL-10 produced was strongly enhanced.

$CD4^{IL-10}$ cells have a cytokine production profile which is comparable to that of naturally derived Tr1 cells.

[00650] Cytokine production profiles of single donor $CD4^{IL-10}$ cells were analyzed after the second (TF2) and third (TF3) restimulation and the results are provided in FIG. 21. Specifically, $CD4^{IL-10}$ cells ($2x10^5$ cells in 200 µl) were restimulated as previously described (Andolfi et al. Mol Ther. 2012;20(9):1778-1790 and Locafaro et al. Mol Ther. 2017;25(10):2254-2269). At day 14, after the 2nd round (TF2) and 3rd round (TF3) of restimulation, $CD4^{IL-10}$ cells were left unstimulated (orange bar) or stimulated with immobilized anti-CD3 and soluble anti-CD28 mAbs (grey bars) for 48hrs. Culture supernatants were collected and levels of IL-10, IL-4, IL-5, IFN-γ, and IL-22 were determined by ELISA. All samples were tested in triplicate. Mean± SD, n=8 donors tested are presented. The results provided in FIG. 21 show that $CD4^{IL-10}$ cells stimulated with immobilized anti-CD3 and soluble anti-CD28 mAbs show a Tr1 cell cytokine production profile.

[00651] Although considerable variations between the different donors were observed, the overall cytokine production profiles after the second (TF2) or the third (TF3) restimulation were comparable and reflected those of Tr1 cells (Roncarolo et al., Immunity, 2018). Like Tr1 cells, the $CD4^{IL-10}$ cells produced high levels of IL-10, IL-5, IFN-γ, and IL-22, but low levels of IL-4 and undetectable levels of IL-2.

$CD4^{IL-10}$ cells express high levels of Granzyme B and selectively kill myeloid leukemia cells

[00652] The $CD4^{IL-10}$ cells were further analyzed after the 2nd round (TF2) of restimulation for expression of granzyme B (GzB). The data in FIG. 22A show that most of the $CD4^{IL-10}$ cells expressed GzB. More than 95 % of all $CD4^{IL-10}$ cells derived from 7 different donors expressed high levels of Granzyme B.

[00653] The $CD4^{IL-10}$ cells from the 2nd round (TF2) of restimulation were further analyzed for their cytotoxic effects against myeloid leukemia cells (ALL-CM) and an erythroid leukemia cell line (K562). $CD4^{IL-10}$ cells (10^5/well) were co-cultured with K562 and ALL-CM cells (10^5/well) at 1:1 ratio for 3 days. Residual leukemic cell lines ($CD45^{low}CD33^{+}$) were counted by FACS for each target cell.

[00654] The $CD4^{IL-10}$ cells selectively killed the myeloid leukemia cells (ALL-CM) as shown in FIG. 22B. The % of killed ALL-CM cells varied between 62% and 100%, whereas the killing of the erythroid leukemia cell line K562 (which are highly sensitive for nonspecific

cytotoxic activities) varied between 0 and 27% (4 different donors tested). Taken together, these data confirm that $CD4^{IL-10}$ cells express Granzyme B and efficiently kill myeloid leukemia cells. As expected, some variations in the killing capacity of the $CD4^{IL-10}$ cells from individual donors was observed.

$CD4^{IL-10}$ cells suppress the proliferative responses of both allogeneic $CD4^+$ and $CD8^+$ T cells

[00655] The $CD4^{IL-10}$ cells were also analyzed for their effects on allogeneic $CD4^+$ T cells or $CD8^+$ T cells. Specifically, allogeneic PBMC cells were labeled with eFluor® 670 (10^5 cells/well) and stimulated with allogeneic mature dendritic (DC) cells ($5x10^4$ cells/well) and soluble anti-CD3 mAbs in the absence or presence of $CD4^{IL-10}$ cells (10^5 cells/well) at a 1:1 Responder:Suppressor ratio. After 4 days of culture, the percentages of proliferating responder cells were determined by eFluor® 670 dilution with flow cytometry after gating on $CD4^+\Delta NGFR^-$ T cells or $CD8^+\Delta NGFR^-$ T cells. FIGs. 23A and 23B show effects of $CD4^{IL-10}$ cells from six different, unpooled, donors (Donor-C, Donor-E, and Donor-F in FIG. 23A and Donor-H, Donor-I, and Donor-L in FIG. 23B) on $CD4^+$ T cells with percentages of proliferation and suppression. FIGs. 24A and 24B show effects of $CD4^{IL-10}$ cells from six different, unpooled, donors (Donor -C, Donor-E, and Donor-F in FIG. 24A and Donor-H, Donor-I, and Donor-L in FIG. 24B) on $CD8^+$ T cells.

[00656] The results demonstrated that $CD4^{IL-10}$ cells from 6 different donors, unpooled and tested separately, downregulated the proliferative responses of both allogeneic $CD4^+$ and $CD8^+$ T cells. The suppressive effects on the $CD4^+$ T-cells varied between 51% and 96%, while the suppressive effects on the $CD8^+$ T-cells varied between 62% and 73 %.

Production and characterization of polydonor $CD4^{IL-10}$ cells

[00657] $CD4^{IL-10}$ cells were generated as described above and FIG. 19 using $CD4^+$ cells from multiple donors. $CD4^{IL-10}$ cells from each donor were stimulated by the second (TF2) and third (TF3) restimulation. After the third stimulation, $CD4^{IL-10}$ cells from the three donors were pooled at a 1:1:1 ratio and stimulated with immobilized anti-CD3 and soluble anti-CD28 mAbs for 48hrs.

Polydonor $CD4^{IL-10}$ cells have a cytokine production profile which is comparable to that of $CD4^{Il-10}$ cells of individual donors and Tr1 cells.

[00658] Culture supernatants were collected and levels of IL-10, IL-4, IL-5, IFN-γ and IL-22 were determined by ELISA. The results provided in FIG. 25 show that the cytokine

production of polydonor $CD4^{IL\text{-}10}$ cells pooled from 3 different allogeneic donors (pooled 1:1:1) (red dot) was comparable to that of $CD4^{IL\text{-}10}$ cells from individual donor (n=8) derived $CD4^{IL\text{-}10}$ cells (gray bars). The polydonor $CD4^{IL\text{-}10}$ cells produced high levels of IL-10, IL-5, IFN-γ and IL-22 and low levels of IL-4 and undetectable levels of IL-2 (not shown). These data indicate that it is feasible to pool $CD4^{IL\text{-}10}$ cells and that these polydonor $CD4^{IL\text{-}10}$ cells maintain the cytokine production signature of single donor derived $CD4^{IL\text{-}10}$ cells and Tr1 cells. Importantly, the pooled allogeneic cell populations contained > 95% viable cells indicating that they did not kill each other.

Polydonor $CD4^{IL\text{-}10}$ cells express high levels of Granzyme B and kill myeloid leukemia cell lines.

[00659] The polydonor $CD4^{IL\text{-}10}$ cells were further analyzed after 3rd round (TF3) of restimulation for expression of granzyme B (GzB). The data in FIG. 26A show that most of the polydonor $CD4^{IL\text{-}10}$ cells express GzB. Over 95 % of the polydonor $CD4^{IL\text{-}10}$ cells expressed Granzyme B, comparable to the GzB expression of single donor derived $CD4^{IL\text{-}10}$ cells.

[00660] The $CD4^{IL\text{-}10}$ cells from 3rd round (TF3) of restimulation were further analyzed for their cytotoxic effects on myeloid leukemia cells (ALL-CM cell line) or K562. The polydonor $CD4^{IL\text{-}10}$ cells (10^5/well) were co-cultured with K562 and ALL-CM cells (10^5/well) at 1:1 ratio for 3 days. Residual leukemic cell lines ($CD45^{low}CD33^+$) were counted by FACS for each target cell. The results provided in FIG. 26B show that some level of cytotoxicity against K562 cells, which are highly sensitive for nonspecific cytotoxicity and NK cell cytotoxicity. Nevertheless, a level of selectivity towards myeloid leukemia cells (ALL-CM) was obtained which is comparable to that of single donor derived $CD4^{IL\text{-}10}$ cells.

Polydonor $CD4^{IL\text{-}10}$ cells suppress the proliferative responses of both allogeneic CD4+ and CD8+ T cells.

[00661] The polydonor $CD4^{IL\text{-}10}$ cells were also analyzed for their effects on allogeneic $CD4^+$ T cells or $CD8^+$ T cells. Specifically, allogeneic PBMC cells were labeled with eFluor® 670 (10^5 cells/well) and stimulated with allogeneic mature dendritic (DC) cells ($5x10^4$ cells/well) and soluble anti-CD3 mAbs in the absence or presence of polydonor $CD4^{IL\text{-}10}$ cells (10^5 cells/well) at a 1:1 Responder:Suppressor ratio. After 4 days of culture, the percentages of proliferating responder cells were determined by eFluor® 670 dilution with flow cytometry after gating on $CD4^+\Delta NGFR^-$ T cells and $CD8^+\Delta NGFR^-$ T cells. FIG. 27A shows results from polydonor $CD4^{IL\text{-}10}$ cells containing $CD4^{IL\text{-}10}$ cells from Donor-C, Donor-E, and Donor-F. FIG.

27B shows results from polydonor $CD4^{IL\text{-}10}$ cells containing $CD4^{IL\text{-}10}$ cells from Donor-H, Donor-I, and Donor-L, which had been frozen, stored and thawed prior to testing.

[00662] FIG. 27A shows that the polydonor $CD4^{IL\text{-}10}$ cells (from 3 different donors) suppress $CD4^+$ and $CD8^+$ T-cell responses by 96% and 74%, respectively. Comparable results were obtained with a second, different batch of polydonor $CD4^{IL\text{-}10}$ cells which was tested after the cells had been frozen, stored and thawed prior to testing (FIG. 27B). Suppression of $CD4^+$ and $CD8^+$ T cell proliferation was 68% and 75 %, respectively. These data indicate that polydonor $CD4^{IL\text{-}10}$ cells can be frozen and stored without loss of function.

[00663] Collectively the data obtained with polydonor $CD4^{IL10}$ cells indicate that these cell preparations can be pooled without any problems. They contain > 95 % viable cells and maintain all the relevant functions (cytokine production, cytotoxic capacity, and suppression of allogeneic T cell responses) of single donor $CD4^{IL\text{-}10}$ cells. The use of larger pools of polydonor $CD4^{IL\text{-}10}$ cells should reduce the natural variations observed between $CD4^{IL\text{-}10}$ cell lots originating from different individual donors, and should provide a large quantity of off-the-shelf $CD4^{IL\text{-}10}$ cells for human therapy.

[00664] A polydonor $CD4^{IL\text{-}10}$ cell product will have significant advantages in terms of a more homogeneous product which will allow the determination of well defined, less lot-to-lot variation, potency, and release criteria. In addition, it will enable the development of a continuous large-scale cell production process.

Other methods for production of polydonor $CD4^{IL\text{-}10}$ cells

[00665] Before the lentiviral transduction, buffy coats from minimally 3-5 different donors were pooled. $CD4^+$ cells were isolated from buffy coats by positive selection using anti-CD4 antibody. Purity of the pooled $CD4^+$ cells was checked by FACS. Alternatively, frozen human $CD4^+$ cells were obtained from minimally 3-5 normal healthy donors. The frozen human $CD4^+$ cells were thawed before use. $CD4^+$ cells from buffy coats or frozen stocks were activated for 24-48 hours by a combination of CD3 and CD28 antibodies or CD3- and CD28 antibody coated beads in the presence of IL-2. In some cases, $CD4^+$ cells from buffy coats or frozen stocks were activated with soluble anti-CD3, soluble anti-CD28 mAbs, and rhIL-2 (50 U/mL) for 48 hours and transduced with a bidirectional lentiviral vector encoding for human IL-10 as described above for production of $CD4^{IL\text{-}10}$ cells.

[00666] In some cases, the HLA haplotype of the T cell donors (or $CD4^+$ cells isolated from the donors) were first determined and $CD4^+$ cells having desired HLA haplotypes are selectively pooled and used.

[00667] Polydonor $CD4^{IL\text{-}10}$ cells were generated by transducing the activated $CD4^+$ cells described above with the lentiviral vector containing human IL-10 and ΔNGFR coding sequences described above.

[00668] On Day 7-11, which is 5-9 days after the transduction, the cells were harvested and successfully transduced T cells purified utilizing an anti-NGFR antibody. This process generally results in 95% pure populations of polydonor $CD4^{IL\text{-}10}$ cells.

[00669] The purified polydonor $CD4^{IL\text{-}10}$ cells were counted and re-stimulated by a mixture of CD3- and CD28 antibodies, CD3- and CD28 antibody coated beads, optionally in the presence of feeder cells for another 8-10 days in the presence of IL-2. In some cases, the purified polydonor $CD4^{IL\text{-}10}$ cells were re-stimulated in the presence of feeder cells.

[00670] After a total culture period of 14-18 days, $CD4^{IL\text{-}10}$ cells were harvested, counted and tested for their capacity to produce IL-10 spontaneously or following activation with CD3 and CD28 antibodies or CD3 and CD28 antibody coated beads. Additionally, the levels of GrzB and perforin were measured. Their capacity to suppress human T cell (PBMC) and purified $CD4^+$ and $CD8^+$ T cell proliferation are also tested.

[00671] In addition, the production of IL-22 is measured both constitutively and following activation of 200,000 $CD4^{IL\text{-}10}$ cells in a volume of 200 microliter using a combination of CD3 and CD28 antibodies as described previously for the production of other cytokines such as IFN-γ, IL-10, IL-4 and IL-5. The pooled $CD4^{IL\text{-}10}$ cells were frozen before storage.

6.9.9. Example 8:Treatment or prevention of GvHD using polydonor $CD4^{IL\text{-}10}$ cells

Effects of polydonor $CD4^{IL\text{-}10}$ cells in vivo.

[00672] A population of polydonor $CD4^{IL\text{-}10}$ cells were tested in a humanized xeno GvHD disease model, an NSG mouse model, for their effect on xeno-GvHD induced by human PBMC as illustrated in FIG. 28. NSG mice were sub-lethally irradiated and intravenously injected with human PBMC ($5x10^6$ cells/mouse), with polydonor (three donors) $CD4^{IL\text{-}10}$ cells ($5x10^6$ cells/mouse), or with human PBMC ($5x10^6$ cells/mouse) in combination with polydonor $CD4^{IL\text{-}10}$

cells (BC-C/E/F) ($5x10^6$ cells/mouse). Xeno-GvHD was evaluated as previously described (Bondanza et al. Blood 2006) based on weight loss (>20% weight loss), skin lesions, fur condition, activity, and hunch.

[00673] FIG. 29 shows % of NSG mice demonstrating xeno-GvHD on each day after injection. Administration of 5x 10^6 human PBMC to irradiated NSG mice resulted unexpectedly in an unusually fulminant GvHD. All mice died at day 10 which reflects very lethal xeno-GvHD. Co-administration of $5x10^6$ polydonor $CD4^{IL-10}$ cells delayed this fulminant GvHD, but the mice were sacrificed at day 14 because they reached the prespecified humane 20 % body weight loss criterion for sacrifice (FIG. 29). Nevertheless, these results indicate that polydonor $CD4^{IL-10}$ can delay very severe xeno-GvHD. Importantly, polydonor $CD4^{IL-10}$ cells administered alone at the same dose as the PBMC ($5x10^6$ cells) failed to induce any sign of xeno-GvHD.

[00674] The presence of human $CD4^{IL-10}$ cells were also tested in the spleen (FIG. 30, left panels) and bone marrow (FIG. 30, right panels) of the NSG mice injected with human PBMC ($5x10^6$ cells/mouse), polydonor (three donors; BC-C/E/F) $CD4^{IL-10}$ cells ($5x10^6$ cells/mouse), or human PBMC ($5x10^6$ cells/mouse) in combination with polydonor $CD4^{IL-10}$ cells (three donors; BC-C/E/F) ($5x10^6$ cells/mouse) at 14 days post injection. The results provided in FIG. 30 show that polydonor $CD4^{IL-10}$ cells migrated to spleen and bone marrow. Low percentages of these cells were found to be present 14 days after infusion of the cells. These results indicate that polydonor $CD4^{IL-10}$ cells delayed fulminant xeno-GvHD induced by human PBMC and that they do not themselves induce any xeno GvHD.

Polydonor $CD4^{IL-10}$ cells inhibit severe xeno GvHD by purified $CD4^+$ cells.

[00675] Polydonor $CD4^{IL-10}$ cells were tested in a humanized xeno GvHD model in which GvHD disease was induced by administration of 2.5 X 10^6 purified human CD4+ T cells as illustrated in FIG. 31. NSG mice were sub-lethally irradiated at day 0 and on day 3 were intravenously injected with human $CD4^+$ T cells ($2.5x10^6$ cells/mouse) alone or in combination with polydonor $CD4^{IL-10}$ cells (three different donors; BC-H/I/L) ($2.5x10^6$ cells/mouse) or with $CD4^{IL-10}$ cells from a single donor (BC-H) from the pool ($2.5x10^6$ cells/mouse). Xeno-GvHD was evaluated as previously described (Bondanza et al. Blood 2006) based on weight loss (>20% weight loss), skin lesions, fur condition, activity, and hunch.

[00676] FIG. 32 shows % of NSG mice demonstrating GvHD on each day after injection. The results show that polydonor $CD4^{IL-10}$ (BC-H/I/L) cells can inhibit the xeno-GvHD mediated by human allogeneic $CD4^+$ T cells. In this experiment, xeno GvHD was very severe, because all

mice in the control group which received CD4+ T cells were dead at day 20. In contrast, co-administration of 2.5 x 10^6 polydonor $CD4^{IL\text{-}10}$ inhibited GvHD by 75 %. Single-donor $CD4^{IL\text{-}10}$ cells were also protective but the effects were less potent.

Other experiments

[00677] Therapeutic effects of the polydonor $CD4^{IL\text{-}10}$ cells are tested in four different groups of mice: (i) mice receiving human PBMC from a donor unrelated to the $CD4^{IL\text{-}10}$ cells (xeno-GvHD positive control); (ii) mice receiving the polydonor $CD4^{IL\text{-}10}$ cells (negative control); (iii) mice receiving a combination of PBMC and the polydonor $CD4^{IL\text{-}10}$ cells at 1:1 ratio; and (iv) mice receiving a combination of PBMC and the polydonor $CD4^{IL\text{-}10}$ cells at 2:1 ratio or at different ratios. Among animals receiving combination of PBMC and the polydonor $CD4^{IL\text{-}10}$ cells, some animals receive PBMC and the polydonor $CD4^{IL\text{-}10}$ cells concurrently, some animals receive polydonor $CD4^{IL\text{-}10}$ cells several days (e.g., 5 days) after receiving PBMC, and some animals receive polydonor $CD4^{IL\text{-}10}$ cells several days (e.g., 5 days) before receiving PBMC.

[00678] The mice are monitored for development of GvHD by measuring weight at weeks 1, 2, 3, 4, and if necessary week 5, after administration of PBMC and/or the polydonor $CD4^{IL\text{-}10}$ cells. In addition to weight loss, the mice will be inspected for skin lesions, fur condition and activity. The mice in the treatment groups are monitored for additional periods to determine effects of the polydonor $CD4^{IL\text{-}10}$ cells on long term survival.

[00679] The amount and localization of the polydonor $CD4^{IL\text{-}10}$ cells are also monitored in peripheral blood and tissues after administration. Specifically, presence of polydonor $CD4^{IL\text{-}10}$ cells are monitored in peripheral blood and at sites of inflammation: lymph nodes, spleen, gut, and bone marrow. The mice in the treatment group(s) are monitored for an additional 3 weeks to determine long-term survival.

[00680] The results demonstrate that polydonor $CD4^{IL\text{-}10}$ cells are effective in reducing and preventing xeno-GvHD.

6.9.10. Example 9: Inhibition of GvHD and treatment of cancer

[00681] A population of polydonor $CD4^{IL\text{-}10}$ cells are tested in an NSG mouse model transplanted with human PBMC and AML tumor cells for their effect on xeno-GvHD induced by human PBMC and anti-tumor effects. AML cells (ALL-CM) are administered i.v. as described

previously in WO 2016/146542. PBMC or polydonor $CD4^{IL-10}$ cells or combinations thereof are administered 3 days later.

[00682] Polydonor $CD4^{IL-10}$ cells are obtained as described in Example 1. Therapeutic effects of the polydonor $CD4^{IL-10}$ cells are tested in four different groups of mice, each having received irradiation and $5x10^6$ ALL-CM cells (AML mice) at day 0: (i) AML mice without additional treatment; (ii) AML mice receiving $5x10^6$ human PBMC from a donor unrelated to the polydonor $CD4^{IL-10}$ cells – the PBMCs cause severe xeno-GvHD; (iii) AML mice receiving $2.5x10^6$ polydonor $CD4^{IL-10}$ cells; and (iv) AML mice receiving combinations of PBMC and the polydonor $CD4^{IL-10}$ cells at 1:1 or 2:1 ratio or at different ratios. One additional group of mice do not receive ALL-CML cells but receive $5x10^6$ human PBMC at day 3 after irradiation.

[00683] Effects of the polydonor $CD4^{IL-10}$ cells on xeno-GvHD induced by human PBMC are tested based on weight loss, skin lesions, fur condition, activity, death rate and long-term survival. Anti-tumor or graft versus leukemia (GvL) effects of the polydonor $CD4^{IL-10}$ cells are tested based on reduction of tumor cells in the circulation and long-term tumor free survival.

[00684] Some mice are monitored for up to 7 weeks in order to monitor long-term survival and complete tumor remissions.

[00685] Results demonstrate that polydonor $CD4^{IL-10}$ cells are effective in both inhibition of xeno-GvHD and treatment of cancer.

6.9.11. Example 10: Treatment of cancer using polydonor $CD4^{IL-10}$ cells

[00686] A population of polydonor $CD4^{IL-10}$ cells are tested in an ALL-CM leukemia model of T cell therapy in NSG mice.

[00687] NSG mice were sub-lethally irradiated and intravenously injected with myeloid leukemia cells (ALL-CM) ($5x10^6$) at day 0 (See FIG. 33A). In the first group of animals, PBMC ($5x10^6$) or single donor (from donor BC-I and donor BC-H) $CD4^{IL-10}$ cells ($2.5x10^6$) were injected at day 3. In the second group of animals, PBMC ($5x10^6$) or polydonor $CD4^{IL-10}$ cells ($2.5x10^6$) were injected at day 3. Graft-versus-leukemia (GvL) effect was tested in the animals based on reduction of circulating leukemia cells and long-term leukemia free survival. Leukemia was measured as previously described (Locafaro G. et al Molecular Therapy 2017).

[00688] As provided in FIG. 33B and FIG. 33C, all of the mice injected with ALL-CM myeloid leukemia cells had extensive leukemia progression at day 17. Administration of $5x10^6$ PBMC resulted in a strong inhibition of leukemia progression. Interestingly, a comparable level

of inhibition of leukemia progression was obtained by lower number ($2.5x10^6$) of single-donor $CD4^{IL10}$ (FIG. 33B) or polydonor $CD4^{IL10}$ (FIG. 33C). These data indicate that single donor and polydonor $CD4^{IL10}$ have strong direct anti myeloid leukemia effects.

[00689] Graft-versus-leukemia (GvL) effects of single-donor $CD4^{IL10}$ and polydonor $CD4^{IL10}$ were further tested in combination with PBMC in mice injected with ALL-CM myeloid leukemia cells (see FIG. 34A). Administration of $5x10^6$ PBMC resulted in a strong inhibition of leukemia progression and administration of $5x10^6$ PBMC combined with single donors $CD4^{IL10}$ ($2.5x10^6$) had synergistic effect (FIG. 34B). Interestingly, administration of $5x10^6$ PBMC combined with $2.5x10^6$ polydonor $CD4^{IL10}$ had a comparable synergistic GvL effect (FIG. 34C). These data indicate that polydonor $CD4^{IL10}$ act in synergy with PBMC to mediate strong GvL effects.

6.9.12. Example 11: Treatment of chronic inflammatory and autoimmune diseases using polydonor $CD4^{IL-10}$ cells

[00690] Activation of the NLPR3 inflammasome has been implicated in many chronic inflammatory and autoimmune diseases. The NLPR3 inflammasome can be activated by "danger signals" which lead to caspase1-mediated production of the pro-inflammatory cytokines IL-1β and IL-18 by monocytes/macrophages. A series of in vitro experiments are performed to investigate the effects of polydonor $CD4^{IL-10}$ cells on the NLPR3 inflammasome and IL-1 β/IL-18 production by human monocytes.

[00691] First, human PBMC are isolated from peripheral blood by standard density centrifugation on Ficoll/Paque (Sigma-Aldrich). Monocytes are isolated from the human PBMC by negative selection using monocyte isolation kit II (Miltenyi) according to the manufacturer's instructions. Negative selection is preferred because positive selection or adherence can lead to undesired activation of the cells. Isolated monocytes are plated at $5x10^4$ cells/200 µl in the presence of $2x10^5$ or $1x10^5$ polydonor $CD4^{IL-10}$ cells /200 µl per well in 96-well microtiter plates in culture medium containing 3% toxin free human AB serum.

[00692] Table 2 summarizes treatment conditions applied to 17 sets of monocytes, each set including 6 wells of cells. It is known that LPS alone can activate human monocytes without a second signal provided by ATP.

Table 2

Group #	Monocytes	CD4 cells/supernatant	YVADfmk*	LPS**	Other

Medium control Incubation time: 1.5 hour; followed by activation by LPS for 4 hours					
1	Monocytes	No	No	No	
2	Monocytes	No	No	LPS	
3	Monocytes	No	YVADfmk	LPS	
Co cultivation of monocytes and polydonor $CD4^{IL-10}$ cells					
4	Monocytes	$CD4^{IL-10}$ cells	No	No	
5	Monocytes	$CD4^{IL-10}$ cells	No	LPS	
6	Monocytes	$CD4^{IL-10}$ cells	YVADfmk	LPS	
Co cultivation of monocytes and control CD4 GFP+ cells					
7	Monocytes	CD4 GFP cells	No	No	
8	Monocytes	CD4 GFP cells	No	LPS	
9	Monocytes	CD4 GFP cells	YVADfmk	LPS	
Cultivation of monocytes in the presence of supernatants* of polydonor $CD4^{IL-10}$ cell or CD4 GFP+ cell culture**					
10	Monocytes	50% supernatant of $CD4^{IL-10}$ cells	No	LPS	
11	Monocytes	25% supernatant of $CD4^{IL-10}$ cells	No	LPS	
12	Monocytes	12.5% supernatant of $CD4^{IL-10}$ cells	No	LPS	
13	Monocytes	50% supernatant of $CD4^{IL-10}$ cells	No	LPS	Anti-IL-10 antibody
14	Monocytes	50% supernatant of CD4 GFP cells	No	LPS	
15	Monocytes	25% supernatant of CD4 GFP cells	No	LPS	
16	Monocytes	12.5% supernatant of CD4 GFP cells	No	LPS	
17	Monocytes	50% supernatant of CD4 GFP cells	No	LPS	Anti-IL-10 antibody

* YVADfmk is an inhibitor specific to caspase 1. 20 mM Z-YVADfmk (Biovision, Enzo Life Sciences, or Axxora Life Sciences) dissolved in DMSO is used.
** LPS (Signa-Aldrich) 100ng/mL
*** Supernatants of $CD4^{IL-10}$ and CD4 GFP or NGFR cultures are obtained by incubating $CD4^{IL-10}$ or CD4 GFP cells at $1X10^6$/mL for 3days and collecting the supernatants. IL-10 production levels are measured by IL-10 specific ELISA.

[00693] After treatments outlined in Table 2, supernatants are collected from 6 wells for each group and IL-1 β/IL-18 production is measured by ELISA specific for mature IL-1β or IL-18 (Biolegend). Cells collected from 6 wells for Group #3, 10, 13, 14, and 17 are analyzed by Western Blot to determine levels of activated caspase 1.

[00694] Data from the experiments show that polydonor $CD4^{IL\text{-}10}$ cells down-regulate IL-1β and IL-18 production by activated monocytes. They further show that polydonor $CD4^{IL\text{-}10}$ cells down-regulate mature caspase-1 production in activated monocytes. Additionally, polydonor $CD4^{IL\text{-}10}$ and IL-10 produced by the polydonor $CD4^{IL\text{-}10}$ down-regulate inflammasome.

[00695] Similar experiments are performed with human macrophages or dendritic cells instead of monocytes. Results from the experiments demonstrate that polydonor $CD4^{IL\text{-}10}$ cells further down-regulate IL-1β, IL-18, and mature caspase-1 production from activated macrophages and dendritic cells.

[00696] These suggest that polydonor $CD4^{IL\text{-}10}$ cells can be used to treat diseases or disorders involving hyperactivation of NLPR3 inflammasome. In particular, polydonor $CD4^{IL\text{-}10}$ cells can be used to treat chronic inflammatory and autoimmune diseases. The NLPR3 inflammasome can be activated by exogenous or endogenous "danger signals", such as Pathogen Associated Molecular Patterns (PAMPs), silica, asbestos, Danger Associated Molecular Patterns (DAMPs) like products from damaged mitochondria, necrotic and stressed cells, and uremic acid crystals.

6.9.13. Example 12:Supernatant of polydonor $CD4^{IL\text{-}10}$ cells inhibit NLPR3 Inflammasome activation and IL-1β and IL-18 production by human monocytes

[00697] $CD14^+$ monocytes were isolated from PBMC using a pan monocyte isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) and plated in 96 flat microtiter wells at $2x10^5$/200µL per well and cultured in the presence of LPS. The cells were cultured further in the presence of Z-YVADfmk (20 µM), MMC950 (10 µM), IL-10 (10ng/mL) or various concentrations of single- or pooled donor $CD4^{IL\text{-}10}$ cell supernatants as summarized in Table 1.

[00698] The supernatants were obtained from single- or pooled donor $CD4^{IL\text{-}10}$ cells activated for 72 hours with a combination of CD3 and CD28 antibodies as described previously. (Andolfi et al. 2012, Mol. Therapy Vol. 20, 1778-1790, Locafaro et al. Mol Ther 2017, 25, 2254) In some cases (FIG. 35C and 35D), the monocytes were incubated with LPS in combination with the NLPR3 inflammasome activator nigericin ("NIG") which was added during the last 30 minutes of the LPS activation.

[00699] The NLPR3 inflammasome was activated by LPS, resulting in the production of mature caspase 1 and the biologically active forms of IL-1β and IL-18. Monocytes plated in the

absence of LPS activation did not produce detectable levels of IL-10 during the incubation period (not shown).

[00700] Addition of the supernatant of single donor derived $CD4^{IL\text{-}10}$ cells (containing 1769 pg IL-10/mL) inhibited IL-1β production by LPS activated monocytes from donor #1 and #2 at concentrations of 50%, 25% and 12.5 % respectively, in a dose dependent fashion (FIG. 35A and FIG. 35B). Supernatant of CD4+ cells transduced with GFP was used as a control. Complete inhibition of IL-1β production is observed at concentrations of 50% and 25%, whereas supernatants of GFP transduced control CD4+ cells at concentrations of 50% were ineffective.

[00701] Various concentrations of $CD4^{IL\text{-}10}$ T cell supernatant (50%, 25% or 12.5%), Z-YVADfmk or MCC950 were further tested on monocytes activated with LPS and nigericin ("NIG"). Supernatants from single donor (BC-E) or pooled donor $CD4^{IL\text{-}10}$ cells contained 5295 or 3532 pg IL-10/mL respectively. Supernatants of single donor $CD4^{IL\text{-}10}$ cells were also very effective in inhibiting LPS induced IL-1β production enhanced by the NLPR3 inflammasome activator nigericin (FIG. 35C and FIG. 35D).

[00702] The data demonstrate that the supernatants from $CD4^{IL\text{-}10}$ cells at concentrations of 50% were as effective as the irreversible caspase 1 inhibitor Z-YVADfmk (Guo et al. 2015, Nature Med 21, 677), the selective NLPR3 inflammasome inhibitor MCC950 (Coll et al. 2019, Nature Chem. Biol 15,556) and recombinant IL-10, indicating that IL-10 containing supernatants inhibit NLPR3 inflammasome activation and mature caspase1 production resulting in strong inhibition of the production of the proinflammatory cytokine IL-1β (FIG. 35A-35D).

[00703] Comparable results were obtained in second series of experiments with supernatants of single donor (BC-E) and pooled $CD4^{IL\text{-}10}$ cells from 2 different donors (BC-C/E). The $CD4^{IL\text{-}10}$ cells were activated by a combination of CD3 and CD28 antibodies as described (Andolfi et al. 2012). After 3 days the supernatants from the $CD4^{IL\text{-}10}$ cells were collected. These supernatants contained 5295 and 3532 pg IL-10/mL respectively, and inhibited LPS induced IL-1β production by monocytes from donor #3 in a dose dependent fashion (FIG. 35E). Supernatants at concentrations of 50% were as effective as Z-YVADfmk and MCC950. The inhibitory effects of the supernatants of the pooled $CD4^{IL\text{-}10}$ cells were completely neutralized by an anti-IL-10 receptor antibody. Similarly, supernatants pooled from 3 different donors containing 2589 pgIL-10/mL, dose dependently inhibited IL-1β production by monocytes from donor #4 (FIG. 35F). The inhibitory effects of the supernatants are completely neutralized by an IL-10 receptor antibody demonstrating that NLPR3 activation is mediated by IL-10. The results

indicate that production of the pro inflammatory cytokine IL-1β is strongly inhibited by IL-10 produced by the polydonor $CD4^{IL-10}$ T cells. As expected, the anti-IL-10 receptor antibody had no effect of the inhibition of IL-1β production mediated by Z-VADfmk and MCC950 (FIG. 35E).

[00704] $CD4^{IL-10}$ T cells were further tested on monocytes from donor #4 activated by LPS and nigericin. Various concentrations of single donor (BC-V) or polydonor (three donors; BC-T/U/V) $CD4^{IL-10}$ cell supernatants containing 2583 or 2589pg IL-10/mL respectively, ZYVADfmk or MCC950 were tested. Data provided in FIG. 35G show that pooled supernatants of 3 different donors (BC T-U-V) down regulate IL-18 production induced by LPS in combination with nigericin.

[00705] Collectively, these data indicate that IL-10 produced by single- and poly donor $CD4^{IL-10}$ cells strongly down regulates the NLPR3 inflammasome resulting in strong inhibition of the pro inflammatory cytokines IL-1β and IL-18.

6.9.14. Example 13: Single-donor and polydonor $CD4^{IL-10}$ cells inhibit xeno GvHD and myeloid tumor growth in vivo

[00706] Functional properties and quality of the single donor (BC-T,BC-V, and BC-E) or polydonor (BC-V/T/E) $CD4^{IL-10}$ cells were tested as described in Andolfi et al. Mol Ther 2012, 20, 177 and Locafaro et al. Mol Ther 2017, 25, 2254. Both single donor- and polydonor $CD4^{IL-10}$ cells produced high levels of IL-10, variable levels of IFN-gamma, very low levels of IL-4 and no detectable IL-2 (the latter not shown), reflecting the characteristic cytokine production profile of Tr1 cells (FIG. 37).

[00707] Further, the suppressive capacity of the single donor (BC-T,BC-V, and BC-E) or polydonor (BC-V/T/E) $CD4^{IL-10}$ cells on CD4+ and CD8+ T cell proliferation was measured in vitro on allogeneic PBMC. PBMC were labeled with eFLuor670 (Invitrogen). Labeled PBMC ($1x10^5$) were activated with immobilized CD3 (10mg/mL) and soluble CD28 antibodies (1mg/mL). Single and polydonor $CD4^{IL-10}$ cells were added at a 1:1 ratio in a final volume of 0.2mL in 96 well round bottom plates. After 4 days of co-culture, their suppressive effects on the proliferation of eFluor670 labeled responder cells was determined by eFluor670 dilution using flow cytometry as described (Locafaro et al. Mol Ther 2017, 25, 2254). FIG. 38 provides results from the flow cytometry. Single- and polydonor $CD4^{IL-10}$ cells strongly inhibited in vitro proliferation of both allogeneic CD4+ and CD8+ T cells by more than 80% (FIG. 38).

[00708] The $CD4^{IL\text{-}10}$ cells were further analyzed for their cytotoxic effects against myeloid leukemia cells (ALL-CM) and an erythroid leukemia cell line (K562). Single (BC-E and BC-V) or polydonor (BC-V/T/E) $CD4^{IL\text{-}10}$ cells were co-cultured at a 1:1 ratio with ALL-CM or K562 cells. After 3 days the cells were harvested and surviving CD45low, CD3- target cells were counted and analyzed by FACS as described ((Locafaro et al. Mol Ther 2017, 25, 2254). The single donor and poly donor $CD4^{IL\text{-}10}$ cells also mediated strong direct cytotoxic effects on ALL-CM myeloid tumor cells, whereas they failed to kill the sensitive K562 cells, which lack Class I MHC expression required for their cytotoxic activity (FIG. 39). Single (BC-E and BC-V) or polydonor (BC-V/T/E) $CD4^{IL\text{-}10}$ cells had comparable cytotoxic activities against these two target cell lines (ALL-CM and K562).

[00709] Cytotoxic effects of single-donor (BC-E) and polydonor (BC-V/T/E) $CD4^{IL\text{-}10}$ cells were also tested in vivo, using a humanized xeno GvHD disease model - an NSG mouse intravenously injected with ALL-CM cells ($2.5x10^6$). Their effect on GvHD induced by human PBMC from an allogeneic donor as well as their effect on the growth of acute myeloid leukemia in cell line ALL CM in a therapeutic setting were tested as illustrated in FIG. 36.

[00710] Eight to ten-week-old female NOD scid gamma, (NSG) mice were obtained from Charles-River Italia (Calco, Italy). The experimental protocol was approved by the internal committee for animal studies of the Ospedale San Raffaele (Institutional Animal Care and Use Committee (IACUC). At day 0, the mice received total body irradiation from a linear accelerator. ALL-CM cells ($2.5x10^6$) were injected at day 0. On day 0, different groups of mice were injected with nothing, allogeneic PBMC ($2.5 x10^6$), single donor (BC-E, $2.5 x10^6$) or polydonor $CD4^{IL\text{-}10}$ cells pooled at 1:1:1 ratio from 3 different donors (BC-V/T/E, $2.5 x10^6$) in combination with allogeneic PBMC ($2.5x10^6$) or polydonor $CD4^{IL\text{-}10}$ cells ($2.5x10^6$) on day 3. All cells were administered i.v. in volumes of 250 µL of Iscove's modified Dulbecco's medium. Mice were monitored 3-4 times per week.

[00711] The NSG mice were divided into five cohorts of 5 mice and each group was treated on day 0 with (i) none as a control; (ii) allogeneic mononuclear cells (PBMC); (iii) allogeneic PBMC and polydonor $CD4^{IL\text{-}10}$ cells (BC-V/T/E); (iv) allogeneic PBMC and single-donor $CD4^{IL\text{-}10}$ cells (BC-E); or (v) polydonor (BC-V/T/E) $CD4^{IL\text{-}10}$ cells administered at day 3 Myeloid leukemia progression was measured as previously described ((Locafaro et al. Mol Ther 2017, 25, 2254).

[00712] Administration of ALL-CM cells to NSG mice resulted in a rapid expansion of these cells and all the mice died or had to be sacrificed on day 20. Injection of PBMC prevented leukemia progression as expected. Both single- and poly- donor $CD4^{IL10}$ cells given in combination with allogeneic PBMC did not interfere with anti myeloid leukemia effects of the PBMC.

[00713] Injection of polydonor $CD4^{IL-10}$ (BC-V/T/E) cells 3 days after administration of the ALL-CM cells (when already massive expansion of these cells is ongoing) resulted in inhibition of tumor growth. These results indicate that polydonor $CD4^{IL-10}$ cells have direct therapeutic anti myeloid leukemia effects in vivo (FIG. 40).

[00714] However, despite their beneficial anti myeloid leukemia effects, the PBMC induced a very severe form of xeno-GvHD and all mice died by day 24 (FIG. 41). In the study, single-donor (BC-E) and polydonor (BC-V/T/E) $CD4^{IL-10}$ cells were tested on their capacity to inhibit xeno-GvHD induced by PBMC following administration in NSG mice. On day 0, the NSG mice were injected with ALL-CM cells ($2.5x10^6$). The mice were divided into five groups and each group was treated with (i) none as a control; (ii) allogeneic mononuclear cells (PBMC); (iii) allogeneic PBMC and polydonor (BC-V/T/E) $CD4^{IL-10}$ cells (BC-E, BC-V, BC-T); (iv) allogeneic PBMC and single-donor $CD4^{IL-10}$ cells (BC-E) or polydonor (BC-V/T/E) $CD4^{IL-10}$ cells injected at day 3. In the animals, xeno-GvHD was measured by survival and weight loss. In addition, hunching, fur condition and skin integrity were monitored as described (Bondanza et al, Blood, 2006, 107, 1828). If weight loss reached more than 20%, the mice were sacrificed for ethical reasons. FIG. 41 shows % of NSG mice free of GvHD in each day following day 1 injection with ALL-CM cells ($2.5x10^6$) and subsequent treatment with PBMC with or without single-donor or polydonor $CD4^{IL-10}$ cells.

[00715] The results show that polydonor $CD4^{IL-10}$ cells did not induce xeno-GvHD, and down regulated xeno-GvHD induced by allogeneic PBMC. Collectively these results indicate that polydonor $CD4^{IL-10}$ cells downregulate severe xeno-GvHD, have direct anti myeloid leukemia effects in a therapeutic setting and do not interfere with the protective anti myeloid leukemia effects of the PBMC.

6.9.15. Example 14: Adoptive transfer of polydonor CD4IL-10 cells derived from four different donors

[00716] Adoptive transfer of polydonor $CD4^{IL-10}$ cells derived from four different donors was tested for the transfer's ability to inhibit PBMC-induced xeno-GvHD.

[00717] In these experiments, single-donor $CD4^{IL-10}$ cells (donor C; lot C) and polydonor $CD4^{IL-10}$ cells derived from 4 different donors(donors C, E, F, and H; lot CEFH) were tested in a humanized mouse model of GvHD induced by allogeneic PBMC. In this model, NSG mice were sub-lethally irradiated at day 0 and injected at day 3 (slow bolus i.v.) with (i) 2.5E+06 allogeneic PBMC, (ii) 2.5E+06 allogeneic PBMC in combination with 2.5E+06 single-donor CD^{4IL-10} cells (lot C), (iii) 2.5E+06 allogeneic PBMC in combination with 2.5E+06 cells polydonor $CD4^{IL-10}$ cells (lot CEFH), or (iv) 2.5E+06 cells polydonor $CD4^{IL-10}$ cells (lot CEFH) alone. GvHD was determined using a composite score of weight loss, fur appearance, skin appearance, hunch, and activity (see Bondanza A, et al. *Blood* 2006;107:1828–36]. As shown in FIG. 42, only mice administered with the polydonor $CD4^{IL-10}$ cells were 100% free of GvHD at the end of the study.

[00718] In summary, this data demonstrated that adoptive transfer of polydonor $CD4^{IL-10}$ cells derived from four different donors inhibits PBMC-induced xeno-GvHD and does not induce xeno GvHD.

6.9.16. Example 15: Generation of a variant of IL-10

[00719] Variants of human IL-10 are generated by introducing amino acid modification(s) (e.g., substitution, insertion, deletion) in view of IL-10 sequences of other species. Modification sites are determined by sequence alignment as provided in FIG. 43A. Amino acid positions having different amino acids among species are identified from the alignment and modified by introducing substitution, insertion, or deletion of amino acids.

[00720] Two examples of the variant of human IL-10 are provided in FIG. 43B. Possible huIL-10 HYBRID #1 (SEQ ID NO: 21) is generated by substituting three amino acids (D, I and A) of human IL-10 with three different amino acids (E, A, and D) of viral IL-10 (EBVB9) at the corresponding positions. Possible huIL-10 HYBRID #2 (SEQ ID NO: 22) is generated by substituting one amino acid (I105) of human IL-10 with another amino acid (A105) of viral IL-10 (EBVB9) at the corresponding position. FIG. 43C shows alignment of human IL-10 (SEQ ID NO: 1) with IL10 EBVB9 (SEQ ID NO: 18) with "*"indicating the one or more amino acid positions that are substituted in IL-10 hybrid #1 and "#"indicating the preferred I105 to A105 amino acid substitution for IL-10 hybrid #2.

[00721] The variants of human IL-10 are cloned into an expression vector as described in the above section and tested for the expression and function of the variant proteins. Selected

variants of human IL-10 are used to generate $CD4^{IL\text{-}10}$ cells. Efficiency of $CD4^{IL\text{-}10}$ cells are tested as provided herein.

6.9.17. Additional Experimental methods and materials for Examples 1-11

[00722] **Cell preparation and cell lines.** Peripheral blood mononuclear cells (PBMC) were prepared by centrifugation over Ficoll-Hypaque gradients. $CD4^+$ T cells were purified with a CD4 T cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) with a resulting purity of >95%. Mature dendritic cells (DC) were generated from peripheral blood $CD14^+$ monocytes positively selected using $CD14^+$ MicroBeads (Miltenyi Biotech, Germany) according to the manufacturer's instructions and cultured in RPMI 1640 (Lonza, Italy) supplemented with 10% fetal bovine serum (FBS; Lonza, Italy), 100 U/mL penicillin/streptomycin (Lonza, Italy), 2 mM L-glutamine (Lonza, Italy), at 37°C in the presence of 10 ng/mL recombinant human (rh) IL-4 (R&D Systems, Minneapolis MN, USA) and 100 ng/mL rhGM-CSF (Genzyme, Seattle, WA, USA) for 5 days and matured with 1 mg/mL of lipopolysaccharide (LPS, Sigma, CA, USA) for an additional two days.

[00723] **Plasmid construction.** The coding sequence of human IL-10 was excised from pH15C (ATCC n° 68192), and the 549bp fragment was cloned into the multiple cloning site of pBluKSM (Invitrogen) to obtain pBluKSM-hIL-10. A fragment of 555bp was obtained by excision of hIL-10 from pBluKSM-hIL-10 and ligation to 1074.1071.hPGK.GFP.WPRE.mhCMV.dNGFR.SV40PA (here named LV-ΔNGFR), to obtain LV-IL-10/ΔNGFR. The presence of the bidirectional promoter (human PGK promoter plus minimal core element of the CMV promoter in opposite direction) allows co-expression of the two transgenes (Locafaro et al. Mol Ther. 2017;25(10):2254-2269). The sequence of LV-IL-10/ΔNGFR was verified by pyrosequencing (Primm).

[00724] **Vector production and titration.** VSV-G-pseudotyped third generation bidirectional lentiviral vectors were produced by Ca_3PO_4 transient four-plasmid co-transfection into 293T cells and concentrated by ultracentrifugation as described (Locafaro et al. Mol Ther. 2017;25(10):2254-2269). Titer was estimated by limiting dilution, vector particles were measured by HIV-1 Gag p24 antigen immune capture (NEN Life Science Products; Waltham, MA), and vector infectivity was calculated as the ratio between titer and particle. Titers ranged from $5x10^8$ to $6x10^9$ transducing units/mL, and infectivity from $5x10^4$ to 10^5 transducing units/ng of p24.

[00725] **Generation of $CD4^{IL\text{-}10}$ cell lines.** Polyclonal CD4-transduced cells were obtained as previously described (Andolfi et al. Mol Ther. 2012;20(9):1778-1790). Briefly, CD4 purified T cells were activated for 48 hours with soluble anti-CD3 monoclonal antibody (mAb, 30 ng/mL, OKT3, Janssen-Cilag, Raritan, NJ, USA), anti-CD28 mAb (1 μg/mL, BD) and rhIL-2 (50 U/mL, PROLEUKIN, Novartis, Italy). T cells were transduced with LV-IL-10/ΔNGFR ($CD4^{IL\text{-}10}$) with multiplicity of infection (MOI) of 20. At day 11, $CD4^{+}\Delta NGFR^{+}$ cells were beads-sorted using $CD271^{+}$ Microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and expanded in X-VIVO15 medium with 5% human serum (BioWhittaker-Lonza, Washington), 100 U/mL penicillin–streptomycin (BioWhittaker), and 50 U/mL rhIL-2 (PROLEUKIN, Novartis, Italy). At day 7 and 10, medium was replaced by fresh medium supplemented with 50U/mL of rhIL-2. At day 14, cells were collected, washed, and restimulated with allogeneic feeder mixture as previously described (Andolfi et al. Mol Ther. 2012;20(9):1778-1790). After 14 days, cells were collected and frozen. Thawed $CD4^{IL\text{-}10}$ cells were restimulated and after the 2^{nd} and 3^{rd} re-stimulation and expansion were functionally characterized *in vitro* and used for *in vivo* experiments.

[00726] **Vector Copy Number Analysis.** Cells were cultured for at 11 days after transduction in order to get rid of non-integrated vector forms. Genomic DNA was isolated with QIAamp DNA Blood Mini Kit (QIAGEN, 51106), according to the manufacturer's instructions. Vector integrations were quantified by QX200 Droplet Digital PCR System (Bio-Rad), according to the manufacturer's instructions.

[00727] **Cytokine determination.** To measure cytokine production, after 2^{nd} and 3^{rd} re-stimulation single donor and polydonor $CD4^{IL\text{-}10}$ cells were left unstimulated or stimulated with immobilized anti-CD3 (10 μg/mL) and soluble anti-CD28 (1μg/mL) mAbs in a final volume of 200 μL of medium (96 well round-bottom plates, $2x10^5$/well). Supernatants were harvested after 48 hours of culture and levels of IL-10, IL-4, IL-5, IFN-γ, and IL-22 were determined by ELISA according to the manufacturer's instructions (BD Biosciences).

[00728] **Flow cytometry analysis.** For the expression of Granzyme B (clone MHGB04, Invitrogen, USA) after surface staining with CD4, $CD4^{IL\text{-}10}$ cells were fixed, permeabilized, and stained using the BD Cytofix/Cytoperm™ Kit according to the manufacturer's instructions (Cat. No. 554714, Biolegend, USA). Stained cells were washed two times with PBS supplemented with 1% FBS and analysed with a BD LSRFortessa analysed utilizing FlowJo 10 software.

[00729] **Killing assays.** After 2nd and 3rd re-stimulation, cytotoxicity of single-donor and polydonor $CD4^{IL\text{-}10}$ cells was analysed in co-culture experiments. Briefly, non-myeloid leukemia and a myeloid leukemia cell lines, K562 and ALL-CM respectively, were used as target cells and plated with $CD4^{IL\text{-}10}$ cells at 1:1 ratio (10^5 target cells and 10^5 $CD4^{IL\text{-}10}$ cells) for 3 days. At the end of co-culture, cells were harvested and K562 and ALL-CM cells were analysed and counted by FACS.

[00730] **Suppression assays.** To measure the suppressive capacity of single donor and polydonor $CD4^{IL\text{-}10}$ cells, allogeneic PBMC were labeled with Cell Proliferation Dye eFluor® 670 (Invitrogen, CA, USA), according to manufacturer's instructions prior to stimulation with allogeneic mature DC ($5x10^4$ cells/well) and soluble anti-CD3 (50 ng/mL) mAb. PBMC and suppressor cells were added at a 1:1 ratio (10^5 PBMC and 10^5 $CD4^{IL\text{-}10}$ cells). After 3 days of culture, proliferation of responder cells was determined by analyzing the eFluor670 dilution of $CD4^+\Delta NGFR^-$ or $CD8^+\Delta NGFR^-$ T cells by FACS.

[00731] **Graft-versus Host Disease models:** In all experiments 6/8 week-old female NSG mice were used. On day 0 mice received total body irradiation with a single dose of 175-200 cGy from a linear accelerator according to the weight of the mice, and were intravenously with PBMC cells ($5x10^6$), or $CD4^{IL\text{-}10}$ cells (single-donors or polydonor - pool of three donors - $5x10^6$ or $2.5x10^6$), or with PBMC ($5x10^6$) in combination with $CD4^{IL\text{-}10}$ cells ($5x10^6$ or $2.5x10^6$). Survival, weight loss, activity, fur, skin, and hunch were monitored at least 3 times per week as previously described (Bondanza et al. Blood. 2006;107(5):1828-1836). Mice were euthanized for ethical reasons when their loss of bodyweight was 20%.

[00732] Alternatively, on day 0 mice received total body irradiation as above. On day 3 mice were injected with $CD4^+$ T cells ($5x10^6$ or $2.5x10^6$), single and polydonor (pool of three donors) $CD4^{IL\text{-}10}$ cells ($5x10^6$ or $2.5x10^6$), or $CD4^+$ T cells $5x10^6$ or $2.5x10^6$) in combination with single and polydonor (pool of three donors) $CD4^{IL\text{-}10}$ cells ($5x10^6$ or $2.5x10^6$). GvHD induction was monitored as indicated above.

7. INCORPORATION BY REFERENCE

[00733] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

8. EQUIVALENTS

[00734] While various specific embodiments have been illustrated and described, the above specification is not restrictive. It will be appreciated that various changes can be made without departing from the spirit and scope of the invention(s). Many variations will become apparent to those skilled in the art upon review of this specification.

9. SEQUENCES

SEQ ID NO: 1 (Human IL-10 amino acid sequence-- Protein Sequence: Ref P22301)

MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNL
LLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLKTLRLRLRRCHR
FLPCENKSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN

SEQ ID NO: 2 (Human IL-10 exemplary polynucleotide sequence)

ATGCACAGCTCAGCACTGCTCTGTTGCCTGGTCCTCCTGACTGGGGTGAGGGCCAGCCCAGGCC
AGGGCACCCAGTCTGAGAACAGCTGCACCCACTTCCCAGGCAACCTGCCTAACATGCTTCGAGA
TCTCCGAGATGCCTTCAGCAGAGTGAAGACTTTCTTTCAAATGAAGGATCAGCTGGACAACTTG
TTGTTAAAGGAGTCCTTGCTGGAGGACTTTAAGGGTTACCTGGGTTGCCAAGCCTTGTCTGAGA
TGATCCAGTTTTACCTGGAGGAGGTGATGCCCCAAGCTGAGAACCAAGACCCAGACATCAAGGC
GCATGTGAACTCCCTGGGGGAGAACCTGAAGACCCTCAGGCTGAGGCTACGGCGCTGTCATCGA
TTTCTTCCCTGTGAAAACAAGAGCAAGGCCGTGGAGCAGGTGAAGAATGCCTTTAATAAGCTCC
AAGAGAAAGGCATCTACAAAGCCATGAGTGAGTTTGACATCTTCATCAACTACATAGAAGCCTA
CATGACAATGAAGATACGAAACTGA

SEQ ID NO: 3 (ΔNGFR amino acid sequence)

MGAGATGRAMDGPRLLLLLLLGVSLGGAKEACPTGLYTHSGECCKACNLGEGVAQPCGANQTVC
EPCLDSVTFSDVVSATEPCKPCTECVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACRVC
EAGSGLVFSCQDKQNTVCEECPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAECEEIP
GRWITRSTPPEGSDSTAPSTQEPEAPPEQDLIASTVAGVVTTVMGSSQPVVTRGTTDNLIPVYC
SILAAVVVGLVAYIAFKRWNRGIL

SEQ ID NO: 4 (ΔNGFR exemplary polynucleotide sequence)

ATGGGGGCAGGTGCCACCGGCCGCGCCATGGACGGGCCGCGCCTGCTGCTGTTGCTGCTTCTGG
GGGTGTCCCTTGGAGGTGCCAAGGAGGCATGCCCCACAGGCCTGTACACACACAGCGGTGAGTG
CTGCAAAGCCTGCAACCTGGGCGAGGGTGTGGCCCAGCCTTGTGGAGCCAACCAGACCGTGTGT
GAGCCCTGCCTGGACAGCGTGACGTTCTCCGACGTGGTGAGCGCGACCGAGCCGTGCAAGCCGT
GCACCGAGTGCGTGGGGCTCCAGAGCATGTCGGCGCCGTGCGTGGAGGCCGACGACGCCGTGTG
CCGCTGCGCCTACGGCTACTACCAGGATGAGACGACTGGGCGCTGCGAGGCGTGCCGCGTGTGC
GAGGCGGGCTCGGGCCTCGTGTTCTCCTGCCAGGACAAGCAGAACACCGTGTGCGAGGAGTGCC
CCGACGGCACGTATTCCGACGAGGCCAACCACGTGGACCCGTGCCTGCCCTGCACCGTGTGCGA
GGACACCGAGCGCCAGCTCCGCGAGTGCACACGCTGGGCCGACGCCGAGTGCGAGGAGATCCCT

GGCCGTTGGATTACACGGTCCACACCCCCAGAGGGCTCGGACAGCACAGCCCCCAGCACCCAGG
AGCCTGAGGCACCTCCAGAACAAGACCTCATAGCCAGCACGGTGGCAGGTGTGGTGACCACAGT
GATGGGCAGCTCCCAGCCCGTGGTGACCCGAGGCACCACCGACAACCTCATCCCTGTCTATTGC
TCCATCCTGGCTGCTGTGGTTGTGGGCCTTGTGGCCTACATAGCCTTCAAGAGGTGGAACAGGG
GGATCCTCTAG

SEQ ID NO: 5 (nucleotide sequence of pLV-IL10):

TGGCCATTGCATACGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACAT
TACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGT
TCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCG
CCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGA
CTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGT
GTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTAT
GCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTA
TTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGG
ATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGAC
TTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGG
AGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGGGGTCTCTCTGGTTAGACCAGATCTGAGCC
TGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGC
TTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTA
GTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGCGAAAGGGAAACCAG
AGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCG
ACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGC
GTCAGTATTAAGCGGGGGAGAATTAGATCGCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAA
AGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAA
TCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAATACTGGGACAGCTACAACCATCCCTT
CAGACAGGATCAGAAGAACTTAGATCATTATATAATACAGTAGCAACCCTCTATTGTGTGCATC
AAAGGATAGAGATAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAG
TAAGACCACCGCACAGCAAGCGGCCGCTGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAA
TTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACC
AAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCCTTG
GGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAG
ACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAG
CATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAA
GATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAACTCATTTGCACCAC
TGCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTGGAACAGATTTGGAATCACACGACC
TGGATGGAGTGGGACAGAGAAATTAACAATTACACAAGCTTAATACACTCCTTAATTGAAGAAT
CGCAAAACCAGCAAGAAAAGAATGAACAAGAATTATTGGAATTAGATAAATGGGCAAGTTTGTG
GAATTGGTTTAACATAACAAATTGGCTGTGGTATATAAAATTATTCATAATGATAGTAGGAGGC
TTGGTAGGTTTAAGAATAGTTTTTGCTGTACTTTCTATAGTGAATAGAGTTAGGCAGGGATATT
CACCATTATCGTTTCAGACCCACCTCCCAACCCCGAGGGGACCCGACAGGCCCGAAGGAATAGA
AGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCTCGACGGTAT
CGGTTAACTTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACA
TAATAGCAACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTAT
CGATCACGAGACTAGCCTCGAGAGATCTGATCATAATCAGCCATACCACATTTGTAGAGGTTTT
ACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTT
GTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAGGCAATAGCATCACAAATTTCA
CAAATAAGGCATTTTTTTCACTGCATTCTAGTTTTGGTTTGTCCAAACTCATCAATGTATCTTA
TCATGTCTGGATCTCAAATCCCTCGGAAGCTGCGCCTGTCTTAGGTTGGAGTGATACATTTTTA
TCACTTTTACCCGTCTTTGGATTAGGCAGTAGCTCTGACGGCCCTCCTGTCTTAGGTTAGTGAA

AAATGTCACTCTCTTACCCGTCATTGGCTGTCCAGCTTAGCTCGCAGGGGAGGTGGTCTGGATC
CACCATGTCTAGAGGATCCCCCTGTTCCACCTCTTGAAGGCTATGTAGGCCACAAGGCCCACAA
CCACAGCAGCCAGGATGGAGCAATAGACAGGGATGAGGTTGTCGGTGGTGCCTCGGGTCACCAC
GGGCTGGGAGCTGCCCATCACTGTGGTCACCACACCTGCCACCGTGCTGGCTATGAGGTCTTGT
TCTGGAGGTGCCTCAGGCTCCTGGGTGCTGGGGGCTGTGCTGTCCGAGCCCTCTGGGGGTGTGG
ACCGTGTAATCCAACGGCCAGGGATCTCCTCGCACTCGGCGTCGGCCCAGCGTGTGCACTCGCG
GAGCTGGCGCTCGGTGTCCTCGCACACGGTGCAGGGCAGGCACGGGTCCACGTGGTTGGCCTCG
TCGGAATACGTGCCGTCGGGGCACTCCTCGCACACGGTGTTCTGCTTGTCCTGGCAGGAGAACA
CGAGGCCCGAGCCCGCCTCGCACACGCGGCACGCCTCGCAGCGCCCAGTCGTCTCATCCTGGTA
GTAGCCGTAGGCGCAGCGGCACACGGCGTCGTCGGCCTCCACGCACGGCGCCGACATGCTCTGG
AGCCCCACGCACTCGGTGCACGGCTTGCACGGCTCGGTCGCGCTCACCACGTCGGAGAACGTCA
CGCTGTCCAGGCAGGGCTCACACACGGTCTGGTTGGCTCCACAAGGCTGGGCCACACCCTCGCC
CAGGTTGCAGGCTTTGCAGCACTCACCGCTGTGTGTGTACAGGCCTGTGGGGCATGCCTCCTTG
GCACCTCCAAGGGACACCCCCAGAAGCAGCAACAGCAGCAGGCGCGGCCCGTCCATGGCGCGGC
CGGTGGCACCTGCCCCCATCGCCCGCCTCCCGCGGCAGCGCTCGACTTCCAGCTCGGTCCGCTT
TGCGGACTGATGGGGCTGCGCTGCGCTGCGCTCCAGCGCCCCCCCCTGCCCGCCGGAGCTGGCCG
CGGCCCGAATTCCTGCAGGAATTCGATGGAGGCTGGATCGGTCCCGGTGTCTTCTATGGAGGTC
AAAACAGCGTGGATGGCGTCTCCAGGCGATCTGACGGTTCACTAAACGAGCTCTGCTTATATAG
GCCTCCCACCGTACACGCCTACCCTCGAGAAGCTTGATATCGAATTCCCACGGGGTTGGGGTTG
CGCCTTTTCCAAGGCAGCCCTGGGTTTGCGCAGGGACGCGGCTGCTCTGGGCGTGGTTCCGGGA
AACGCAGCGGCGCCGACCCTGGGTCTCGCACATTCTTCACGTCCGTTCGCAGCGTCACCCGGAT
CTTCGCCGCTACCCTTGTGGGCCCCCCGGCGACGCTTCCTGCTCCGCCCCTAAGTCGGGAAGGT
TCCTTGCGGTTCGCGGCGTGCCGGACGTGACAAACGGAAGCCGCACGTCTCACTAGTACCCTCG
CAGACGGACAGCGCCAGGGAGCAATGGCAGCGCGCCGACCGCGATGGGCTGTGGCCAATAGCGG
CTGCTCAGCGGGGCGCGCCGAGAGCAGCGGCCGGGAAGGGGCGGTGCGGGAGGCGGGGTGTGGG
GCGGTAGTGTGGGCCCTGTTCCTGCCCGCGCGGTGTTCCGCATTCTGCAAGCCTCCGGAGCGCA
CGTCGGCAGTCGGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCCAGGGGGATCCCCGGTC
TGCAGGAATTCATGCACAGCTCAGCACTGCTCTGTTGCCTGGTCCTCCTGACTGGGGTGAGGGC
CAGCCCAGGCCAGGGCACCCAGTCTGAGAACAGCTGCACCCACTTCCCAGGCAACCTGCCTAAC
ATGCTTCGAGATCTCCGAGATGCCTTCAGCAGAGTGAAGACTTTCTTTCAAATGAAGGATCAGC
TGGACAACTTGTTGTTAAAGGAGTCCTTGCTGGAGGACTTTAAGGGTTACCTGGGTTGCCAAGC
CTTGTCTGAGATGATCCAGTTTTACCTGGAGGAGGTGATGCCCCAAGCTGAGAACCAAGACCCA
GACATCAAGGCGCATGTGAACTCCCTGGGGGAGAACCTGAAGACCCTCAGGCTGAGGCTACGGC
GCTGTCATCGATTTCTTCCCTGTGAAAACAAGAGCAAGGCCGTGGAGCAGGTGAAGAATGCCTT
TAATAAGCTCCAAGAGAAAGGCATCTACAAAGCCATGAGTGAGTTTGACATCTTCATCAACTAC
ATAGAAGCCTACATGACAATGAAGATACGAAACTGAGTCGAGAATCAACCTCTGGATTACAAAA
TTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGC
TTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAA
TCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCA
CTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGG
GACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGC
TGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCT
TTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCC
TTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCG
CGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCCTGGAATTC
GAGCTCGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAA
GAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAACGAAGACAAGATCTGCTTTTTGCTTGTA
CTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACT
GCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGAC
TCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTAGTAGTT

CATGTCATCTTATTATTCAGTATTTATAACTTGCAAAGAAATGAATATCAGAGAGTGAGAGGAA
CTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAA
GCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCT
GGCTCTAGCTATCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCAT
TCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTG
AGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCGTCGAGACGTACCC
AATTCGCCCTATAGTGAGTCGTATTACGCGCGCTCACTGGCCGTCGTTTTACAACGTCGTGACT
GGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCG
TAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAAGCGGCCGCACGCTCAGTGGAACGAAAA
CTCACGTTAAGGGATTTTGGTCATGAACAATAAAACTGTCTGCTTACATAAACAGTAATACAAG
GGGTGTTATGAGCCATATTCAACGGGAAACGTCTTGCTCTAGGCCGCGATTAAATTCCAACATG
GATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCT
ATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGC
CAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACC
ATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGGAAAA
CAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAACATTGTTGATGCGCTGGCAGT
GTTCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTT
CGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACG
AGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACC
GGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTA
ATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTAT
GGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGA
TAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAAGAATTAATT
CATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGACTATGT
CTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCC
GTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAA
CAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCC
GAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTA
GGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAG
TGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGA
TAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACC
TACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAA
AGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGG
GGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTT
TTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGT
TCCTGGCCTTTTGCTGGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGA
GCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAG
AGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGTATGCTTCCGGCTCGTATGTTGTGTGG
AATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCCGAAT
TAACCCTCACTAAAGGGAACAGCTAGC

SEQ ID NO: 6 (Viral interleukin-10 homolog aka interleukin-10 BCRF1 aka IL10H_EBVB9) Protein Sequence: Ref: P03180

MERRLVVTLQCLVLLYLAPECGGTDQCDNFPQMLRDLRDAFSRVKTFFQTKDEVDNLLLKESLL
EDFKGYLGCQALSEMIQFYLEEVMPQAENQDPEAKDHVNSLGENLKTLRLRLRRCHRFLPCENK
SKAVEQIKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTIKAR

SEQ ID NO: 7 (Viral interleukin-10 homolog cDNA sequence)

Nucleotide sequence (cDNA): Ref: NC_007605.1

5′_ATGGAGCGAAGGTTAGTGGTCACTCTGCAGTGCCTGGTGCTGCTTTACCTGGCACCTGAGT
GTGGAGGTACAGACCAATGTGACAATTTTCCCCAAATGTTGAGGGACCTAAGAGATGCCTTCAG
TCGTGTTAAAACCTTTTTCCAGACAAAGGACGAGGTAGATAACCTTTTGCTCAAGGAGTCTCTG
CTAGAGGACTTTAAGGGCTACCTTGGATGCCAGGCCCTGTCAGAAATGATCCAATTCTACCTGG
AGGAAGTCATGCCACAGGCTGAAAACCAGGACCCTGAAGCCAAAGACCATGTCAATTCTTTGGG
TGAAAATCTAAAGACCCTACGGCTCCGCCTGCGCAGGTGCCACAGGTTCCTGCCGTGTGAGAAC
AAGAGTAAAGCTGTGGAACAGATAAAAAATGCCTTTAACAAGCTGCAGGAAAAAGGAATTTACA
AAGCCATGAGTGAATTTGACATTTTTATTAACTACATAGAAGCATACATGACAATTAAAGCCAG
GTGA_3′

SEQ ID NO: 9 (anti-CD19 CAR amino acid sequence)

MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDG
TVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEI
TGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWL
GVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ
GTSVTVSSAAAPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFDTALAAVICSALAT
VLLALLILCVIYCKRQPRRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRS
ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAY
SEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 10 (anti-CD19 CAR polynucleotide sequence)

ATGCTGCTGCTGGTCACCAGCCTGCTGCTGTGCGAGCTCCCTCACCCCGCCTTTCTGCTTATCC
CGGACATTCAGATGACACAGACCACCTCGAGCTTGTCCGCGTCGCTGGGCGATCGCGTGACCAT
CTCCTGCCGGGCCTCCCAAGACATTTCAAAGTATCTCAACTGGTACCAGCAGAAGCCGGACGGA
ACCGTGAAACTGCTGATCTACCATACCAGCCGCCTGCACTCCGGCGTGCCGTCCCGCTTCTCCG
GATCGGGTTCCGGAACTGACTACTCACTGACTATCTCCAACTTGGAACAAGAGGACATCGCCAC
TTACTTCTGTCAACAAGGAAATACCCTTCCCTACACCTTCGGGGGGGGTACCAAGCTGGAGATC
ACTGGGGGCGGAGGCTCCGGTGGAGGCGGATCCGGCGGTGGAGGGAGCGAAGTCAAGCTGCAGG
AATCAGGACCAGGACTCGTGGCGCCATCCCAGTCCCTGTCGGTGACCTGTACTGTCTCCGGAGT
CAGCCTCCCCGATTACGGAGTGTCATGGATTAGGCAACCCCCAAGAAAAGGGCTGGAATGGCTC
GGAGTGATCTGGGGCTCCGAAACCACCTACTACAACTCGGCGCTGAAGTCCCGGCTGACCATCA
TCAAGGACAACTCCAAGAGCCAAGTGTTCTTGAAGATGAACAGCTTGCAGACCGACGATACCGC
AATCTACTACTGTGCCAAGCACTATTACTACGGGGGGTCTTACGCCATGGACTACTGGGGACAG
GGCACCTCCGTGACTGTGTCGTCCGCGGCCGCGCCCGCCCCTCGGCCCCCGACTCCTGCCCCGA
CGATCGCTTCCCAACCTCTCTCGCTGCGCCCGGAAGCATGCCGGCCCGCCGCCGGTGGCGCTGT
CCACACTCGCGGACTGGACTTTGATACCGCACTGGCGGCCGTGATCTGTAGCGCCCTGGCCACC
GTGCTGCTGGCGCTGCTCATCCTTTGCGTGATCTACTGCAAGCGGCAGCCTAGGCGAAAGAAGC
TCCTCTACATTTTCAAGCAACCCTTCATGCGCCCCGTGCAAACCACCCAGGAGGAGGATGGATG
CTCATGCCGGTTCCCTGAGGAAGAAGAGGGCGGTTGCGAGCTCAGAGTGAAATTCAGCCGGTCG
GCTGACGCCCCGGCGTACCAGCAGGGCCAGAACCAGCTGTACAATGAGCTCAACCTGGGGCGCC
GCGAAGAGTACGACGTGCTGGACAAGAGGAGAGGCAGAGATCCGGAAATGGGCGGAAAGCCAAG
GCGGAAGAACCCGCAGGAAGGTCTTTACAACGAACTGCAGAAGGACAAGATGGCCGAGGCCTAC
TCCGAGATTGGGATGAAGGGAGAAAGACGGAGGGGAAAGGGACATGACGGACTTTACCAGGGCC
TGAGCACTGCCACGAAGGACACCTATGATGCCCTGCACATGCAGGCGCTGCCGCCTCGG

SEQ ID NO: 11 (anti-CD19 scFV amino acid sequence for FMC63)

MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDG
TVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEI
TGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWL
GVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ
GTSVTVSS

SEQ ID NO: 12 (anti-CD19 scFV polynucleotide sequence)

ATGCTTCTCCTGGTCACCTCCCTGCTCCTCTGCGAACTGCCTCACCCTGCCTTCCTTCTGATTC
CTGACATTCAGATGACTCAGACCACCTCTTCCTTGTCCGCGTCACTGGGAGACAGAGTGACCAT
CTCGTGTCGCGCAAGCCAGGATATCTCCAAGTACCTGAACTGGTACCAACAGAAGCCCGACGGG
ACTGTGAAGCTGCTGATCTACCACACCTCACGCCTGCACAGCGGAGTGCCAAGCAGATTCTCCG
GCTCCGGCTCGGGAACCGATTACTCGCTTACCATTAGCAACCTCGAGCAGGAGGACATCGCTAC
CTACTTCTGCCAGCAAGGAAATACCCTGCCCTACACCTTCGGCGGAGGAACCAAATTGGAAATC
ACCGGCGGAGGAGGCTCCGGGGGAGGAGGTTCCGGGGGCGGGGGTTCCGAAGTGAAGCTCCAGG
AGTCCGGCCCCGGCCTGGTGGCGCCGTCGCAATCACTCTCTGTGACCTGTACCGTGTCGGGAGT
GTCCCTGCCTGATTACGGCGTGAGCTGGATTCGGCAGCCGCCGCGGAAGGGCCTGGAATGGCTG
GGTGTCATCTGGGGATCCGAGACTACCTACTACAACTCGGCCCTGAAGTCCCGCCTGACTATCA
TCAAAGACAACTCGAAGTCCCAGGTCTTTCTGAAGATGAACTCCCTGCAAACTGACGACACCGC
CATCTATTACTGTGCTAAGCACTACTACTACGGTGGAAGCTATGCTATGGACTACTGGGGGCAA
GGCACTTCGGTGACTGTGTCAAGCGCGGCCGCAACTACCACCCCTGCCCCTCGGCCGCCGACTC
CGGCCCCAACCATCGCAAGCCAACCCCTCTCCTTGCGCCCCGAAGCTTGCCGCCCGGCCGCGGG
TGGAGCCGTGCATACCCGGGGGCTGGACTTTGCCTGCGATATCTACATTTGGGCCCCGCTGGCC
GGCACTTGCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGAAGA
AGCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGACGG
ATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGCGTCAAGTTCTCACGG
TCCGCCGACGCCCCCGCATATCAACAGGGCCAGAATCAGCTCTACAACGAGCTGAACCTGGGAA
GGAGAGAGGAGTACGACGTGCTGGACAAGCGACGCGGACGCGACCCGGAGATGGGGGGGAAACC
ACGGCGGAAAAACCCTCAGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCC
TACTCAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTGTACCAGG
GACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGCACTCCCACCCCGG

SEQ ID NO: 13 (anti-CD19 scFV VH for FMC63)

MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDG
TVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEI
T

SEQ ID NO: 14 (anti-CD19 scFV VL for FMC63)
EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALK
SRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSS

SEQ ID NO: 16 (anti-CD20 CAR amino acid sequence)

MLLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPG
QGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSADYYCARSNYYGSSY
WFFDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVLTQSPAILSASPGEKVTMTCRASSSVNYMD
WYQKKPGSSPKPWIYATSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPPTF
GGGTKLEIKAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPL
AGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS

RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 17 (anti-CD20 CAR polynucleotide sequence)

ATGCTCCTTCTCGTGACCTCCCTGCTTCTCTGCGAACTGCCCCATCCTGCCTTCCTGCTGATTC
CCGAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGGAGCCAGCGTGAAGATGAG
CTGCAAGGCCTCCGGTTACACCTTCACCTCCTACAACATGCACTGGGTGAAACAGACCCCGGGA
CAAGGGCTCGAATGGATTGGCGCCATCTACCCCGGGAATGGCGATACTTCGTACAACCAGAAGT
TCAAGGGAAAGGCCACCCTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTC
CCTGACCTCCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTAC
TGGTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGCGGAGGATCCG
GTGGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACTCAGTCCCCGGCAATCCTGTC
GGCCTCACCGGGCGAAAAGGTCACGATGACTTGTAGAGCGTCGTCCAGCGTGAACTACATGGAT
TGGTACCAAAAGAAGCCTGGATCGTCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCT
CCGGCGTGCCAGCGCGGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCG
CGTGGAGGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACTTTT
GGAGGCGGTACTAAGCTGGAGATCAAAGCGGCCGCAACTACCACCCCTGCCCCTCGGCCGCCGA
CTCCGGCCCCAACCATCGCAAGCCAACCCCTCTCCTTGCGCCCCGAAGCTTGCCGCCCGGCCGC
GGGTGGAGCCGTGCATACCCGGGGGCTGGACTTTGCCTGCGATATCTACATTTGGGCCCCGCTG
GCCGGCACTTGCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGAGGGGCCGGA
AGAAGCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGA
CGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGCGTCAAGTTCTCA
CGGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAATCAGCTCTACAACGAGCTGAACCTGG
GAAGGAGAGAGGAGTACGACGTGCTGGACAAGCGACGCGGACGCGACCCGGAGATGGGGGGGAA
ACCACGGCGGAAAAACCCTCAGGAAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAA
GCCTACTCAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTGTACC
AGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGCACTCCCACCCCG
G

SEQ ID NO: 18 (anti-CD20 scFV amino acid sequence)

EVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMEIWVKQTPGQGLEWIGAIYPGNGDTSYNQK
FKGKATLTADKSSSTAYMQLSSLTSEDSADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGGS
GGGGSGGGGSDIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLA
SGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIK

SEQ ID NO: 19 (anti-CD20 scFV polynucleotide sequence)

GAGGTGCAGTTGCAACAGTCAGGAGCTGAACTGGTCAAGCCAGGAGCCAGCGTGAAGATGAGCT
GCAAGGCCTCCGGTTACACCTTCACCTCCTACAACATGCACTGGGTGAAACAGACCCCGGGACA
AGGGCTCGAATGGATTGGCGCCATCTACCCCGGGAATGGCGATACTTCGTACAACCAGAAGTTC
AAGGGAAAGGCCACCCTGACCGCCGACAAGAGCTCCTCCACCGCGTATATGCAGTTGAGCTCCC
TGACCTCCGAGGACTCCGCCGACTACTACTGCGCACGGTCCAACTACTATGGAAGCTCGTACTG
GTTCTTCGATGTCTGGGGGGCCGGCACCACTGTGACCGTCAGCTCCGGGGGCGGAGGATCCGGT
GGAGGCGGAAGCGGGGGTGGAGGATCCGACATTGTGCTGACTCAGTCCCCGGCAATCCTGTCGG
CCTCACCGGGCGAAAAGGTCACGATGACTTGTAGAGCGTCGTCCAGCGTGAACTACATGGATTG
GTACCAAAAGAAGCCTGGATCGTCACCCAAGCCTTGGATCTACGCTACATCTAACCTGGCCTCC
GGCGTGCCAGCGCGGTTCAGCGGGTCCGGCTCGGGCACCTCATACTCGCTGACCATCTCCCGCG

TGGAGGCTGAGGACGCCGCGACCTACTACTGCCAGCAGTGGTCCTTCAACCCGCCGACTTTTGG
AGGCGGTACTAAGCTGGAGATCAAA

SEQ ID NO: 20 (anti-CD20 scFV VH)

EVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMEIWVKQTPGQGLEWIGAIYPGNGDTSYNQK
FKGKATLTADKSSSTAYMQLSSLTSEDSADYYCARSNYYGSSYWFFDVWGAGTTVTVSS

SEQ ID NO: 21 (anti-CD20 scFV VL)

DIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFSGS
GSGTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIK

SEQ ID NO: 22 (anti-CD22 CAR amino acid sequence)

MLLLVTSLLLCELPHPAFLLIPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQS
PSRGLEWLGRTYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCAREVTGD
LEDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQTIWS
YLNWYQQRPGKAPNLLIYAASSLQSGVPSRFSGRGSGTDFTLTISSLQAEDFATYYCQQSYSIP
QTFGQGTKLEIKAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV
KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK
MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 23 (anti-CD22 CAR polynucleotide sequence)

ATGCTTCTTTTGGTGACTTCCCTTTTGCTGTGCGAGTTGCCACACCCCGCCTTCCTGCTTATTC
CCCAGGTACAGCTCCAGCAGAGTGGCCCAGGGCTCGTGAAGCCAAGCCAGACGCTGTCCCTGAC
TTGTGCAATTTCAGGGGATTCAGTTTCATCAAATAGCGCGGCGTGGAATTGGATTCGACAATCT
CCTTCCCGAGGGTTGGAATGGCTTGGACGAACATATTACAGATCCAAATGGTATAACGACTATG
CGGTATCAGTAAAGTCAAGAATAACCATTAACCCCGACACAAGCAAGAACCAATTCTCTTTGCA
GCTTAACTCTGTCACGCCAGAAGACACGGCAGTCTATTATTGCGCTCGCGAGGTAACGGGTGAC
CTGGAAGACGCTTTTGACATTTGGGGGCAGGGTACGATGGTGACAGTCAGTTCAGGGGGCGGTG
GGAGTGGGGGAGGGGGTAGCGGGGGGGGAGGGTCAGACATTCAGATGACCCAGTCCCCTTCATC
CTTGTCTGCCTCCGTCGGTGACAGGGTGACAATAACATGCAGAGCAAGCCAAACAATCTGGAGC
TATCTCAACTGGTACCAGCAGCGACCAGGAAAAGCGCCAAACCTGCTGATTTACGCTGCTTCCT
CCCTCCAATCAGGCGTGCCTAGTAGATTTAGCGGTAGGGGCTCCGGCACCGATTTTACGCTCAC
TATAAGCTCTCTTCAAGCAGAAGATTTTGCGACTTATTACTGCCAGCAGTCCTATAGTATACCT
CAGACTTTCGGACAGGGTACCAAGTTGGAGATTAAGGCGGCCGCAACTACCACCCCTGCCCCTC
GGCCGCCGACTCCGGCCCCAACCATCGCAAGCCAACCCCTCTCCTTGCGCCCCGAAGCTTGCCG
CCCGGCCGCGGGTGGAGCCGTGCATACCCGGGGGCTGGACTTTGCCTGCGATATCTACATTTGG
GCCCCGCTGGCCGGCACTTGCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGA
GGGGCCGGAAGAAGCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCCGTGCAGACGACTCA
GGAAGAGGACGGATGCTCGTGCAGATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGCGTC
AAGTTCTCACGGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAATCAGCTCTACAACGAGC
TGAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGGACAAGCGACGCGGACGCGACCCGGAGAT
GGGGGGGAAACCACGGCGGAAAAACCCTCAGGAAGGACTGTACAACGAACTCCAGAAAGACAAG
ATGGCGGAAGCCTACTCAGAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACG
GGCTGTACCAGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGCACT
CCCACCCCGG

SEQ ID NO: 24 (anti-CD22 scFV amino acid sequence)

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSK
WYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCAREVTGDLEDAFDIWGQ
GTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQTIWSYLNW
YQQRPGKAPNLLIYAASSLQSGVPSRFSGRGSGTDFTLTISSLQAEDFATYYCQQSYSIPQ
TFGQGTKLEIKAAA

SEQ ID NO: 25 (anti-CD22 scFV polynucleotide sequence)

CAGGTACAGCTCCAGCAGAGTGGCCCAGGGCTCGTGAAGCCAAGCCAGACGCTGTCCCTGACTT
GTGCAATTTCAGGGGATTCAGTTTCATCAAATAGCGCGGCGTGGAATTGGATTCGACAATCTCC
TTCCCGAGGGTTGGAATGGCTTGGACGAACATATTACAGATCCAAATGGTATAACGACTATGCG
GTATCAGTAAAGTCAAGAATAACCATTAACCCCGACACAAGCAAGAACCAATTCTCTTTGCAGC
TTAACTCTGTCACGCCAGAAGACACGGCAGTCTATTATTGCGCTCGCGAGGTAACGGGTGACCT
GGAAGACGCTTTTGACATTTGGGGGCAGGGTACGATGGTGACAGTCAGTTCAGGGGGCGGTGGG
AGTGGGGGAGGGGGTAGCGGGGGGGGAGGGTCAGACATTCAGATGACCCAGTCCCCTTCATCCT
TGTCTGCCTCCGTCGGTGACAGGGTGACAATAACATGCAGAGCAAGCCAAACAATCTGGAGCTA
TCTCAACTGGTACCAGCAGCGACCAGGAAAAGCGCCAAACCTGCTGATTTACGCTGCTTCCTCC
CTCCAATCAGGCGTGCCTAGTAGATTTAGCGGTAGGGGCTCCGGCACCGATTTTACGCTCACTA
TAAGCTCTCTTCAAGCAGAAGATTTTGCGACTTATTACTGCCAGCAGTCCTATAGTATACCTCA
GACTTTCGGACAGGGTACCAAGTTGGAGATTAAGGCGGCCGCA

SEQ ID NO: 26 anti-CD22 scFV VH)

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYNDYA
VSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCAREVTGDLEDAFDIWGQGTMVTVSS

SEQ ID NO: 27 (anti-CD22 scFV VL)

DIQMTQSPSSLSASVGDRVTITCRASQTIWSYLNWYQQRPGKAPNLLIYAASSLQSGVPSRFSG
RGSGTDFTLTISSLQAEDFATYYCQQSYSIPQTFGQGTKLEIKAAA

SEQ ID NO: 28 (human CD8 hinge region)

AAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLL
SLVITLYC

SEQ ID NO: 29 (TNFRSF 19 transmembrane domain)

DTALAAVICSALATVLLALLILCVIYCKRQ

SEQ ID NO: 30 (CD3zeta)

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK
DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 31 (4-1BB co-stimulatory domain)

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

SEQ ID NO: 32 (CD28 co-stimulatory domain)

RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS

SEQ ID NO: 33 (2A sequence)

RAKRGSGATNFSLLKQAGDVEENPGPRAKR

SEQ ID NO: 34 (anti-B7-H3 CAR amino acid sequence)

MLLLVTSLLLCELPHPAFLLIPQVQLQQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPG
QGLEWMGGIIPILGIANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGGAGGSGS
YYPLIWGQGTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCGASQSVSSSY
LAWYQQKPGQAPRLLIYDASSRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSSWPP
TWTFGQGTKLEIKRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVG
GVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKF
SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA
EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 35 (anti-B7-H3 CAR polynucleotide sequence)

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCGTTTCTGCTGATTC
CGCAGGTGCAGCTGCAGCAGAGCGGCGCGGAAGTGAAAAAACCGGGCAGCAGCGTGAAAGTGAG
CTGCAAAGCGAGCGGCGGCACCTTTAGCAGCTATGCGATTAGCTGGGTGCGCCAGGCGCCGGGC
CAGGGCCTGGAATGGATGGGCGGCATTATTCCGATTCTGGGCATTGCGAACTATGCGCAGAAAT
TTCAGGGCCGCGTGACCATTACCGCGGATGAAAGCACCAGCACCGCGTATATGGAACTGAGCAG
CCTGCGCAGCGAAGATACCGCGGTGTATTATTGCGCGCGCGGCGGCGCGGGCGGCAGCGGCAGC
TATTATCCGCTGATTTGGGGCCAGGGCACCACCGTGACCGTGAGCAGCGGCGGCGGCGGCAGCG
GCGGCGGCGGCAGCGGCGGCGGCGGCAGCGAAATTGTGCTGACCCAGAGCCCGGCGACCCTGAG
CCTGAGCCCGGGCGAACGCGCGACCCTGAGCTGCGGCGCGAGCCAGAGCGTGAGCAGCAGCTAT
CTGGCGTGGTATCAGCAGAAACCGGGCCAGGCGCCGCGCCTGCTGATTTATGATGCGAGCAGCC
GCGCGACCGGCATTCCGGCGCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGACCAT
TAGCAGCCTGGAACCGGAAGATTTTGCGGTGTATTATTGCCAGCAGCGCAGCAGCTGGCCGCCG
ACCTGGACCTTTGGCCAGGGCACCAAACTGGAAATTAAACGCGCGGCGGCGATTGAAGTGATGT
ATCCGCCGCCGTATCTGGATAACGAAAAAAGCAACGGCACCATTATTCATGTGAAAGGCAAACA
TCTGTGCCCGAGCCCGCTGTTTCCGGGCCCGAGCAAACCGTTTTGGGTGCTGGTGGTGGTGGGC
GGCGTGCTGGCGTGCTATAGCCTGCTGGTGACCGTGGCGTTTATTATTTTTTGGGTGCGCAGCA
AACGCAGCCGCCTGCTGCATAGCGATTATATGAACATGACCCCGCGCCGCCCGGGCCCGACCCG
CAAACATTATCAGCCGTATGCGCCGCCGCGCGATTTTGCGGCGTATCGCAGCCGCGTGAAATTT
AGCCGCAGCGCGGATGCGCCGGCGTATCAGCAGGGCCAGAACCAGCTGTATAACGAACTGAACC
TGGGCCGCCGCGAAGAATATGATGTGCTGGATAAACGCCGCGGCCGCGATCCGGAAATGGGCGG
CAAACCGCGCCGCAAAAACCCGCAGGAAGGCCTGTATAACGAACTGCAGAAAGATAAAATGGCG
GAAGCGTATAGCGAAATTGGCATGAAAGGCGAACGCCGCCGCGGCAAAGGCCATGATGGCCTGT
ATCAGGGCCTGAGCACCGCGACCAAAGATACCTATGATGCGCTGCATATGCAGGCGCTGCCGCC
GCGC

SEQ ID NO: 36 (anti-B7-H3 scFV amino acid sequence)

QVQLQQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPILGIANYAQKF
QGRVTITADESTSTAYMELSSLRSEDTAVYYCARGGAGGSGSYYPLIWGQGTTVTVSSGGGGSG
GGGSGGGGSEIVLTQSPATLSLSPGERATLSCGASQSVSSSYLAWYQQKPGQAPRLLIYDASSR
ATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSSWPPTWTFGQGTKLEIKRAAA

SEQ ID NO: 37 (anti-B7-H3 scFV polynucleotide sequence)

CAGGTGCAGCTGCAGCAGAGCGGCGCGGAAGTGAAAAAACCGGGCAGCAGCGTGAAAGTGAGCT
GCAAAGCGAGCGGCGGCACCTTTAGCAGCTATGCGATTAGCTGGGTGCGCCAGGCGCCGGGCCA
GGGCCTGGAATGGATGGGCGGCATTATTCCGATTCTGGGCATTGCGAACTATGCGCAGAAATTT
CAGGGCCGCGTGACCATTACCGCGGATGAAAGCACCAGCACCGCGTATATGGAACTGAGCAGCC
TGCGCAGCGAAGATACCGCGGTGTATTATTGCGCGCGCGGCGGCGCGGGCGGCAGCGGCAGCTA
TTATCCGCTGATTTGGGGCCAGGGCACCACCGTGACCGTGAGCAGCGGCGGCGGCGGCAGCGGC
GGCGGCGGCAGCGGCGGCGGCGGCAGCGAAATTGTGCTGACCCAGAGCCCGGCGACCCTGAGCC
TGAGCCCGGGCGAACGCGCGACCCTGAGCTGCGGCGCGAGCCAGAGCGTGAGCAGCAGCTATCT
GGCGTGGTATCAGCAGAAACCGGGCCAGGCGCCGCGCCTGCTGATTTATGATGCGAGCAGCCGC
GCGACCGGCATTCCGGCGCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGACCATTA
GCAGCCTGGAACCGGAAGATTTTGCGGTGTATTATTGCCAGCAGCGCAGCAGCTGGCCGCCGAC
CTGGACCTTTGGCCAGGGCACCAAACTGGAAATTAAACGCGCGGCGGCG

SEQ ID NO: 38 (anti-B7-H3 scFV VH)

QVQLQQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPILGIANYAQKF
QGRVTITADESTSTAYMELSSLRSEDTAVYYCARGGAGGSGSYYPLIWGQGTTVTVSS

SEQ ID NO: 39 (anti-B7-H3 scFV VL)

EIVLTQSPATLSLSPGERATLSCGASQSVSSSYLAWYQQKPGQAPRLLIYDASSRATGIPARFS
GSGSGTDFTLTISSLEPEDFAVYYCQQRSSWPPTWTFGQGTKLEIKRAAA

SEQ ID NO: 40 (CD8α transmembrane region)

IYIWAPLAGTCGVLLLSLVITLYC

SEQ ID NO: 41 (anti-BCMA CAR amino acid sequence)

MALPVTALLLPLALLLHAARPDIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIYWYQQK
PGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTK
LEIKGSTSGSGKPGSGEGSTKGQIQLVQSGPELKKPGETVKISCKASGYTFRHYSMNWVKQAPG
KGLKWMGRINTESGVPIYADDFKGRFAFSVETSASTAYLVINNLKDEDTASYFCSNDYLYSLDF
WGQGTALTVSSFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
CDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPR
DFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 42 (anti-BCMA CAR amino acid sequence)

MALPVTALLLPLALLLHAARPDIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIHWYQQK
PGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTK
LEIKGSTSGSGKPGSGEGSTKGQIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPG

KGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLKYEDTATYFCALDYSYAMDY
WGQGTSVTVSSFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
CDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPR
DFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 43 (anti-BCMA CAR amino acid sequence)

MALPVTALLLPLALLLHAARPDIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIYWYQQK
PGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTK
LEIKGSTSGSGKPGSGEGSTKGQIQLVQSGPELKKPGETVKISCKASGYTFTHYSMNWVKQAPG
KGLKWMGRINTETGEPLYADDFKGRFAFSLETSASTAYLVINNLKNEDTATFFCSNDYLYSCDY
WGQGTTLTVSSFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
CDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPR
DFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 44 (anti-BCMA CAR amino acid sequence)

MLLLVTSLLLCELPHPAFLLIPDIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIHWYQQ
KPGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGT
KLEIKGSTSGSGKPGSGEGSTKGQIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAP
GKGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLKYEDTATYFCALDYSYAMD
YWGQGTSVTVSSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG
LDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPY
APPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 45 (anti-BCMA CAR amino acid sequence)

MALPVTALLLPLALLLHAARPDIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIHWYQQK
PGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTK
LEIKGSTSGSGKPGSGEGSTKGQIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPG
KGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLKYEDTATYFCALDYSYAMDY
WGQGTSVTVSSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL
DFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA
PPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 46 (anti-BCMA CAR amino acid sequence)

MALPVTALLLPLALLLHAARPDIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIHWYQQK
PGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTK
LEIKGSTSGSGKPGSGEGSTKGQIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPG
KGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLKYEDTATYFCALDYSYAMDY
WGQGTSVTVSSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL
DFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRFSVVKRGRKKLLYIFKQPFMRPVQTTQEED
GCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK
PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 47 (anti-BCMA CAR amino acid sequence)

MALPVTALLLPLALLLHAARPDIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIHWYQQK
PGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTK
LEIKGSTSGSGKPGSGEGSTKGQIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPG
KGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLKYEDTATYFCALDYSYAMDY
WGQGTSVTVSSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL
DFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRDQRLPPDAHKPPGGGSFRTPIQEEQADAH
STLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGL
YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 48 (anti-BCMA CAR amino acid sequence)

MALPVTALLLPLALLLHAARPDIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIHWYQQK
PGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTK
LEIKGSTSGSGKPGSGEGSTKGQIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPG
KGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLKYEDTATYFCALDYSYAMDY
WGQGTSVTVSSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL
DFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRFSVVKRGRKKLLYIFKQPFMRPVQTTQEED
GCSCRFPEEEEGGCELRRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSADAP
AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 49 (anti-BCMA CAR amino acid sequence)

DIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIHWYQQKPGQPPTLLIQLASNVQTGVPA
RFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTKLEIKGSTSGSGKPGSGEGSTK
GQIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPGKGLKWMGWINTETREPAYAYD
FRGRFAFSLETSASTAYLQINNLKYEDTATYFCALDYSYAMDYWGQGTSVTVSSAAATTTPAPR
PPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKR
GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNEL
NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG
LYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 50 (anti-BCMA Heavy Chain amino acid sequence)

QLQLQESGPGLVKPSETLSLTCTVSGGSISSGSYFWGWIRQPPGKGLEWIGSIYYSGITYYNPS
LKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARHDGAVAGLFDYWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV
PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 51 (anti-BCMA Light Chain amino acid sequence)

SYVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQPPGQAPVVVVYDDSDRPSGIPERFSGS
NSGNTATLTISRVEAGDEAVYYCQVWDSSSDHVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQ
ANKATLVCLISDFYPGAVTVAWKGDSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRS
YSCQVTHEGSTVEKTVAPTECS

SEQ ID NO: 52 (anti-BCMA CAR Heavy Chain amino acid sequence)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSNYWMHWVRQAPGQGLEWMGATYRGHSDTYYNQKF
KGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGAIYDGYDVLDNWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV
PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

SEQ ID NO: 53 (anti-BCMA Light Chain amino acid sequence)

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKLLIYYTSNLHSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQYRKLPWTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 54 (anti-BCMA CAR amino acid sequence)

MALPVTALLLPLALLLHAARPQVKLEESGGGLVQAGRSLRLSCAASEHTFSSHVMGWFRQAPGK
ERESVAVIGWRDISTSYADSVKGRFTISRDNAKKTLYLQMNSLKPEDTAVYYCAARRIDAADFD
SWGQGTQVTVSSGGGGSEVQLVESGGGLVQAGGSLRLSCAASGRTFTMGWFRQAPGKEREFVAA
ISLSPTLAYYAESVKGRFTISRDNAKNTVVLQMNSLKPEDTALYYCAADRKSVMSIRPDYWGQG
TQVTVSSTSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGT
CGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSA
DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYS
EIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 55 (nucleic acid sequence comprising anti-BCMA CAR coding sequence)

TGTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCTT
ACAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTGGAAGTAAGGTGGTACGATCGTGCCTTA
TTAGGAAGGCAACAGACGGGTCTGACATGGATTGGACGAACCACTGAATTGCCGCATTGCAGAG
ATATTGTATTTAAGTGCCTAGCTCGATACATAAACGGGTCTCTCTGGTTAGACCAGATCTGAGC
CTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTG
CTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTT
AGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGCGAAAGGGAAACCA
GAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGC
GACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAG
CGTCAGTATTAAGCGGGGGAGAATTAGATCGCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGA
AAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTA
ATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAATACTGGGACAGCTACAACCATCCCT
TCAGACAGGATCAGAAGAACTTAGATCATTATATAATACAGTAGCAACCCTCTATTGTGTGCAT
CAAAGGATAGAGATAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAA
GTAAGACCACCGCACAGCAAGCGGCCGGCCGCTGATCTTCAGACCTGGAGGAGGAGATATGAGG
GACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCAC
CCACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTT
CCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAG

GCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGC
AACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGT
GGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAACTCATTTGC
ACCACTGCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTGGAACAGATTTGGAATCACA
CGACCTGGATGGAGTGGGACAGAGAAATTAACAATTACACAAGCTTAATACACTCCTTAATTGA
AGAATCGCAAAACCAGCAAGAAAAGAATGAACAAGAATTATTGGAATTAGATAAATGGGCAAGT
TTGTGGAATTGGTTTAACATAACAAATTGGCTGTGGTATATAAAATTATTCATAATGATAGTAG
GAGGCTTGGTAGGTTTAAGAATAGTTTTTGCTGTACTTTCTATAGTGAATAGAGTTAGGCAGGG
ATATTCACCATTATCGTTTCAGACCCACCTCCCAACCCCGAGGGGACCCGACAGGCCCGAAGGA
ATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCTCGAC
GGTATCGCCTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACA
TAATAGCAACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCG
GGTTTATTACAGGGACAGCAGAGATCCAGTTTATCGATGAGTAATTCATACAAAAGGACTCGCC
CCTGCCTTGGGGAATCCCAGGGACCGTCGTTAAACTCCCACTAACGTAGAACCCAGAGATCGCT
GCGTTCCCGCCCCCTCACCCGCCCGCTCTCGTCATCACTGAGGTGGAGAAGAGCATGCGTGAGG
CTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGG
GTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGT
ACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAA
CGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGG
CCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACGCCCCTGGCTGCAG
TACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTT
AAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCTTGGGCGCTGGGGCCGCCGCGTGCG
AATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTT
TGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGC
ACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATG
TTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGC
CGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGG
CCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTC
AAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCC
TTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCG
ATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGA
GTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCC
TTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAA
GTTTTTTTCTTCCATTTCAGGTGTCGTGAGGATCGCTAGCGCTACCGGACTCAGATCTCGAGCT
CAAGCTTCGAATTCGCCGCCACCATGGCTCTGCCCGTCACCGCTCTGCTGCTGCCTCTGGCTCT
GCTGCTGCACGCTGCTCGCCCTCAGGTCAAACTGGAAGAATCTGGCGGAGGCCTGGTGCAGGCA
GGACGGAGCCTGCGCCTGAGCTGCGCAGCATCCGAGCACACCTTCAGCTCCCACGTGATGGGCT
GGTTTCGGCAGGCCCCAGGCAAGGAGAGAGAGAGCGTGGCCGTGATCGGCTGGAGGGACATCTC
CACATCTTACGCCGATTCCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACGCCAAGAAGACA
CTGTATCTGCAGATGAACAGCCTGAAGCCCGAGGACACCGCCGTGTACTATTGCGCAGCAAGGA
GAATCGACGCAGCAGACTTTGATTCCTGGGGCCAGGGCACCCAGGTGACAGTGTCTAGCGGAGG
AGGAGGATCTGAGGTGCAGCTGGTGGAGAGCGGAGGCGGCCTGGTGCAGGCCGGAGGCTCTCTG
AGGCTGAGCTGTGCAGCATCCGGAAGAACCTTCACAATGGGCTGGTTTAGGCAGGCACCAGGAA
AGGAGAGGGAGTTCGTGGCAGCAATCAGCCTGTCCCCTACCCTGGCCTACTATGCCGAGAGCGT
GAAGGGCAGGTTTACCATCTCCCGCGATAACGCCAAGAATACAGTGGTGCTGCAGATGAACTCC
CTGAAACCTGAGGACACAGCCCTGTACTATTGTGCCGCCGATCGGAAGAGCGTGATGAGCATTA
GACCAGACTATTGGGGGCAGGGAACACAGGTGACCGTGAGCAGCACTAGTACCACGACGCCAGC
GCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCG
TGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATATCTACA
TCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCCTTTACTG

CAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTATGAGACCAGTACAAACT
ACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGA
GAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAA
CGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCT
GAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAG
ATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCA
CGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG
GCCCTGCCCCCTCGCTAATCTAGATCCGCGTCTGGAACAATCAACCTCTGGATTACAAAATTTG
TGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTA
ATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCT
GGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGT
GTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACT
TTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGA
CAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAGCTGACGTCCTTTCC
ATGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCG
GCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTC
TTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCCTGGAATTAATTC
TGCAGTCGAGACCTAGAAAAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTG
ATTGTGCCTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACC
TTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGAGGGGA
CTGGAAGGGCTAATTATCTGAGCCTGGGAGCTCTCTCTGCTTTTTGCTTGTACTGGGTCTCTCT
GGTTAGACCAGCACTCCCAACGAAGACAAGATGGCTAACTAGGGAACCCACTGCTTAAGCCTCA
ATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAG
AGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGT

SEQ ID NO: 56 (exemplary human IL-10 variant substitutions based on viral IL-10)

MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQTKDEVDNL
LLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPEAKDHVNSLGENLKTLRLRLRRCHR
FLPCENKSKAVEQIKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN

SEQ ID NO: 57 (exemplary human IL-10 variant with amino acid substitutions based on viral IL-10)

MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNL
LLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPD**A**KAHVNSLGENLKTLRLRLRRCHR
FLPCENKSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN

SEQ ID NO: 58 (Mus musculus; "MOUSE")

MPGSALLCCLLLLTGMRISRGQYSREDNNCTHFPVGQSHMLLELRTAFSQVKTFFQTKDQLDNI
LLTDSLMQDFKGYLGCQALSEMIQFYLVEVMPQAEKHGPEIKEHLNSLGEKLKTLRMRLRRCHR
FLPCENKSKAVEQVKSDFNKLQDQGVYKAMNEFDIFINCIEAYMMIKMKS

SEQ ID NO: 59 (Rattus norvegicus; "RAT")

MPGSALLCCLLLLAGVKTSKGHSIRGDNNCTHFPVSQTHMLRELRAAFSQVKTFFQKKDQLDNI
LLTDSLLQDFKGYLGCQALSEMIKFYLVEVMPQAENHGPEIKEHLNSLGEKLKTLWIQLRRCHR
FLPCENKSKAVEQVKNDFNKLQDKGVYKAMNEFDIFINCIEAYVTLKMKN

SEQ ID NO: 60 (Macaca mulatta; "MACMU")

MHSSALLCCLVLLTGVRASPGQGTQSENSCTRFPGNLPHMLRDLRDAFSRVKTFFQMKDQLDNI
LLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENHDPDIKEHVNSLGENLKTLRLRLRRCHR
FLPCENKSKAVEQVKNAFSKLQEKGVYKAMSEFDIFINYIEAYMTMKIQN

SEQ ID NO: 61 (Gorilla gorilla; "GORILLA")

MHSSALLCCLVLLTGVRASPGHGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNL
LLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLKTLRLRLRRCHR
FLPCENKSKAVEQVKNAFNKLQEKGVYKAMSEFDIFINYIEAYMTMKIRN

SEQ ID NO: 62 (Macaca fascicularis; "CYNO")

MHSSALLCCLVLLTGVRASPGQGTQSENSCTRFPGNLPHMLRDLRDAFSRVKTFFQMKDQLDNI
LLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENHDPDIKEHVNSLGENLKTLRLRLRRCHR
FLPCENKSKAVEQVKNAFSKLQEKGVYKAMSEFDIFINYIEAYMTMKIQN

SEQ ID NO: 63 (Papio Anubis; "OLIVE BABOON")

MHSSALLCCLVVLTGVRASPGQGTQSENSCTRFPGNLPHMLRDLRDAFSRVKTFFQMKDQLDNI
LLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENHDPDIKEHVNSLGENLKTLRLRLRRCHR
FLPCENKSKAVEQVKNAFSKLQEKGVYKAMSEFDIFINYIEAYMTMKIQN

SEQ ID NO: 64 (Pan paniscus; "BONOBO")

MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNL
LLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKVHVNSLGENLKTLRLRLRRCHR
FLPCENKSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN

SEQ ID NO: 65 (Pan troglodytes; "CHIMP")

MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNL
LLKESLLEDFKGYLGCQALXEMIQFYLEEVMPQAENQDPDIKVHVNSLGENLKTLRLRLRRCHR
FLPCENKSKAVEQVKNAFNKLQEKGIVKAMSEFDIFINYIEAYMTMKIRN

SEQ ID NO: 66 (EBVB9)

MERRLVVTLQCLVLLYLAPECGGTDQCDNFPQMLRDLRDAFSRVKTFFQTKDEVDNLLLKESLL
EDFKGYLGCQALSEMIQFYLEEVMPQAENQDPEAKDHVNSLGENLKTLRLRLRRCHRFLPCENK
SKAVEQIKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTIKAR

SEQ ID NO: 67 huIL-10 HYBRID#1

MHSSALLCCLVLLTGRASPGQGTQSENSCTHFPGNIPNMLRDIRDAFSRVKTEFQTKDEVDNLL
LKESLLEDEKGYLGCQALSEMIQFYLEEVMPQAENQDPEAKDHVNSLGENLKTLRLRIRRCHRF
LPCENKSKAVEQIKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN

SEQ ID NO: 68 huIL-10 HYBRID#2

MHSSALLCCLVLLTGRASPGQGTQSENSCTHFPGNIPNMLRDIRDAFSRVKTEFQMKDQLDNLL
LKESLLEDEKGYLGCQALSEMIQFYLEEVMPQAENQDPDAKAHVNSLGENLKTLRLRIRRCHRF
LPCENKSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN

WHAT IS CLAIMED IS:

1. A genetically modified CD4$^+$ T cell (CD4$^{IL\text{-}10/CAR}$) comprising:

(a) a first exogenous polynucleotide segment encoding a chimeric antigen receptor (CAR); and

(b) a second exogenous polynucleotide segment encoding interleukin-10 (IL-10).

2. The genetically modified CD4$^+$ T cell of claim 1, wherein the first exogenous polynucleotide segment comprises a first regulatory element operably linked to a coding sequence of the CAR.

3. The genetically modified CD4$^+$ T cell of claim 2, wherein the regulatory element drives constitutive expression of the CAR.

4. The genetically modified CD4$^+$ T cell of any one of claims 1-3, wherein the CAR comprises an antigen-binding domain, a hinge region, a transmembrane domain, and an intracellular signaling domain.

5. The genetically modified CD4$^+$ T cell of claim 4, wherein the antigen-binding domain is a single chain antibody fragment.

6. The genetically modified CD4$^+$ T cell of claim 5, wherein the single chain antibody fragment comprises a single chain Fv (scFv).

7. The genetically modified CD4$^+$ T cell of any one of claims 4-6, wherein the antigen-binding domain targets an antigen associated with an autoimmune disease, inflammatory disorder, or cancer.

8. The genetically modified CD4$^+$ T cell of claim 7, wherein the antigen is selected from the group consisting of: CD19, CD20, CD22, CD27, CD38, B7-H3, CD23, Lyml, Lym2, CLEC5A, CDH179b, FLT3, GCC, Muc, BCMA, CSF2RA, GFRa4, CD32, CD33, CEA, IL1lRa, IL13Ra, NYBRI, SLea, CD200R, TGFBetaR2, CD276, TROP2, LAMP1, PTK7, DLL3, CDH1, CDH6, CDH17, CDH19, TSHR, tyrosinase, HLA-A*02, HLA-A*24 or citrullinated peptides, insulin, MOG, GAD65, IA2, gliadin, and desmoglein.

9. The genetically modified CD4$^+$ T cell of any one of claims 4-8, wherein the antigen-binding domain comprises an anti-CD19 antigen-binding domain.

10. The genetically modified $CD4^+$ T cell of claim 9, wherein the anti-CD19 antigen-binding domain has the sequence of SEQ ID NO: 11.

11. The genetically modified $CD4^+$ T cell of any one of claims 4-8, wherein antigen-binding domain is an anti-BCMA antigen-binding domain.

12. The genetically modified $CD4^+$ T cell of claim 10, wherein the anti-BCMA antigen-binding domain comprises the sequence of SEQ ID NOs: 50-53.

13. The genetically modified $CD4^+$ T cell of any one of claims 1-12, wherein the hinge region is selected from a human CD8α hinge region, a human CD28 hinge region, a IgG1 hinge region, or a IgG4 hinge region.

14. The genetically modified $CD4^+$ T cell of claim 13, wherein the hinge region is derived from human CD8α.

15. The genetically modified $CD4^+$ T cell of any one of claims 1-14, wherein the transmembrane domain is selected from a TNFRSF 19 transmembrane domain, a CD3zeta transmembrane domain, a CD8α transmembrane domain, a CD4 transmembrane domain, a CD28 transmembrane domain, or an B7-family inducible costimulatory (ICOS) transmembrane domain.

16. The genetically modified $CD4^+$ T cell of claim 15, wherein the transmembrane domain is derived from CD8α.

17. The genetically modified $CD4^+$ T cell of any one of claims 1-16, wherein the CAR further comprises one or more co-stimulatory domains.

18. The genetically modified $CD4^+$ T cell of any claim 17, wherein the CAR comprises two co-stimulatory domains.

19. The genetically modified $CD4^+$ T cell of any one of claims 1-18, wherein the one or more co-stimulatory domains are selected from the group consisting of 4-1BB, CD28, OX40, ICOS, CD27, MYD88-CD40, and KIR2DS2.

20. The genetically modified $CD4^+$ T cell of claim 19, wherein one of the one or more co-stimulatory domain is derived from CD28.

21. The genetically modified CD4+ T cell of any one of claims 18-20, wherein a second co-stimulatory domain is derived from 4-1BB.

22. The genetically modified CD4+ T cell of any one of claims 1-21, wherein the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM).

23. The genetically modified CD4+ T cell of any one of claims 1-22, the immunoreceptor tyrosine-based activation motif (ITAM) is derived from CD3zeta.

24. The genetically modified CD4+ T cell of any one of claims 1-23, wherein the CAR comprises:

an anti-CD19 antigen binding domain, an anti-BCMA antigen binding domain, or an anti-CD20 antigen binding domain;

a human CD8α hinge region;

a human CD8α transmembrane region;

a CD28 co-stimulatory domain; and

a CD3zeta chain intracellular signaling domain.

25. The genetically modified CD4+ T cell of any one of claims 1-24, wherein the CAR comprises the sequence of SEQ ID NOs: 9, 16, 22, 34, 41-49, or 54.

26. The genetically modified CD4+ T cell of claim 25, wherein the first exogenous polynucleotide segment comprises the sequence of SEQ ID NOs: 10, 17, 23, 35, or 55.

27. The genetically modified CD4+ T cell of any one of claims 1-26, wherein the first exogenous polynucleotide segment is integrated into the T cell nuclear genome.

28. The genetically modified CD4+ T cell of any one of claims 1-27, wherein the first exogenous polynucleotide segment is not integrated into the T cell nuclear genome.

29. The genetically modified CD4+ T cell of claim 27 or 28, wherein the first exogenous polynucleotide segment is in a vector.

30. The genetically modified CD4+ T cell of any one of claims 1-29, wherein the second exogenous polynucleotide segment comprises a second regulatory element operably linked to the coding sequence of IL-10.

31. The genetically modified $CD4^+$ T cell of any one of claims 1-30, wherein the IL-10 is a human IL-10.

32. The genetically modified $CD4^+$ T cell of any one of claims 1-30, wherein the IL-10 is a viral IL-10.

33. The genetically modified $CD4^+$ T cell of any one of claims 1-30, wherein the IL-10 is a protein having the sequence of SEQ ID NO: 1.

34. The genetically modified $CD4^+$ T cell of claim 33, wherein the second exogenous polynucleotide segment having the sequence of SEQ ID NO: 2.

35. The genetically modified $CD4^+$ T cell of any one of claims 30-34, wherein the second regulatory element drives constitutive or inducible expression of the IL-10.

36. The genetically modified $CD4^+$ T cell of any one of claims 1-35, wherein the second exogenous polynucleotide segment is integrated into the T cell nuclear genome.

37. The genetically modified $CD4^+$ T cell of any one of claims 1-35, wherein the second exogenous polynucleotide segment is not integrated into the T cell nuclear genome.

38. The genetically modified $CD4^+$ T cell of claim 36 or 37, wherein the second exogenous polynucleotide segment is in a vector.

39. The genetically modified $CD4^+$ T cell of any one of claims 1-38, wherein the first exogenous polynucleotide segment and the second exogenous polynucleotide segment are in the same vector.

40. The genetically modified $CD4^+$ T cell of any claim 29, 38 or 39, wherein the vector is a viral vector.

41. The genetically modified $CD4^+$ T cell of claim 40, wherein the vector is a lentiviral vector.

42. The genetically modified $CD4^+$ T cell of any one claim 1-41, wherein the $CD4^+$ T cell constitutively expresses at least 100pg IL-10 per 10^6 of the $CD4^+$ T cells/mL of culture medium.

43. The genetically modified $CD4^+$ T cell of claim 42, wherein the $CD4^+$ T cell constitutively expresses at least 100 pg, 200 pg, 500pg, 1ng, 5ng, 10ng, or 50ng IL-10 per 10^6 of the $CD4^+$ T cells/mL.

44. The genetically modified $CD4^+$ T cell of any one of claims 1-43, wherein the $CD4^+$ T cell expresses at least 1ng IL-10 per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies.

45. The genetically modified $CD4^+$ T cell of claim 44, wherein the $CD4^+$ T cells expresses at least 2ng, 5ng, 10ng, 100ng, 200ng, or 500ng IL-10 per 10^6 of the $CD4^+$ T cells/mL after activation with anti-CD3 and anti-CD28 antibodies.

46. The genetically modified $CD4^+$ T cell of any one of claims 1-45, wherein the $CD4^+$ T cell expresses IL-10 at a level at least 5-fold higher than unmodified $CD4^+$ T cells.

47. The genetically modified $CD4^+$ T cell of claim 46, wherein the $CD4^+$ T cell expresses IL-10 at a level at least 10-fold higher than unmodified $CD4^+$ T cells.

48. The genetically modified $CD4^+$ T cell of any one of claims 1-47, wherein the first exogenous polynucleotide segment, second exogenous polynucleotide segment, or both, further comprises a sequence encoding a selection marker.

49. The genetically modified $CD4^+$ T cell of claim 48, wherein the selection marker is ΔNGFR.

50. The genetically modified $CD4^+$ T cell of claim 49, wherein the ΔNGFR has the sequence of SEQ ID NO: 3.

51. The genetically modified $CD4^+$ T cell of claim 50, wherein the second exogenous polynucleotide segment comprises a sequence of SEQ ID NO: 4.

52. The genetically modified $CD4^+$ T cell of claim 48, wherein the selection marker is a truncated form of an EGFR polypeptide.

53. The genetically modified $CD4^+$ T cell of any one of claims 9-10, or 13-52 as dependent from claim 9, wherein the $CD4^+$ T cell is capable of in vitro cytotoxicity to a $CD19^+$ target cell.

54. The genetically modified $CD4^+$ T cell of any one of claims 9-10, or 13-52 as dependent from claim 9, wherein the $CD4^+$ T cell is capable of in vivo cytotoxicity to a $CD19^+$ target cell.

55. The genetically modified $CD4^+$ T cell of claim 53 or 54, wherein the $CD19^+$ target cell is an autoantibody producing B cell.

56. The genetically modified $CD4^+$ T cell of claim 53 or 54, wherein the $CD19^+$ target cell is a $CD19^+$ cancer cell.

57. The genetically modified $CD4^+$ T cell of any one of claims 1-56, wherein the $CD4^+$ T cell is capable of in vitro cytotoxicity to a myeloid target cell.

58. The genetically modified $CD4^+$ T cell of any one of claims 1-56, wherein the $CD4^+$ T cell is capable of in vivo cytotoxicity to a myeloid target cell.

59. The genetically modified $CD4^+$ T cell of any one of claims 53-58, wherein the $CD4^+$ T cell is capable of cytotoxicity to a $CD19^+$ target cell and a myeloid target cell.

60. The genetically modified $CD4^+$ T cell of any one of claims 57-59, wherein the myeloid target cell express one or more of Class I MHC, CD13, CD54 and CD112.

61. The genetically modified $CD4^+$ T cell of any one of claims 53-60, wherein the cytotoxicity to a $CD19^+$ target cell is maintained after one or more in vitro restimulations.

62. The genetically modified $CD4^+$ T cell of any one of claims 53-60, wherein the cytotoxicity to a myeloid target cell is maintained after one or more in vitro restimulations.

63. The genetically modified $CD4^+$ T cell of any one of claims 1-62, wherein the $CD4^+$ T cell is capable of suppressing allogeneic $CD4^+$ T cell proliferation.

64. The genetically modified $CD4^+$ T cell of any one of claims 1-63, wherein the $CD4^+$ T cell is capable of suppressing allogeneic $CD8^+$ T cell proliferation.

65. The genetically modified $CD4^+$ T cell of any one of claims 1-64, wherein the $CD4^+$ T cell is capable of suppressing allogeneic $CD4^+$ T cell proliferation, allogenic $CD8^+$ T cell proliferation, and allogenic PBMC.

66. The genetically modified $CD4^+$ T cell of any one of claims 63-65, wherein the suppressive property is maintained after one or more in vitro restimulations.

67. A population of $CD4^+$ T cells comprising the genetically modified $CD4^+$ cell of any one of claims 1-66.

68. The population $CD4^+$ T cells of claim 67, wherein the $CD4^+$ T cells before the genetic modification were obtained from one, two, three, four, five, six, seven, eight, nine, or ten T cell donors and pooled.

69. The population of $CD4^+$ T cells claim 68, wherein the $CD4^+$ T cells in the population collectively have six, seven, eight, nine, ten, eleven, twelve, or more different HLA haplotypes.

70. The population of $CD4^+$ T cells of any one of claims 67-69, wherein all the $CD4^+$ T cells in the population have at least 1/10, 2/10, 3/10, 4/10, 5/10, 6/10, 7/10, 8/10, or 9/10 match at the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci to each other.

71. The population of $CD4^+$ T cells of any one of claims 67-70, wherein all the $CD4^+$ T cells in the population have at least 1/8, 2/8, 3/8, 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci to each other.

72. The population of $CD4^+$ T cells of any one of claims 67-71, wherein all the $CD4^+$ T cells in the population have 2/2 match at the HLA-A locus to each other.

73. The population of $CD4^+$ T cells of any one of claims 67-72, wherein all the $CD4^+$ T cell in the population have 2/2 match at the HLA-B locus to each other.

74. The population of $CD4^+$ T cells of any one of claims 67-73, wherein all the $CD4^+$ T cell in the population have 2/2 match at the HLA-C locus to each other.

75. The population of $CD4^+$ T cells of any one of claims 67-74, wherein all the $CD4^+$ T cells in the population have at least 3/4 or 4/4 match at the HLA-DRB1 and HLA-DQB1 loci with each other.

76. The population of $CD4^+$ T cells of any one of claims 67-69, wherein all the $CD4^+$ T cells in the population have less than 5/10, 6/10, 7/10, 8/10, or 9/10 match at the HLA-A, HLA-B, HLA C, HLA-DRB1, and HLA-DQB1 loci to each other.

77. The population of $CD4^+$ T cells of claim 76, wherein all the $CD4^+$ T cells in the population have less than 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA C, and HLA DRB1 loci to each other.

78. The population of $CD4^+$ T cells of any one of claims 76-77, wherein all the $CD4^+$ T cells in the population have less than 2/2 match at the HLA-A locus to each other.

79. The population of $CD4^+$ T cells of any one of claims 76-78, wherein all the $CD4^+$ T cell in the population have less than 2/2 match at the HLA-B locus to each other.

80. The population of $CD4^+$ T cells of any one of claims 76-79, wherein all the $CD4^+$ T cell in the population have less than 2/2 match at the HLA-C locus to each other.

81. The population of $CD4^+$ T cells of any one of claims 76-80, wherein all the $CD4^+$ T cells in the population have less than 2/4, 3/4 or 4/4 match at the HLA-DRB1 and HLA DQB1 loci with each other.

82. The population of $CD4^+$ T cells of any one of the above claims 67-81, wherein all the $CD4^+$ T cells in the population have an A*02 allele or are all A*02 negative.

83. The population of $CD4^+$ T cells of any one of the above claims 67-81, wherein all the $CD4^+$ T cells in the population have an A*24 allele or are all A*24 negative

84. The population of $CD4^+$ T cells of any one of claims 67-83, wherein at least 30% of the $CD4^+$ T cells within the population of $NGFR^+$ cells express the CAR.

85. The population of $CD4^+$ T cells of claim 84, wherein at least 60% of the $CD4^+$ T cells within the population of $NGFR^+$ cells express the CAR.

86. The population $CD4^+$ T cells of claim 85, wherein at least 90% of the $CD4^+$ T cells within the population of $NGFR^+$ cells express the CAR.

87. The population $CD4^+$ T cells of any one of claims 67-86, wherein the $CD4^+$ T cells are in a frozen suspension.

88. The population $CD4^+$ T cells of any one of claims 67-86, wherein the $CD4^+$ T cells are in a liquid suspension.

89. The population $CD4^+$ T cells of claim 88, wherein the liquid suspension has previously been frozen.

90. A pharmaceutical composition comprising the genetically modified $CD4^+$ T cell of any one of claims 1-66 or the population of $CD4^+$ T cells of any one of claims 67-89.

91. A method of making genetically modified $CD4^+$ T cell ($CD4^{IL\text{-}10/CAR}$) cells, comprising the steps of:
(a) obtaining primary $CD4^+$ T cells from one or more T cell donors; and
(b) modifying the $CD4^+$ T cells by introducing
(i) a first exogenous polynucleotide segment encoding a chimeric antigen receptor (CAR), and
(ii) a second exogenous polynucleotide segment encoding IL-10.

92. The method of claim 91, wherein in step (a), the primary $CD4^+$ T cells are obtained from two, three, four, five, six, seven, eight, nine, or ten different T cell donors.

93. The method of claim 92, further comprising pooling the genetically modified $CD4^+$ T cells from step (b).

94. The method of claim 91, wherein in step (a), the primary $CD4^+$ T cells are autologous to a patient.

95. The method of any one of claims 91-94, further comprising the step, after step (a) and before step (b), or after step (b) of:
incubating the $CD4^+$ T cells in the presence of an anti-CD3 antibody, and anti-CD28 antibody or anti-CD3 antibody and CD28 antibody coated beads or a polymeric nanomatrix reagent to activate and expand human T cells via CD3 and CD28 or in the presence of other T-cell specific immune cell culture media, activators, and supplements.

96. The method of claim 95, incubating the $CD4^+$ T cells further in the presence of IL-2.

97. The method of any one of claims 91-96, wherein the first exogenous polynucleotide segment, second exogenous polynucleotide segment, or both are introduced into the primary $CD4^+$ T cells using one or more viral vectors.

98. The method of claim 97, wherein the viral vector is a lentiviral vector.

99. The method of any one of claims 91-98, wherein the CAR is specific to a target antigen associated with an autoimmune disease, inflammatory disorder or cancer.

100. The method of claim 99, wherein the target antigen is associated with an autoimmune disease, inflammatory disorder or cancer.

101. The method of any one of claims 91-100, wherein the CAR is an anti-CD19 CAR, an anti-CD20 CAR, an anti-CD22 CAR, an anti BCMA CAR, an anti-B7-H3 CAR, an anti-CD27 CAR, or an anti-CD38 CAR.

102. The method of any one of claims 91-101, wherein the CAR has the sequence of SEQ ID NOs: 9, 16, 22, 34, 41-49, or 54.

103. The method of any one of claims 91-102, wherein the first exogenous polynucleotide segment has the sequence of SEQ ID NOs: 10, 17, 23, 35, or 55.

104. The method of any one of claims 91-103, wherein the IL-10 has the sequence of SEQ ID NO: 1.

105. The method of any one of claims 91-104, wherein the second exogenous polynucleotide segment has the sequence of SEQ ID NO: 2.

106. The method of any one of claims 91-105, wherein the first exogenous polynucleotide segment, second exogenous polynucleotide segment, or both further comprise a segment encoding a selection marker.

107. The method of claim 106, wherein the encoded selection marker is ΔNGFR.

108. The method of claim 107, wherein the encoded selection marker has the sequence of SEQ ID NO: 3.

109. The method of claim 106, wherein the selection marker is a truncated form of EGFR polypeptide.

110. The method of any one of claims 91-109, further comprising the step, after step (b), of:

(c) isolating the genetically-modified $CD4^+$ T cells expressing the selection marker, thereby generating an enriched population of genetically-modified $CD4^+$ T cells.

111. The method of claim 110, wherein at least 30% or at least 60% of the genetically-modified $CD4^+$ T cells in the enriched population express IL-10 and the CAR.

112. The method of claim 111, wherein at least 90% of the genetically-modified $CD4^+$ T cells in the enriched population express IL-10 and the CAR.

113. The method of any one of claims 110-112, wherein at least 75% of the genetically-modified $CD4^+$ T cells in the enriched population express the selection marker.

114. The method of claim 113, wherein at least 90% of the genetically-modified $CD4^+$ T cells in the enriched population express the selection marker.

115. The method of any one of claims 110-114, further comprising, after step (c), the step of incubating the enriched population of genetically-modified $CD4^+$ T cells.

116. The method of claim 115, wherein the step of incubating the enriched population of genetically-modified $CD4^+$ T cells is performed in the presence (i) anti-CD3 antibody and anti-CD28 antibody or (ii) beads coated with anti-CD3 antibody and anti-CD28 antibody in the presence of IL-2 or a polymeric nanomatrix reagent to activate and expand human T cells via CD3 and CD28 or (iii) in the presence of other T-cell specific immune cell culture media, activators, and supplements.

117. The method of any one of claims 91-116, further comprising the later step of freezing the genetically-modified $CD4^+$ T cells.

118. The method of any one of claim 92-117, wherein the at least two T cell donors have at least 1/10, 2/10, 3/10, 4/10, 5/10, 6/10, 7/10, 8/10, or 9/10 match at the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci to each other.

119. The method of any one of claims 92-118, wherein the at least two T cell donors have at least 1/8, 2/8, 3/8, 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci to each other.

120. The method of any one of claims 92-119, wherein the at least two T cell donors have 2/2 match at the HLA-A locus to each other.

121. The method of any one of claims 92-120, wherein the at least two T cell donors have 2/2 match at the HLA-B locus to each other.

122. The method of any one of claims 92-121, wherein the at least two T cell donors have 2/2 match at the HLA-C locus to each other.

123. The method of any one of claims 92-122, wherein the at least two T cell donors have at least 3/4 or 4/4 match at the HLA-DRB1 and HLA-DQB1 loci to each other.

124. The method of any one of claims 92-117, wherein the at least two T cell donors have less than 5/10, 6/10, 7/10, 8/10, or 9/10 match at the HLA-A, HLA-B, HLA C, HLA-DRB1, and HLA-DQB1 loci to each other.

125. The method of claim 124, wherein the at least two T cell donors have less than 4/8, 5/8, 6/8, 7/8, or 8/8 match at the HLA-A, HLA-B, HLA C, and HLA DRB1 loci to each other.

126. The method of any one of claims 124-125, wherein the at least two T cell donors have less than 2/2 match at the HLA-A locus to each other.

127. The method of any one of claims 124-126, wherein the at least two T cell donors have less than 2/2 match at the HLA-B locus to each other.

128. The method of any one of claims 124-127, wherein the at least two T cell donors have less than 2/2 match at the HLA-C locus to each other.

129. The method of any one of claims 124-128, wherein the at least two T cell donors have less than 4/2, 3/4 or 4/4 match at the HLA-DRB1 and HLA DQB1 loci with each other.

130. The method of any one of claims 92-129, wherein each of the at least two T cell donors has an A*02 allele or are A*02 negative.

131. The method of any one of claims 92-129, wherein each of the at least two T cell donors has an A*24 allele or are A*24 negative.

132. The method of any one of claims 92-131, wherein in step (a), the primary $CD4^+$ T cells are obtained from one or more frozen stocks.

133. The method of any one of claims 92-132, wherein in step (a), the primary $CD4^+$ T cells are obtained from unfrozen peripheral blood mononuclear cells of the at least two different T cell donors.

134. The method of claim 133, further comprising the step of isolating $CD4^+$ T cells from the peripheral blood mononuclear cells.

135. A method for treating a hematological cancer, comprising:
administering to a hematological cancer patient a therapeutically effective amount of $CD4^{IL\text{-}10/CAR}$ cells of any one of claims 1-66, population of $CD4^{IL\text{-}10/CAR}$ cells of any one of claims 67-89, or the pharmaceutical composition of claim 90 sufficient to induce anti-cancer effect.

136. The method of claim 135, further comprising the step of administering allo-HSCT graft to the patient prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$.

137. A method of treating a patient with a malignancy, comprising:
administering an allo-HSCT graft to the patient, and
administering a therapeutically effective amount of $CD4^{IL\text{-}10/CAR}$ cells of any one of claims 1-66, population of $CD4^{IL\text{-}10/CAR}$ cells of any one of claims 67-89, or the pharmaceutical composition of claim 90.

138. The method of claim 137, wherein the amount of $CD4^{IL\text{-}10/CAR}$ cells is further sufficient to suppress or prevent graft versus host disease (GvHD) without suppressing graft versus leukemia (GvL) or graft versus tumor (GvT) efficacy of the allo-HSCT.

139. The method of any one of claims 135-138, wherein the malignancy or hematological cancer is a myeloid leukemia.

140. The method of any one of claims 135-139, wherein the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells that express CD13.

141. The method of any one of claims 135-140, wherein the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells that express HLA-class I.

142. The method of any one of claims 135-141, wherein the myeloid leukemia is acute myeloid leukemia (AML).

143. The method of any one of claims 135-138, wherein the malignancy or hematological cancer is a $CD19^+$, $CD20^+$, $CD22^+$, $BCMA^+$, $CD27^+$, $CD38^+$or $B7\text{-}H3^+$ hematological cancer.

144. The method of claim 143, wherein the $CD19^+$, $CD20^+$, $CD22^+$, $BCMA^+$, $CD27^+$, $CD38^+$, or $B7\text{-}H3^+$ hematological cancer is selected from chronic lymphocytic leukemia, acute lymphoblastic leukemia (ALL), and non-Hodgkin's lymphomas.

145. The method of any one of claims 136-144, wherein the allo-HSCT graft is obtained from a related or unrelated donor with respect to the patient.

146. The method of any one of claims 135-144, wherein the $CD4^{IL\text{-}10/CAR}$ cells are non-autologous to the patient.

147. The method of any one of claims 135-144, wherein the $CD4^{IL\text{-}10/CAR}$ cells are autologous to the patient.

148. The method of any one of claims 135-144, wherein the $CD4^{IL\text{-}10/CAR}$ cells are allogeneic to the patient.

149. The method of any one of claims 135-144, wherein the $CD4^{IL\text{-}10/CAR}$ cells are not anergized to host allo-antigens prior to administration to the patient.

150. The method of any one of claims 135-144, wherein the $CD4^{IL\text{-}10/CAR}$ cells are Tr1-like cells.

151. The method of any one of claims 135-144, wherein the $CD4^{IL\text{-}10/CAR}$ cells are polyclonal.

152. The method of any one of claims 135-144, wherein the $CD4^{IL\text{-}10/CAR}$ cells are polyclonal and non-autologous to the patient.

153. The method of any one of claims 135-144, wherein the $CD4^{IL\text{-}10/CAR}$ cells are polyclonal and autologous to the patient.

154. The method of any one of claims 135-144, wherein the $CD4^{IL\text{-}10/CAR}$ cells are isolated from at least two donors prior to being genetically modified.

155. The method of claim 154, wherein none of the at least two donors is the same donor as the allo-HSCT donor.

156. The method of any one of claims 135-155, wherein the allo-HSCT graft is obtained from a matched or mismatched donor with respect to the patient.

157. The method of any one of claims 135-156, wherein the $CD4^{IL\text{-}10/CAR}$ cells target and kill cells that express CD19, CD20 or BCMA.

158. The method of any one of claims 135-157, wherein the $CD4^{IL\text{-}10/CAR}$ cells target and kill cells that express CD54.

159. The method of any one of claims 135-158, wherein the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells that express HLA-class I and CD54.

160. The method of any one of claims 135-159, wherein the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells that express CD112 and CD155.

161. The method of any one of claims 135-160, wherein the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells that express CD58.

162. The method of any one of claims 135-161, wherein the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells in the patient.

163. The method of any one of claims 135-162, wherein the $CD4^{IL\text{-}10/CAR}$ cells target and kill solid tumor cells in the patient.

164. The method of any one of claims 136-163, wherein the $CD4^{IL\text{-}10/CAR}$ cells suppress allogeneic $CD4^+$ T cell proliferation.

165. The method of any one of claims 136-164, wherein the $CD4^{IL\text{-}10/CAR}$ cells suppress allogeneic $CD8^+$ T cell proliferation.

166. The method of any one of claims 136-163, wherein the $CD4^{IL\text{-}10/CAR}$ cells suppress allogeneic $CD4^+$ T cell proliferation, allogeneic $CD8^+$ T cell proliferation, and PBMC.

167. A method of treating a hematological cancer by allogeneic hematopoietic stem cell transplant (allo-HSCT), comprising:

administering allo-HSCT graft to a patient;

administering to the patient an amount of $CD4^{IL\text{-}10/CAR}$ cells, optionally wherein the amount is sufficient to suppress or prevent graft-versus-host disease (GvHD) without suppressing graft-versus-leukemia (GvL) or graft-versus-tumor (GvT) efficacy of the allo-HSCT graft,

wherein the $CD4^{IL\text{-}10/CAR}$ cells comprise $CD4^+$ T cells genetically modified by vector-mediated gene transfer of the coding sequence of human IL-10 under control of a constitutive or inducible promoter and vector-mediated gene transfer of the coding sequence of a CAR under control of a constitutive or inducible promoter;

wherein the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells in the patient;

wherein the wherein the $CD4^{IL\text{-}10/CAR}$ cells are not anergized to host allo-antigens prior to administration to the patient; and

wherein the $CD4^{IL\text{-}10/CAR}$ cells are non-autologous to the patient, and are Tr1-like.

168. A method of treating a hematological cancer by allogeneic hematopoietic stem cell transplant (allo-HSCT), comprising:

administering allo-HSCT graft to a patient;

administering to the patient an amount of $CD4^{IL\text{-}10/CAR}$ cells, optionally wherein the amount is sufficient to suppress or prevent graft-versus-host disease (GvHD) without suppressing graft-versus-leukemia (GvL) or graft-versus-tumor (GvT) efficacy of the allo-HSCT graft,

wherein the $CD4^{IL\text{-}10/CAR}$ cells comprise $CD4^+$ T cells genetically modified by vector-mediated gene transfer of the coding sequence of human IL-10 under control of a constitutive or inducible promoter and vector-mediated gene transfer of the coding sequence of a CAR under control of a constitutive or inducible promoter;

wherein the $CD4^{IL\text{-}10/CAR}$ cells target and kill cancer cells in the patient;

wherein the wherein the $CD4^{IL\text{-}10/CAR}$ cells are not anergized to host allo-antigens prior to administration to the patient; and

wherein the $CD4^{IL\text{-}10/CAR}$ cells are autologous to the patient, and are Tr1-like.

169. A method for preventing relapse of a $CD19^+$ or $CD20^+$, $CD22^+$, $BCMA^+$, $CD27^+$, $CD38^+$, or B7-$H3^+$ hematological cancer in a patient, comprising:

administering to a patient, identified as being at risk of having a relapse of a $CD19^+$, $CD20^+$, $CD22^+$, $BCMA^+$, $CD27^+$, $CD38^+$, or B7-$H3^+$ hematological cancer, a therapeutically effective amount of $CD4^{IL\text{-}10/CAR}$ cells of any one of claims 1-66, population of $CD4^{IL\text{-}10/CAR}$ cells of any one of claims 67-89, or the pharmaceutical composition of claim 90, wherein the therapeutically effective amount is sufficient to induce anti-cancer effect.

170. A method for preventing relapse of a B7-H3$^+$ cancer in a patient, comprising:

administering to a patient, identified as having had a B7-H3$^+$ cancer, a therapeutically effective amount of $^{CD4IL\text{-}10/CAR}$ cells of any one of claims 1-66, population of CD4$^{IL\text{-}10/CAR}$ cells of any one of claims 67-89, or the pharmaceutical composition of claim 90 sufficient to induce anti-cancer effect.

171. The method of claim 170, wherein the B7-H3$^+$ cancer is solid tumor.

172. The method of claim 171, wherein the solid tumor is selected from the group consisting of: breast cancer, brain cancer, lung cancer, liver cancer, stomach cancer, spleen cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, uterine cancer, skin cancer, head cancer, neck cancer, sarcomas, neuroblastomas and ovarian cancer.

173. A method for treating a cancer patient with minimal residual disease, comprising:

administering to a patient, identified as having minimal residual disease or at risk of having minimal residual disease, a therapeutically effective amount of CD4$^{IL\text{-}10/CAR}$ cells of any one of claims 1-66, population of CD4$^{IL\text{-}10/CAR}$ cells of any one of claims 67-89, or the pharmaceutical composition of claim 90, wherein the therapeutically effective amount is sufficient to induce anti-cancer effect.

174. A method of treating a patient, comprising:

administering a therapeutically effective amount of CD4$^{IL\text{-}10/CAR}$ cells of any one of claims 1-66, the population of CD4$^{IL\text{-}10/CAR}$ of any one of claims 67-89, or the pharmaceutical composition of claim 90, to a patient in need of immune tolerization.

175. The method of claim 174, further comprising the preceding step of thawing a frozen suspension of CD4$^{IL\text{-}10/CAR}$ cells or the population of CD4$^{IL\text{-}10/CAR}$ cells.

176. The method of claim 174 or 175, wherein the patient has an inflammatory or autoimmune disease.

177. The method of claim 176, wherein the inflammatory or autoimmune disease is selected from the group consisting of: type-1 diabetes, autoimmune uveitis, autoimmune hepatitis, rheumatoid arthritis, psoriasis, psoriatic arthritis, vitiligo, alopecia areata ,multiple sclerosis, systemic lupus, inflammatory bowel disease, Addison's disease, Graves' disease, Sjögren's

syndrome, Hashimoto's thyroiditis, myasthenia gravis, autoimmune vasculitis, pernicious anemia, ulcerative colitis, bullous diseases, scleroderma, and celiac disease.

178. The method of claim 177, wherein the inflammatory or autoimmune disease is Crohn's disease, ulcerative colitis, celiac disease, type-1 diabetes, lupus, psoriasis, psoriatic arthritis, ankylosing spondylitis, or rheumatoid arthritis.

179. The method of claim 174, wherein the patient has a disease or disorder involving hyperactivity of NLPR3 inflammasome.

180. The method of claim 174, wherein the patient has type 2 diabetes, neurodegenerative disease, cardiovascular disease or inflammatory bowel disease.

181. The method of claim 174, wherein the patient has a disease or disorder involving increased IL-1β production by activated monocytes, macrophages or dendritic cells.

182. The method of claim 174, wherein the patient has a disease or disorder involving increased IL-18 production by activated monocytes, macrophages or dendritic cells.

183. The method of claim 174, wherein the patient has a disease or disorder involving increased mature caspase 1 production by activated monocytes, macrophages or dendritic cells.

184. The method of claim 174, wherein the patient has an allergic or atopic disease.

185. The method of claim 184, wherein the allergic or atopic disease is selected from the group consisting of: asthma, atopic dermatitis, and rhinitis.

186. The method of claim 174, wherein the patient has a food allergy.

187. The method of claim 174, wherein the patient has a solid tumor.

188. The method of claim 187, wherein the solid tumor is selected from the group consisting of: breast cancer, brain cancer, lung cancer, liver cancer, stomach cancer, spleen cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, uterine cancer, skin cancer, head cancer, neck cancer, sarcomas, neuroblastomas and ovarian cancer.

189. The method of claim 174, further comprising the step of organ transplantation to the patient, either prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells or the pharmaceutical composition.

190. The method of claim 189, wherein the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition prevents or reduces severity of host rejection of the organ transplantation.

191. The method of claim 174, further comprising the step of transplanting iPS cell-derived cells or tissues to the patient, either prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition.

192. The method of claim 191, wherein the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition prevents or reduces severity of host rejection of the cell transplantation.

193. The method of claim 174, further comprising the step of administering a recombinant AAV to the patient, either prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition.

194. The method of claim 193, wherein the $CD4^{IL\text{-}10/CAR}$ cells, the population of $^{CD4IL\text{-}10/CAR}$ cells, or the pharmaceutical composition reduces immune responses against the recombinant AAV.

195. The method of claim 174, further comprising the step of administering a recombinant viral vector other than AAV to the patient, either prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition.

196. The method of claim 195, wherein the $CD4^{IL\text{-}10/CAR}$ cells, the population of $^{CD4IL\text{-}10/CAR}$ cells, or the pharmaceutical composition reduces immune responses against the recombinant viral vector other than AAV.

197. The method of claim 196, further comprising administering an immunogenic therapeutic protein to the patient, either prior to or subsequent to administration of the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition.

198. The method of claim 197, wherein the $CD4^{IL\text{-}10/CAR}$ cells, the population of $CD4^{IL\text{-}10/CAR}$ cells, or the pharmaceutical composition reduces immune responses against the immunogenic therapeutic protein.

199. The method of claims 197 or 198, wherein the immunogenic therapeutic protein is selected from a therapeutic antibody, a factor VIII replacement, a cytokine, and a cytokine mutein.

200. The method of claim 174, wherein the patient has an excessive immune response against viral or bacterial infection.

201. The method of claim 200, wherein the patient has a coronavirus infection.

202. The method of claim 174, further comprising the step of detecting a selection marker in a biological sample obtained from the patient, thereby detecting presence or absence of $CD4^{IL\text{-}10/CAR}$ cells.

203. The method of claim 202, wherein the biological sample is a biopsy or blood from the patient.

204. A method of treating or inhibiting autoimmune disease, allergic disease, or inflammatory disease in a patient, comprising:

administering to a patient, identified as having autoimmune disease, allergic disease, or inflammatory disease, a therapeutically effective amount of $CD4^{IL\text{-}10/CAR}$ cells of any one of claims 1-66, population of $CD4^{IL\text{-}10/CAR}$ cells of any one of claims 67-89, or the pharmaceutical composition of claim 90 sufficient to treat or inhibit the autoimmune disease, allergic disease, or inflammatory disease.

205. A method for reducing transplant rejection in a patient transplanted with hematopoietic stem cells, bone marrow cells, cord blood cells, tissue stem cells, or a solid organ, comprising:

administering to a patient, identified as having rejection of transplanted hematopoietic stem cells, bone marrow cells, or a solid organ, a therapeutically effective amount of $CD4^{IL\text{-}10/CAR}$ cells of any one of claims 1-66, population of $CD4^{IL\text{-}10/CAR}$ cells of any one of claims 67-89, or the pharmaceutical composition of claim 90 sufficient to reduce transplant rejection.

206. A method for treating graft-versus-host disease (GvHD) in a patient, comprising:

administering to a patient, identified as having graft-versus-host disease (GvHD) or at risk of having graft-versus-host disease (GvHD), a therapeutically effective amount of $CD4^{IL\text{-}10/CAR}$ cells of any one of claims 1-66, population of $CD4^{IL\text{-}10/CAR}$ cells of any one of claims 67-89, or the pharmaceutical composition of claim 90 sufficient to suppress or prevent GvHD.

207. The method of claim 206, wherein the graft-versus-host disease (GvHD) comprises acute GvHD.

208. The method of claim 206, wherein the graft-versus-host disease (GvHD) comprises chronic GvHD.

209. A method for treating tissue or organ damage in a patient, comprising:

administering to a patient, identified as having tissue or organ damage or at risk of having tissue or organ damage, a therapeutically effective amount of $CD4^{IL\text{-}10/CAR}$ cells of any one of claims 1-66, population of $CD4^{IL\text{-}10/CAR}$ cells of any one of claims 67-89, or the pharmaceutical composition of claim 90 sufficient to induce repair of tissue or organ damage.

210. A polynucleotide construct comprising:

(a) a first polynucleotide segment encoding a chimeric antigen receptor (CAR); and

(b) a second polynucleotide segment encoding interleukin-10 (IL-10).

211. The polynucleotide construct of claim 210, wherein the first polynucleotide segment comprises a first regulatory element operably linked to a coding sequence of the CAR.

212. The polynucleotide construct of claim 211, wherein the first regulatory element drives constitutive expression of the CAR.

213. The polynucleotide construct of any one of claims 210-212, wherein the second polynucleotide segment comprises a second regulatory element operably linked to a coding sequence of the IL-10.

214. The polynucleotide construct of claim 213, wherein the second regulatory element drives constitutive expression of the IL-10.

215. The polynucleotide construct of any one of claims 210-214, further comprising an internal ribosome entry site (IRES) or a self-cleaving peptide between the first polynucleotide segment and the second polynucleotide segment.

216. The polynucleotide construct of claim 215, wherein the self-cleaving peptide is selected from the group consisting of F2A, P2A, T2A and E2A.

217. The polynucleotide construct of any one of claims 210-216, wherein the CAR comprises an antigen-binding domain, a hinge region, a transmembrane domain, and an intracellular signaling domain.

218. The polynucleotide construct of claim 217, wherein the antigen-binding domain comprises a single chain antibody fragment.

219. The polynucleotide construct of claim 218, wherein the single chain antibody fragment comprises a single chain Fv (scFv).

220. The polynucleotide construct of any one of claims 210-219, wherein the antigen-binding domain targets an antigen associated with an autoimmune disease, inflammatory disorder, or cancer.

221. The polynucleotide construct of claim 220, wherein the antigen is selected from the group consisting of: CD19, CD20, CD22, BCMA, CD27, CD38, CEA, B7-H3, CD23, Lyml, Lym2, CLEC5A, CDH179b, FLT3, GCC, Muc, CSF2RA, GFRa4, CD32, CD33, IL1lRa, IL13Ra, NYBRI, SLea, CD200R, TGFBetaR2, CD276, TROP2, LAMP1, PTK7, DLL3, CDH1, CDH6, CDH17, CDH19, TSHR, tyrosinase, HLA-A*02,HLA-A*24 citrullinated peptides, insulin, MOG, GAD65, IA2, gliadin, and desmoglein.

222. The polynucleotide construct of any one of claims 210-221, wherein the antigen-binding domain is an anti-CD19 antigen binding domain.

223. The polynucleotide construct of claim 222, wherein the anti-CD19 antigen-binding domain has the sequence of SEQ ID NO: 11.

224. The polynucleotide construct of any one of claims 210-221, wherein the antigen-binding domain is an anti-BCMA antigen binding domain.

225. The polynucleotide construct of claim 224, wherein the anti-BCMA antigen-binding domain has the sequence of any one of SEQ ID NOs: 50-53.

226. The polynucleotide construct of any one of claims 210-225, wherein the hinge region is selected from a human CD8α hinge region, a human CD28 hinge region, a IgG1 hinge region, or a IgG4 hinge region.

227. The polynucleotide construct of claim 226, wherein the hinge region is derived from human CD8α.

228. The polynucleotide construct of any one of claims 210-227, wherein the transmembrane domain is selected from a TNFRSF 19 transmembrane domain, a CD3zeta transmembrane domain, CD8α transmembrane domain, a CD4 transmembrane domain, a CD28 transmembrane domain, or an B7-family inducible costimulatory (ICOS) transmembrane domain.

229. The polynucleotide construct of claim 228, wherein the transmembrane domain is derived from C CD8α.

230. The polynucleotide construct of any one of claims 210-229, wherein the CAR further comprises one or more co-stimulatory domains.

231. The polynucleotide construct of any claim 230, wherein the CAR comprises two co-stimulatory domains.

232. The polynucleotide construct of any one of claims 210-231, wherein the one or more co-stimulatory domains are selected from the group consisting of 4-1BB, CD28, OX40, ICOS, CD27, MYD88-CD40, and KIR2DS2.

233. The polynucleotide construct of claim 232, wherein one of the one or more co-stimulatory domain is derived from CD28.

234. The polynucleotide construct of any one of claims 231-233, wherein a second co-stimulatory domain is derived from 4-1BB.

235. The polynucleotide construct of any one of claims 210-234, wherein the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM).

236. The polynucleotide construct of any one of claims 210-235, the immunoreceptor tyrosine-based activation motif (ITAM) is derived from CD3zeta.

237. The polynucleotide construct of any one of claims 210-236, wherein the CAR comprises:

an anti-CD19 antigen binding domain, an anti-BCMA antigen binding domain, or an anti-CD20 antigen-binding;

a human CD8 hinge region;

a CD8 transmembrane region;

a CD28 co-stimulatory domain; and

a CD3zeta chain intracellular signaling domain.

238. The polynucleotide construct of any one of claims 210-237, wherein the CAR comprises the sequence of SEQ ID NOs: 9, 16, 22, 34, 41-49, or 54.

239. The polynucleotide construct of claim 238, wherein the first polynucleotide segment comprises the sequence of SEQ ID NOs: 10, 17, 23, 35, or 55.

240. The polynucleotide construct of any one of claims 210-239, wherein the IL-10 is a human IL-10.

241. The polynucleotide construct of any one of claims 210-239, wherein the IL-10 is a viral IL-10.

242. The polynucleotide construct of any one of claims 210-239, wherein the IL-10 is a protein having the sequence of SEQ ID NO: 1.

243. The polynucleotide construct of claim 242, wherein the second polynucleotide segment having the sequence of SEQ ID NO: 2.

244. The polynucleotide construct of any one of claims 210-243, wherein the first polynucleotide segment or the second polynucleotide segment further comprises a sequence encoding a selection marker.

245. The polynucleotide construct of claim 244, wherein the selection marker is ΔNGFR.

246. The polynucleotide construct of claim 245, wherein the ΔNGFR has the sequence of SEQ ID NO: 3.

247. The polynucleotide construct of claim 245, wherein the polynucleotide construct comprises the sequence of SEQ ID NO: 4.

248. The polynucleotide construct of any one of claims 244-247, wherein the selection marker is a truncated form of an EGFR polypeptide.

249. The polynucleotide construct of any one of claims 210-248, wherein the construct is a vector.

250. The polynucleotide construct of claim 249, wherein the vector is a viral vector.

251. The polynucleotide construct of claim 250, wherein the vector is a lentiviral vector.

252. A polynucleotide construct, comprising:

(a) a first polynucleotide segment having the sequence of SEQ ID NOs: 10, 17, 23, 35, or 55; and

(b) a second polynucleotide segment having the sequence of SEQ ID NO: 2.

253. A polynucleotide construct, comprising:

(a) a first polynucleotide segment having the sequence of SEQ ID NOs: 10, 17, 23, 35, or 55;

(b) a second polynucleotide segment having the sequence of SEQ ID NO: 2; and

(c) a third polynucleotide segment having the sequence of SEQ ID NO: 33 in between the first polynucleotide segment and the second polynucleotide segment.

254. A polynucleotide construct, comprising:

(a) a first polynucleotide segment having the sequence of SEQ ID NOs: 10, 17, 23, 35, or 55;

(b) a second polynucleotide segment having the sequence of SEQ ID NO: 2;

(c) a third polynucleotide segment having the sequence of SEQ ID NO: 27 in between the first polynucleotide segment and the second polynucleotide segment; and

(d) a fourth polynucleotide segment having the sequence of SEQ ID NO: 4.

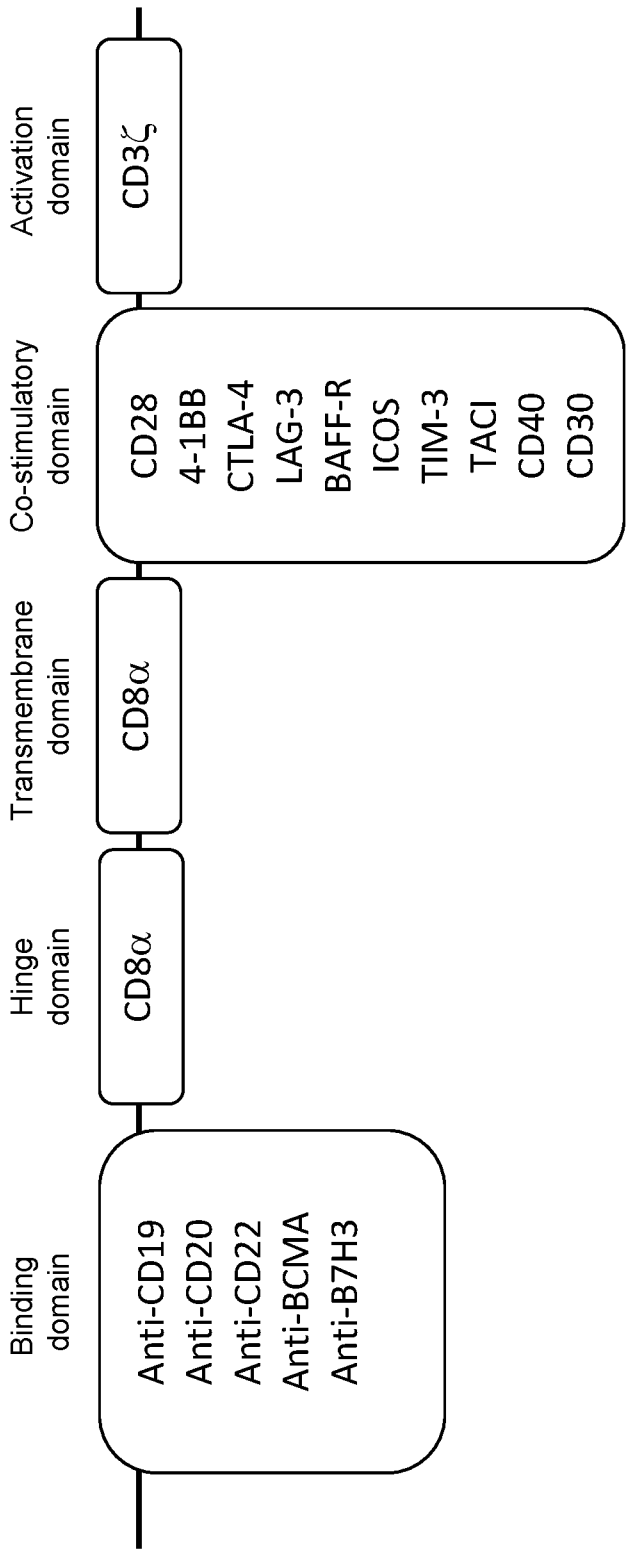

Binding domain
Anti-CD19
Anti-CD20
Anti-CD22
Anti-BCMA
Anti-B7H3
Hinge domain
CD8α
Transmembrane domain
CD8α
Co-stimulatory domain
CD28
4-1BB
CTLA-4
LAG-3
BAFF-R
ICOS
TIM-3
TACI
CD40
CD30
Activation domain
CD3ζ

FIG. 1

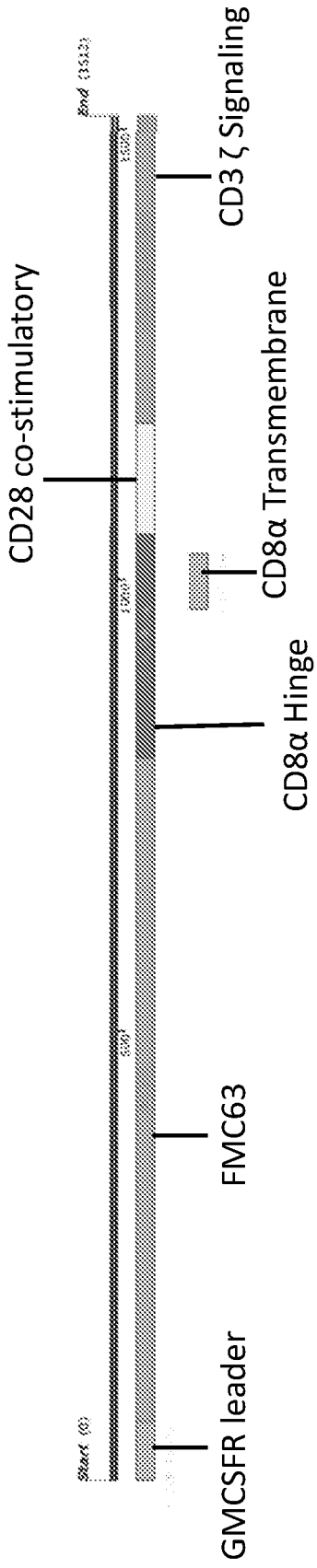

GMCSFR leader
FMC63
CD8α Hinge
CD8α Transmembrane
CD28 co-stimulatory
CD3 ζ Signaling

FIG. 2A

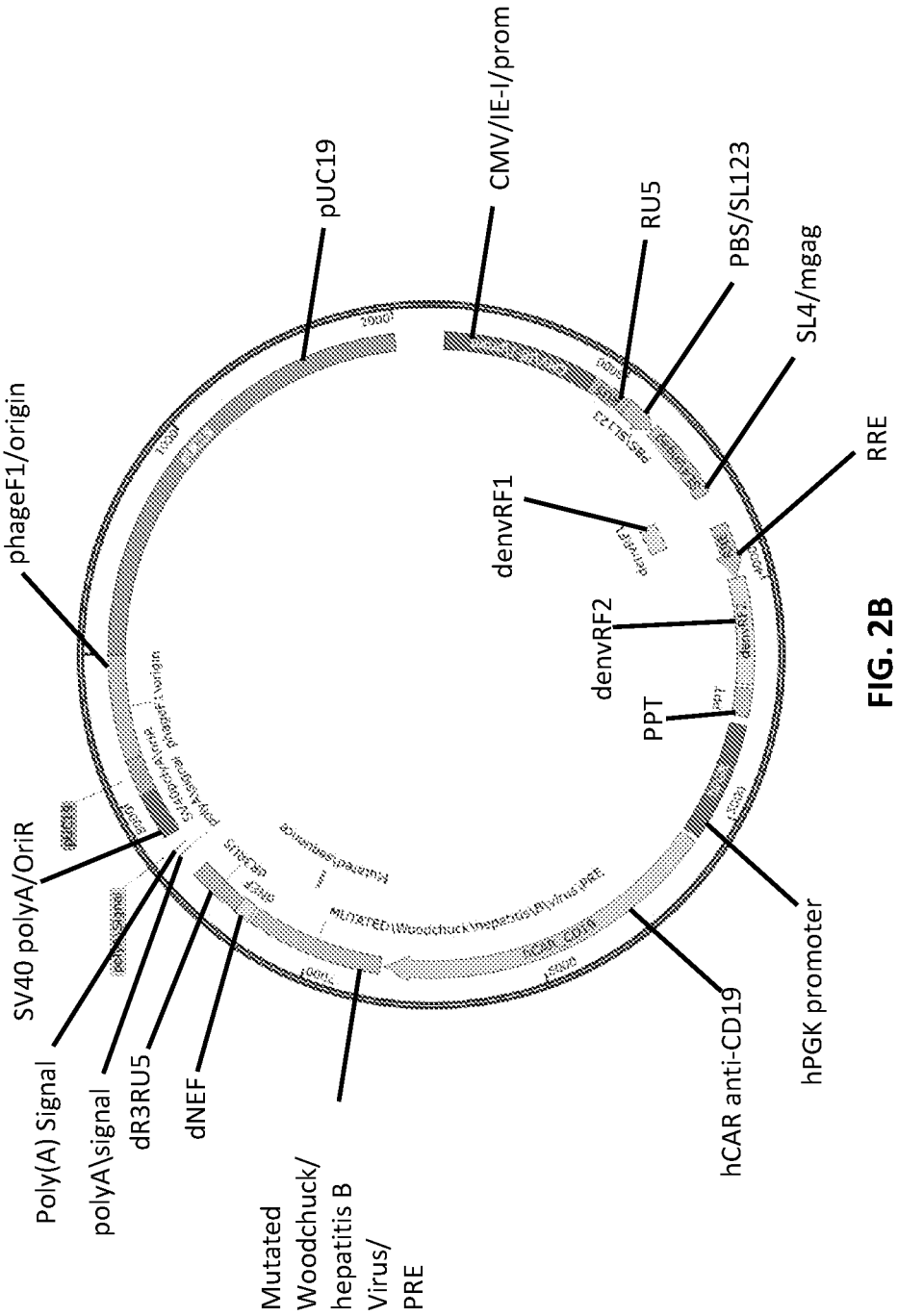

pUC19
CMV/IE-I/prom
RU5
PBS/SL123
SL4/mgag
RRE
denvRF1
denvRF2
PPT
hPGK promoter
hCAR anti-CD19
Mutated Woodchuck/ hepatitis B Virus/ PRE
dNEF
dR3RU5
polyA\signal
Poly(A) Signal
SV40 polyA/OriR
phageF1/origin

FIG. 2B

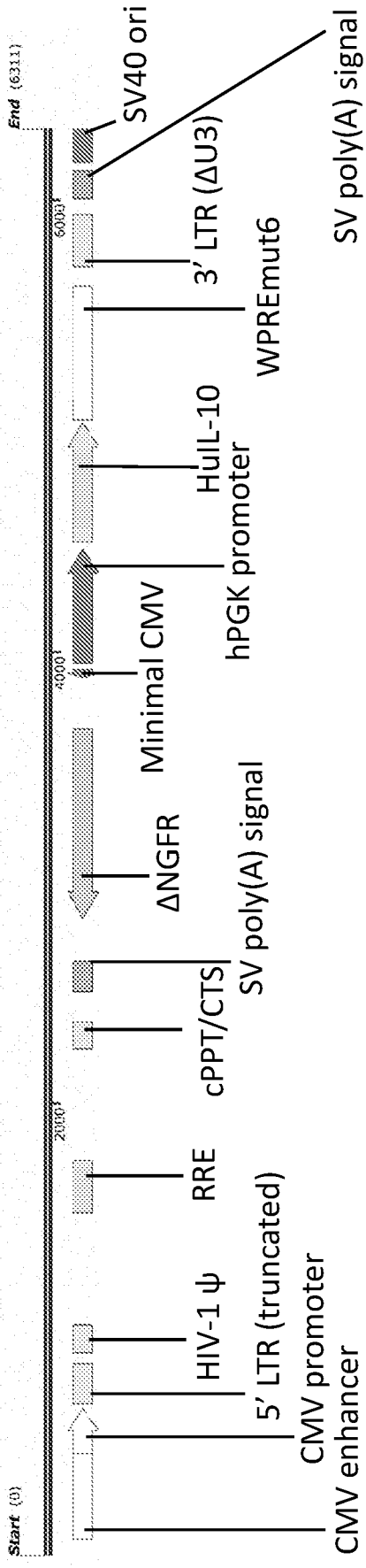

Start (0)
End (6311)
2000
4000
6000
CMV enhancer
CMV promoter
5' LTR (truncated)
HIV-1 ψ
RRE
cPPT/CTS
SV poly(A) signal
ΔNGFR
Minimal CMV
hPGK promoter
HuIL-10
WPREmut6
3' LTR (ΔU3)
SV40 ori
SV poly(A) signal

FIG. 3A

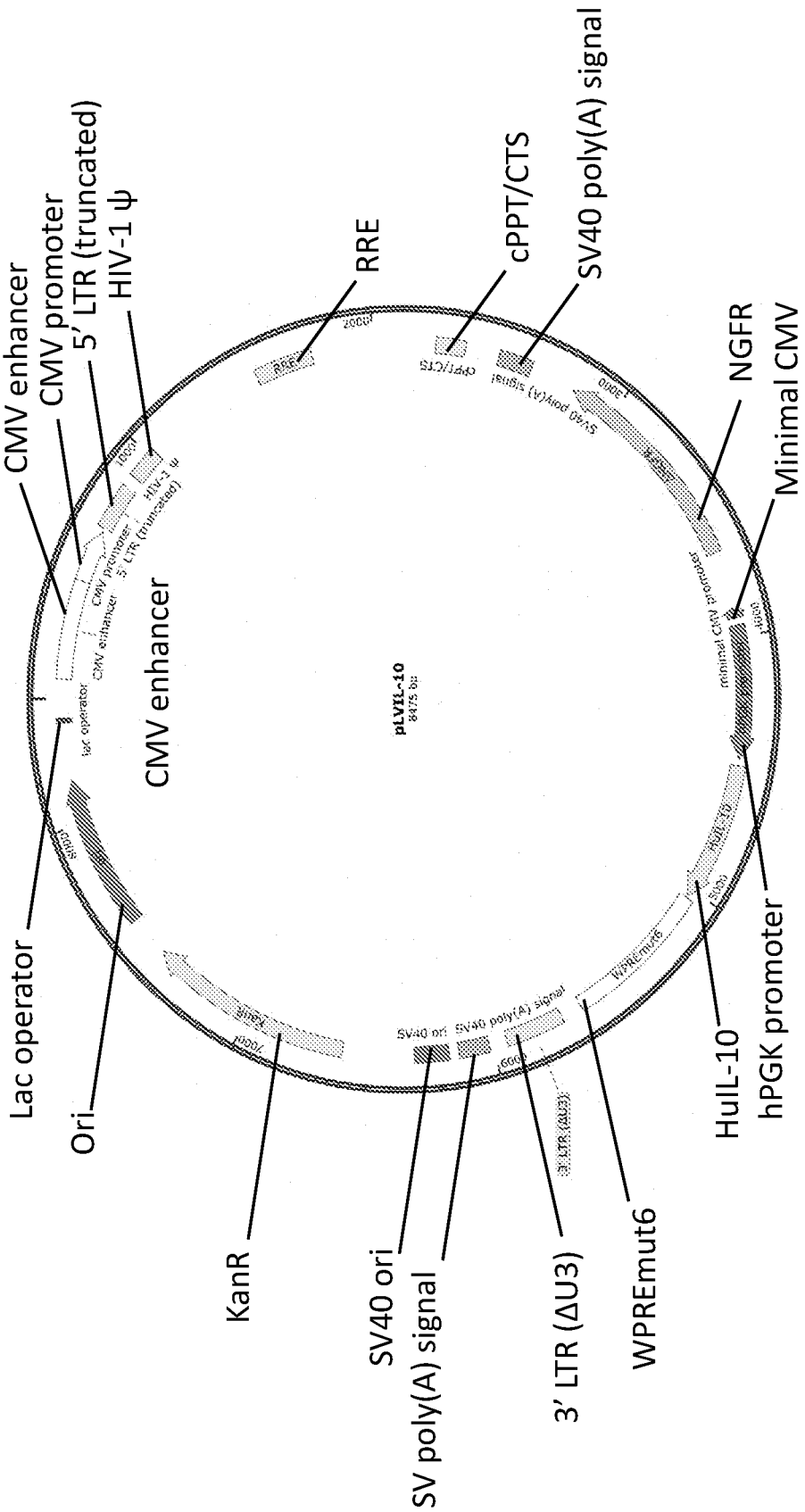

CMV enhancer
CMV promoter
5' LTR (truncated)
HIV-1 ψ
RRE
cPPT/CTS
SV40 poly(A) signal
NGFR
Minimal CMV
hPGK promoter
HuIL-10
WPREmut6
3' LTR (ΔU3)
SV poly(A) signal
SV40 ori
KanR
Ori
Lac operator
CMV enhancer
pLVIL-10
8475 bp

FIG. 3B

Expression of CD19, CD20, CD22, CD27, CD38, and BCMA during B-cell differentiation in bone marrow and periphery

FIG. 4

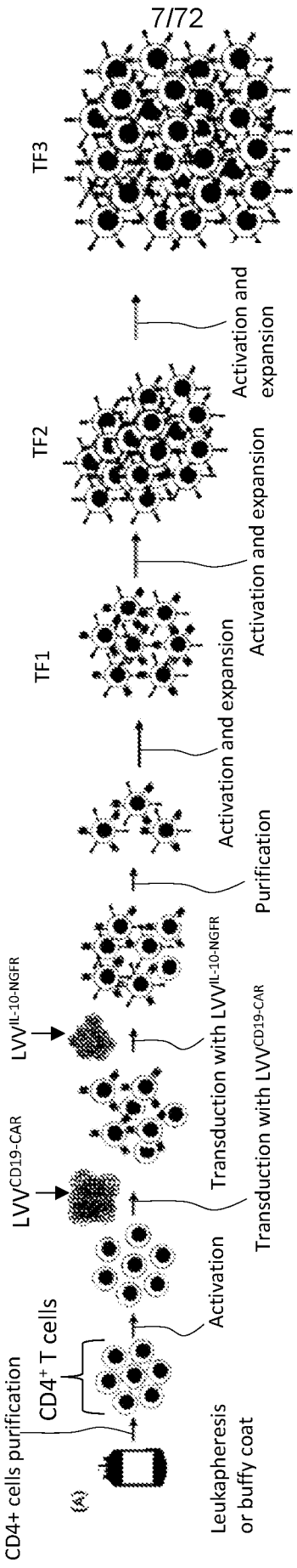

TF3
TF2
TF1
Activation and expansion
Activation and expansion
Activation and expansion
Purification
Transduction with LVVIL-10-NGFR
Transduction with LVVCD19-CAR
LVVIL-10-NGFR
LVVCD19-CAR
Activation
CD4+ cells purification
CD4+ T cells
Leukapheresis
or buffy coat

FIG. 5A

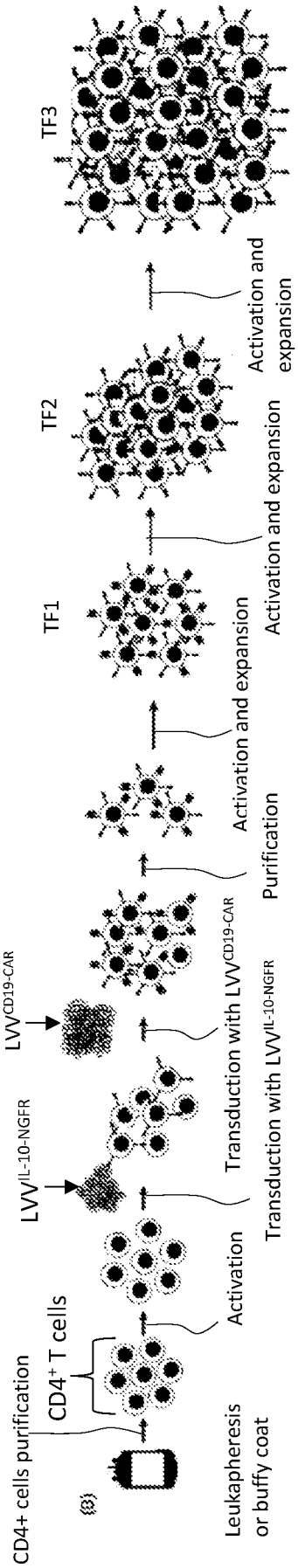

TF3
TF2
TF1
Activation and expansion
Activation and expansion
Activation and expansion
Purification
Transduction with LVVCD19-CAR
Transduction with LVVIL-10-NGFR
Activation
LVVCD19-CAR
LVVIL-10-NGFR
CD4+ cells purification
CD4+ T cells
Leukapheresis
or buffy coat

FIG. 5B

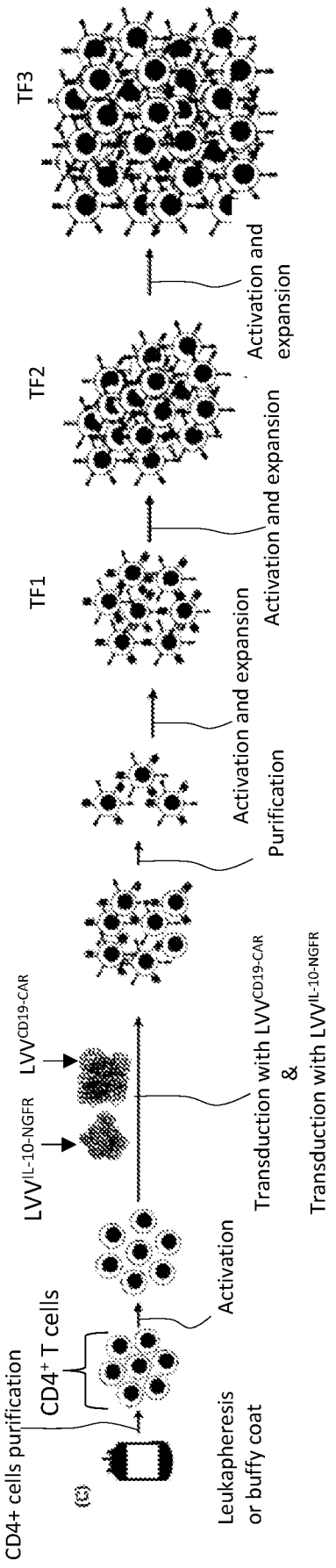

CD4+ cells purification
CD4+ T cells
Leukapheresis or buffy coat
Activation
LVVIL-10-NGFR
LVVCD19-CAR
Transduction with LVVCD19-CAR & Transduction with LVVIL-10-NGFR
Purification
Activation and expansion
TF1
Activation and expansion
TF2
Activation and expansion
TF3

FIG. 5C

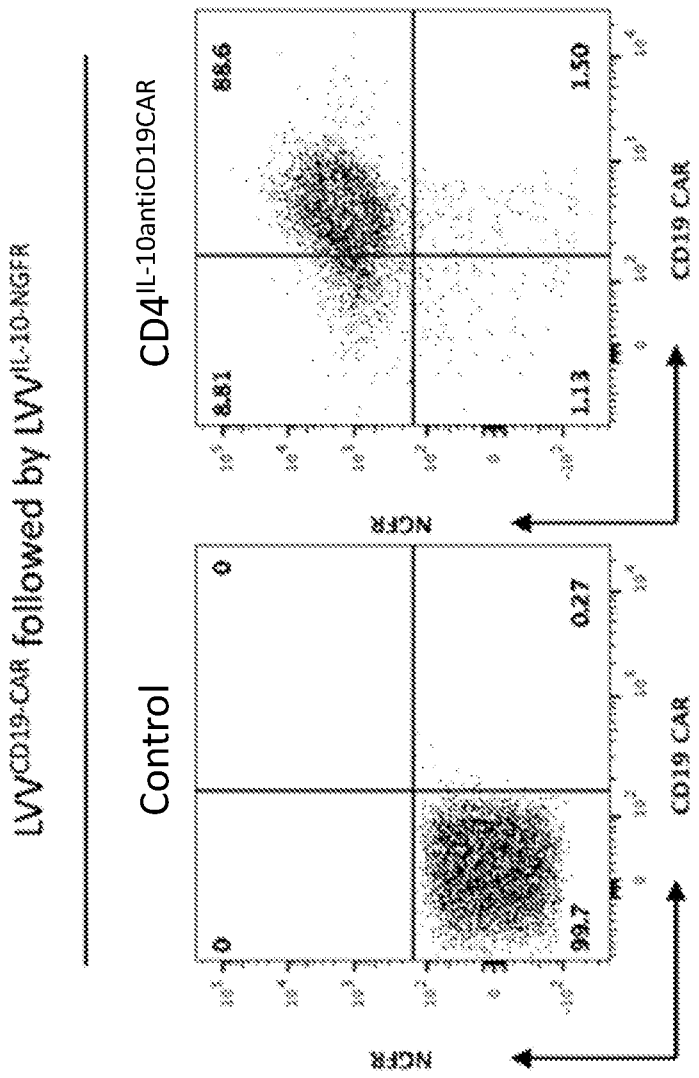

LVVCD19-CAR followed by LVVIL-10-NGFR
Control
CD4IL-10antiCD19CAR
NGFR
CD19 CAR
0
0
0.27
99.7
88.6
1.50
4.81
1.13

FIG. 6A

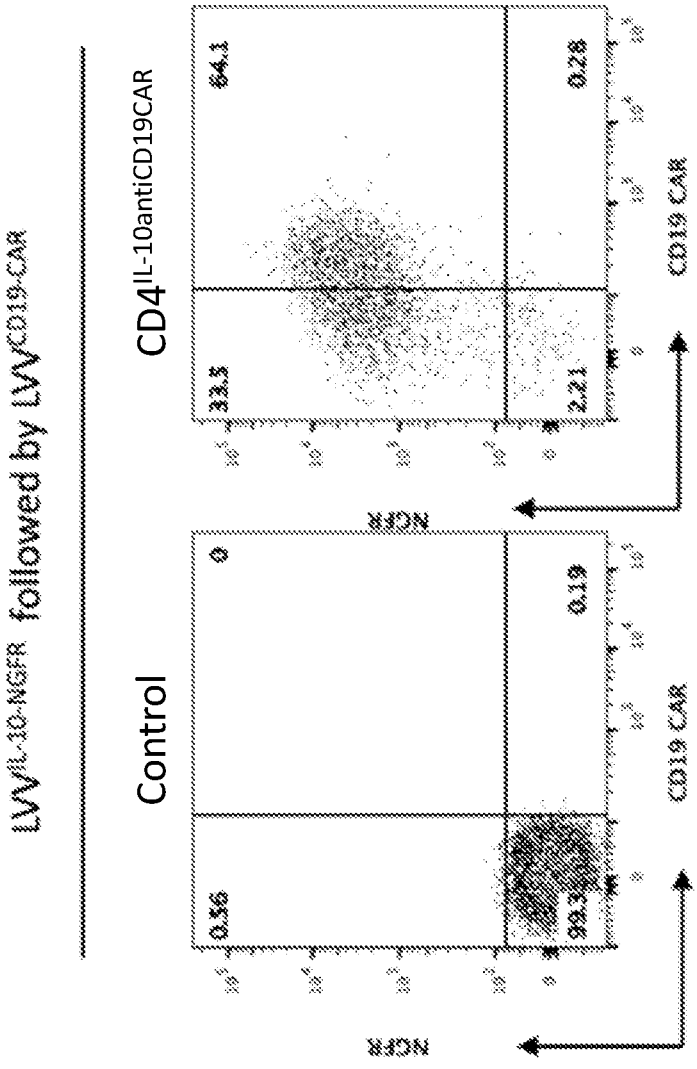

LVVIL-10-NGFR followed by LVVCD19-CAR
Control
CD4IL-10antiCD19CAR
0
0.19
0.56
99.3
64.1
0.28
33.3
2.21
NGFR
CD19 CAR

FIG. 6B

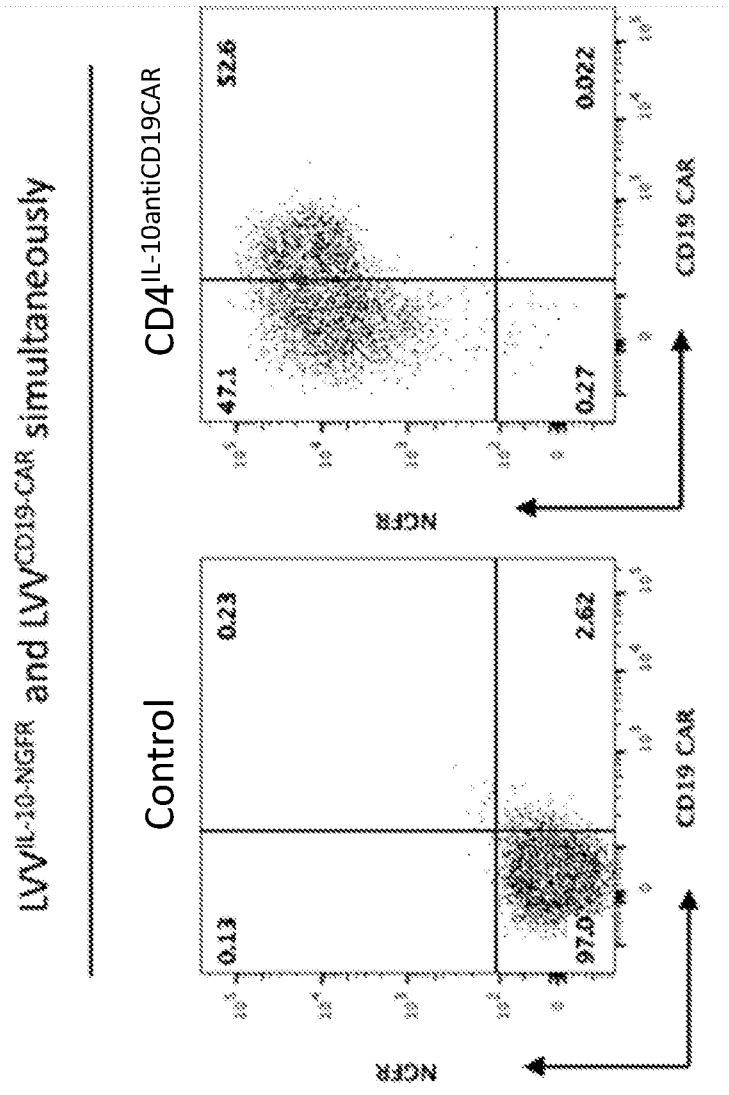

LVV$^{IL\text{-}10\text{-}NGFR}$ and LVV$^{CD19\text{-}CAR}$ simultaneously
Control
CD4$^{IL\text{-}10}$antiCD19CAR
0.13
0.23
97.0
2.62
47.1
32.6
0.27
0.022
NGFR
CD19 CAR

FIG. 6C

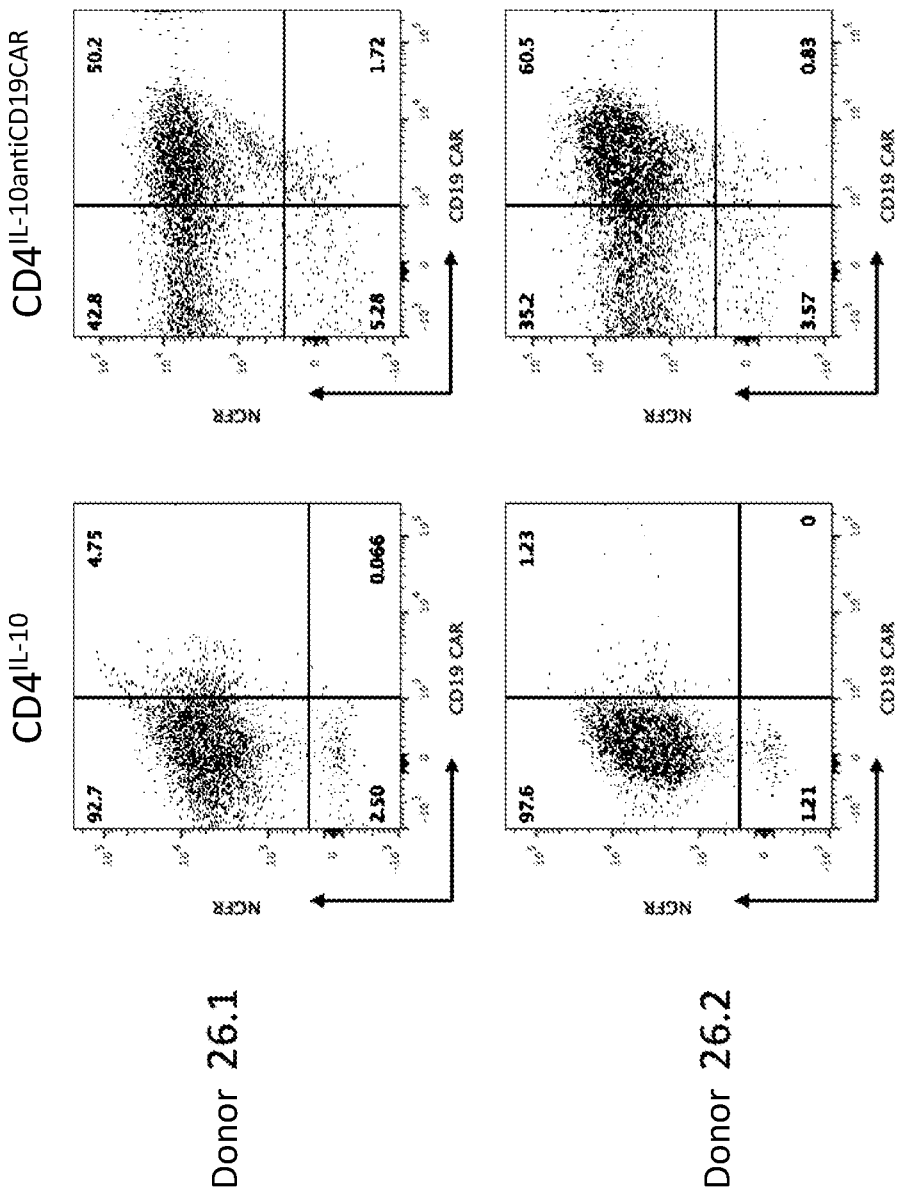

CD4IL-10
CD4IL-10antiCD19CAR
Donor 26.1
Donor 26.2
NGFR
CD19 CAR
92.7
4.75
2.50
0.066
97.6
1.23
1.21
0
42.8
50.2
5.28
1.72
35.2
60.5
3.57
0.83

FIG. 7

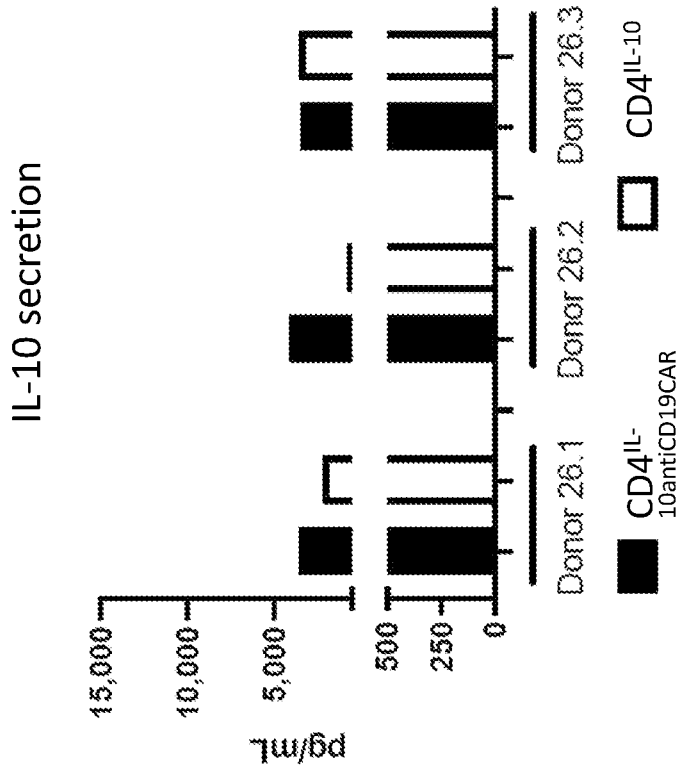

IL-10 secretion
pg/mL
15,000
10,000
5,000
500
250
0
Donor 26.1
Donor 26.2
Donor 26.3
CD4IL-10antiCD19CAR
CD4IL-10

FIG. 8A

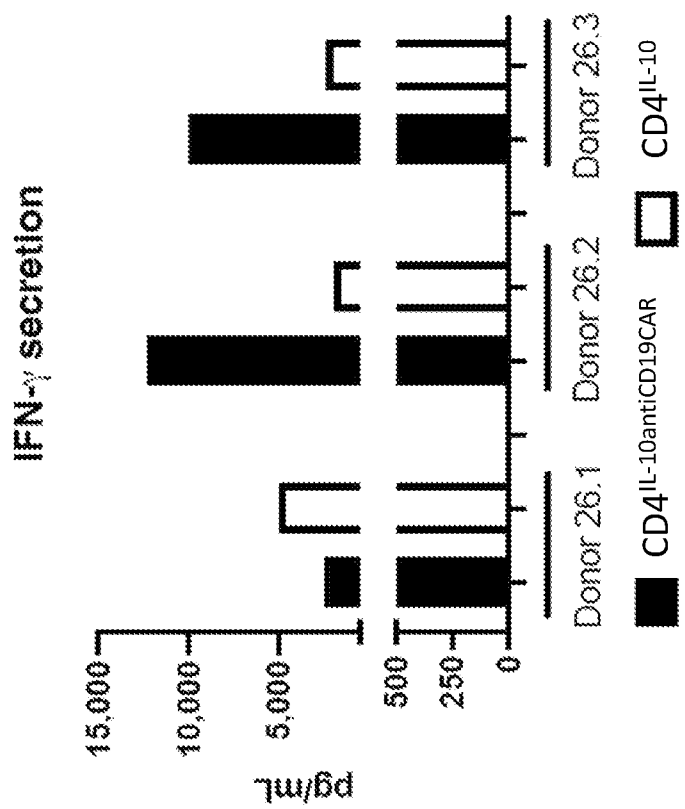

IFN-γ secretion
pg/mL
15,000
10,000
5,000
500
250
0
Donor 26.1
Donor 26.2
Donor 26.3
CD4IL-10antiCD19CAR
CD4IL-10

FIG. 8B

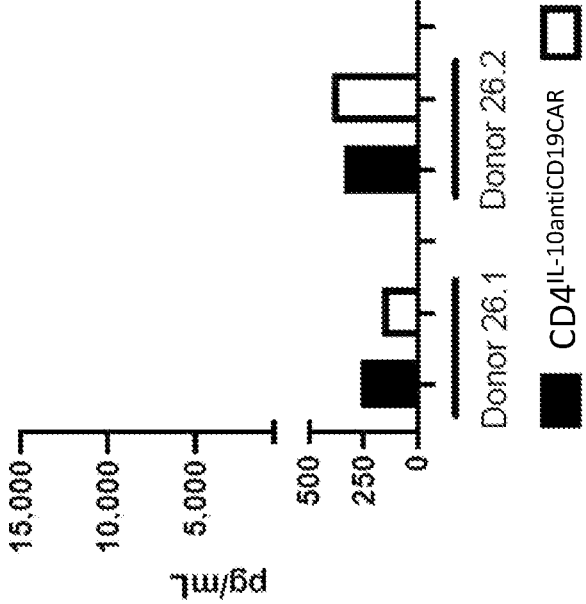

IL-4 secretion
pg/mL
15,000
10,000
5,000
500
250
0
Donor 26.1
Donor 26.2
Donor 26.3
CD4IL-10antiCD19CAR
CD4IL-10

FIG. 8C

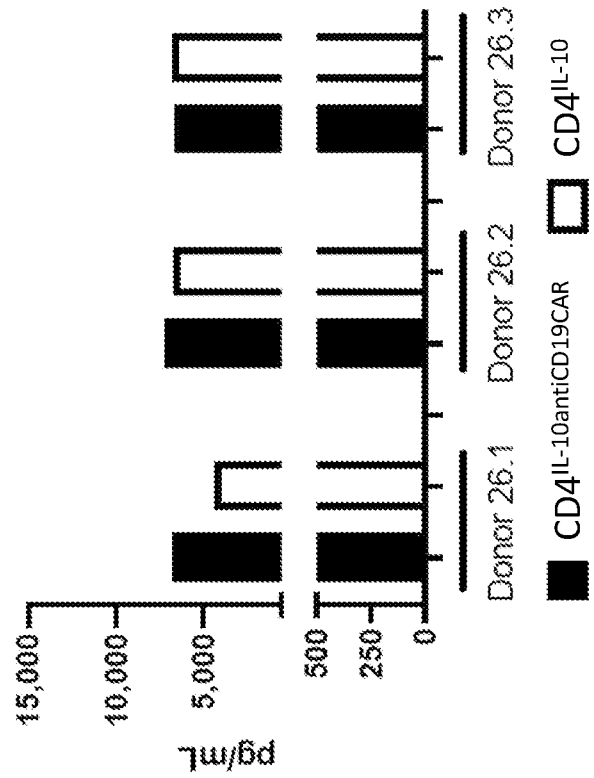

IL-5 secretion
pg/mL
15,000
10,000
5,000
500
250
0
Donor 26.1
Donor 26.2
Donor 26.3
CD4IL-10antiCD19CAR
CD4IL-10

FIG. 8D

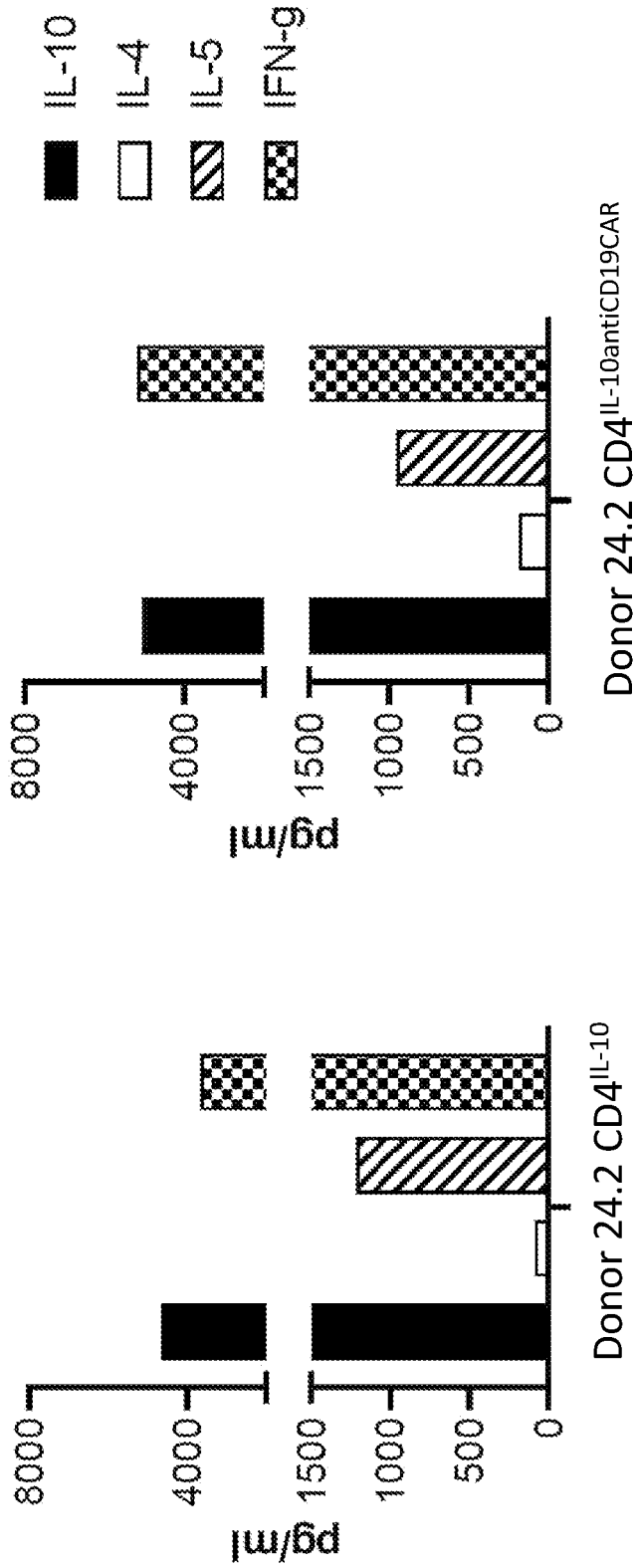

IL-10
IL-4
IL-5
IFN-g
pg/ml
8000
4000
1500
1000
500
0
Donor 24.2 CD4IL-10antiCD19CAR
Donor 24.2 CD4IL-10

FIG. 9

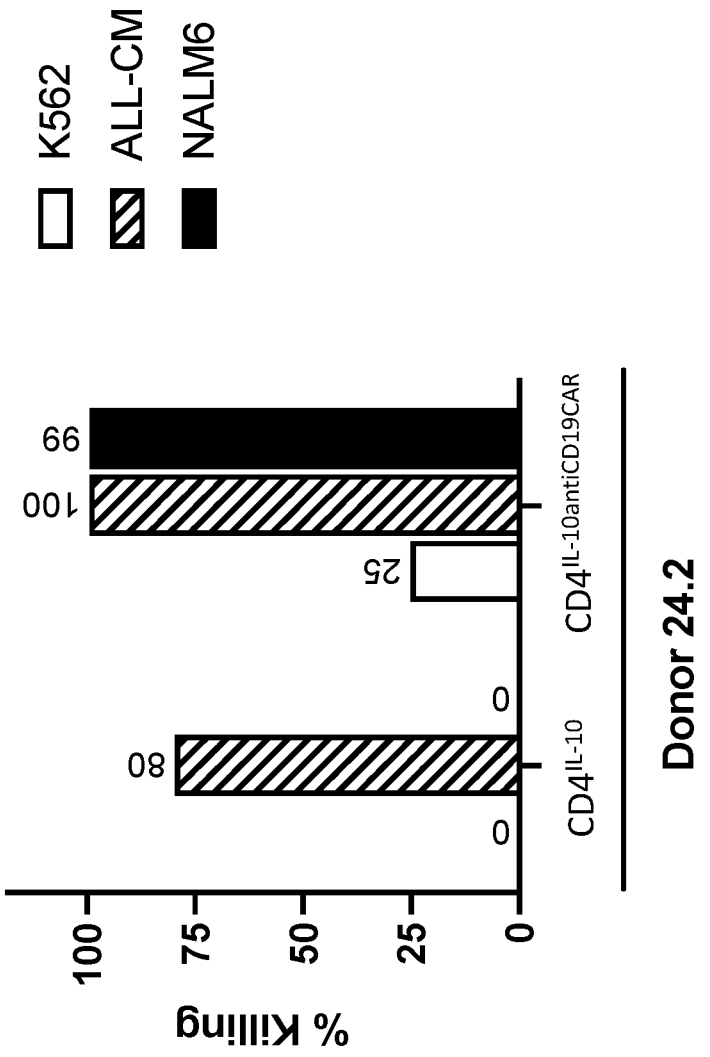

K562
ALL-CM
NALM6
% Killing
100
75
50
25
0
99
100
25
0
80
0
CD4IL-10
CD4IL-10antiCD19CAR
Donor 24.2

FIG. 10

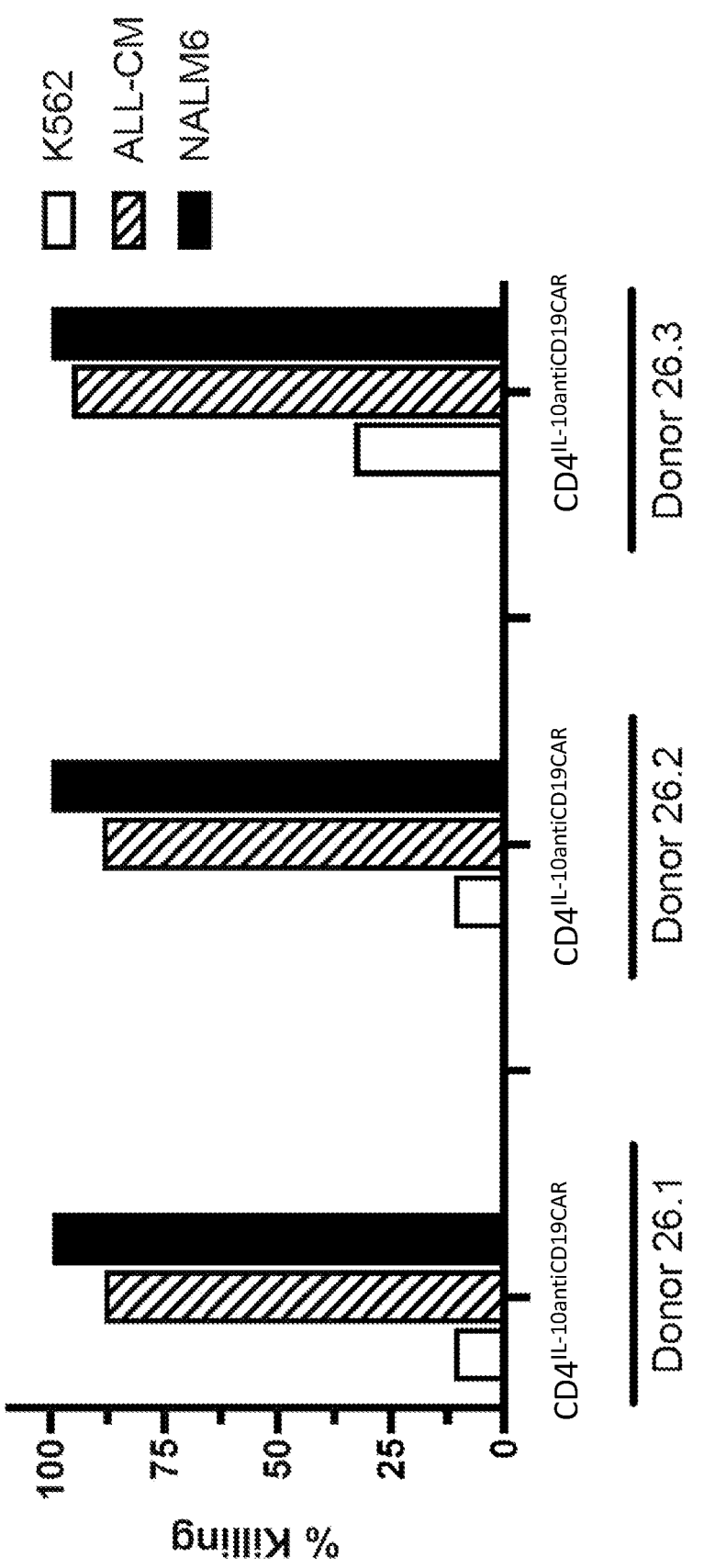

K562
ALL-CM
NALM6
% Killing
100
75
50
25
0
CD4IL-10antiCD19CAR
CD4IL-10antiCD19CAR
CD4IL-10antiCD19CAR
Donor 26.1
Donor 26.2
Donor 26.3

FIG. 11A

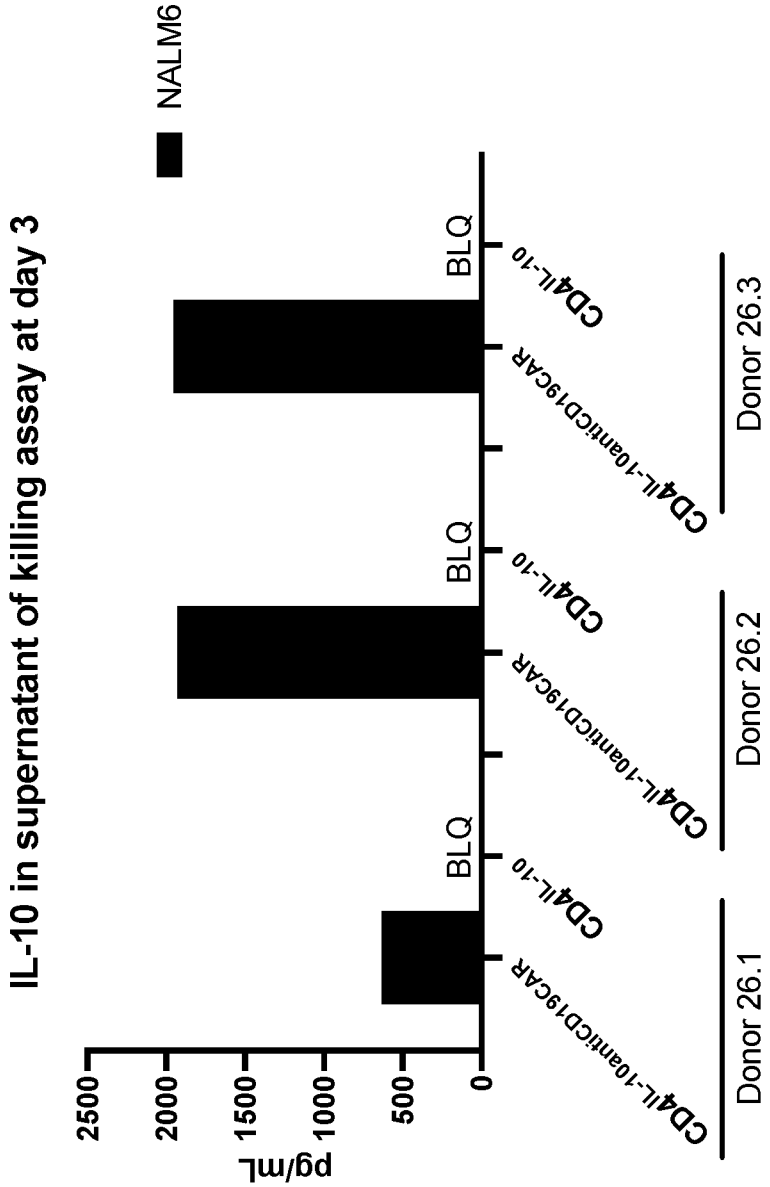

IL-10 in supernatant of killing assay at day 3
NALM6
pg/mL
2500
2000
1500
1000
500
0
BLQ
BLQ
BLQ
CD4IL-10
CD4IL-10antiCD19CAR
CD4IL-10
CD4IL-10antiCD19CAR
CD4IL-10
CD4IL-10antiCD19CAR
Donor 26.1
Donor 26.2
Donor 26.3

FIG. 11B

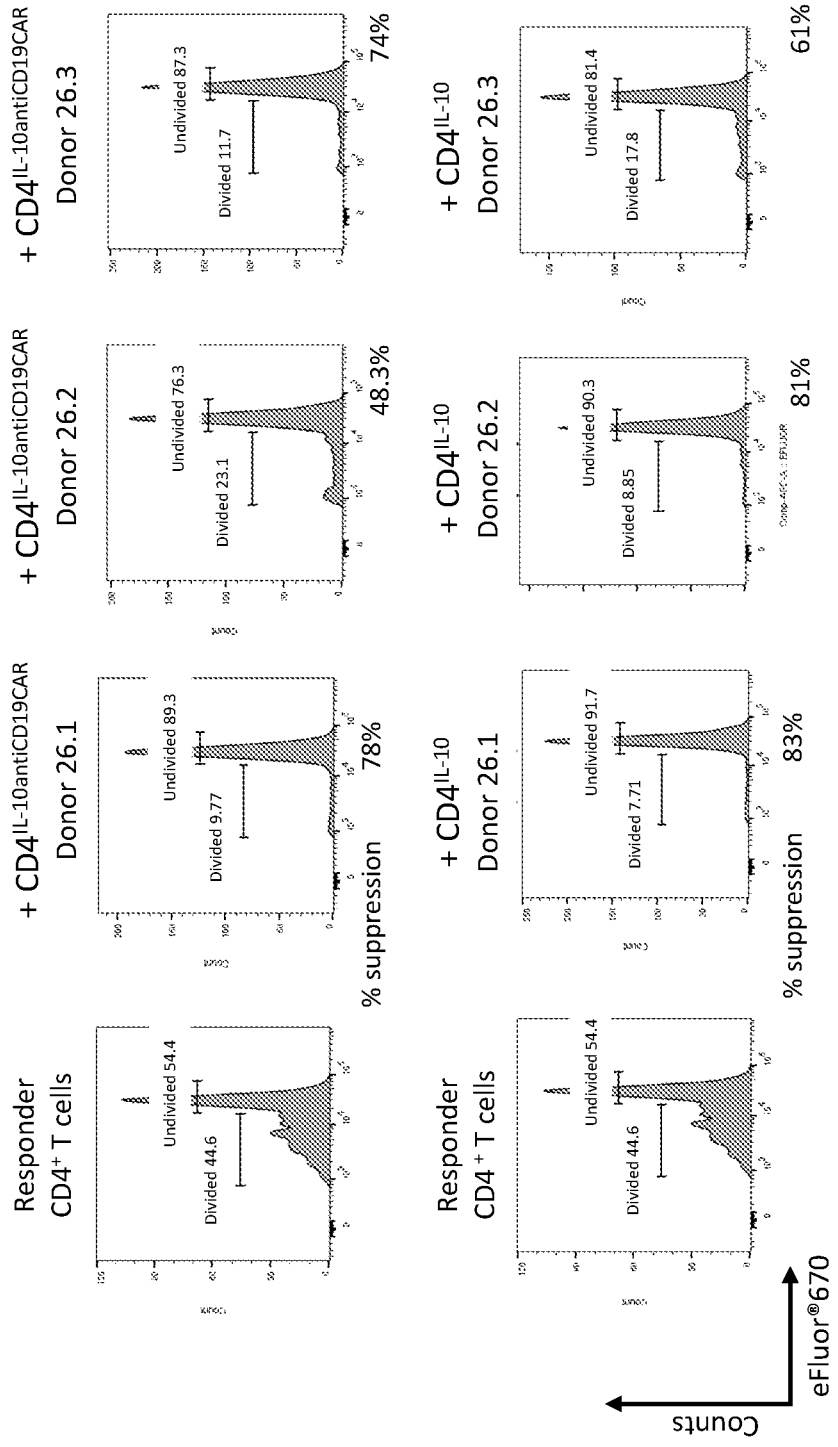

Responder
CD4+ T cells
+ CD4IL-10antiCD19CAR
Donor 26.1
Undivided 54.4
Divided 44.6
Undivided 89.3
Divided 9.77
% suppression 78%
+ CD4IL-10antiCD19CAR
Donor 26.2
Undivided 76.3
Divided 23.1
48.3%
+ CD4IL-10antiCD19CAR
Donor 26.3
Undivided 87.3
Divided 11.7
74%
Responder
CD4+ T cells
+ CD4IL-10
Donor 26.1
Undivided 54.4
Divided 44.6
Undivided 91.7
Divided 7.71
% suppression 83%
+ CD4IL-10
Donor 26.2
Undivided 90.3
Divided 8.85
81%
+ CD4IL-10
Donor 26.3
Undivided 81.4
Divided 17.8
61%
Counts
eFluor®670

FIG. 12

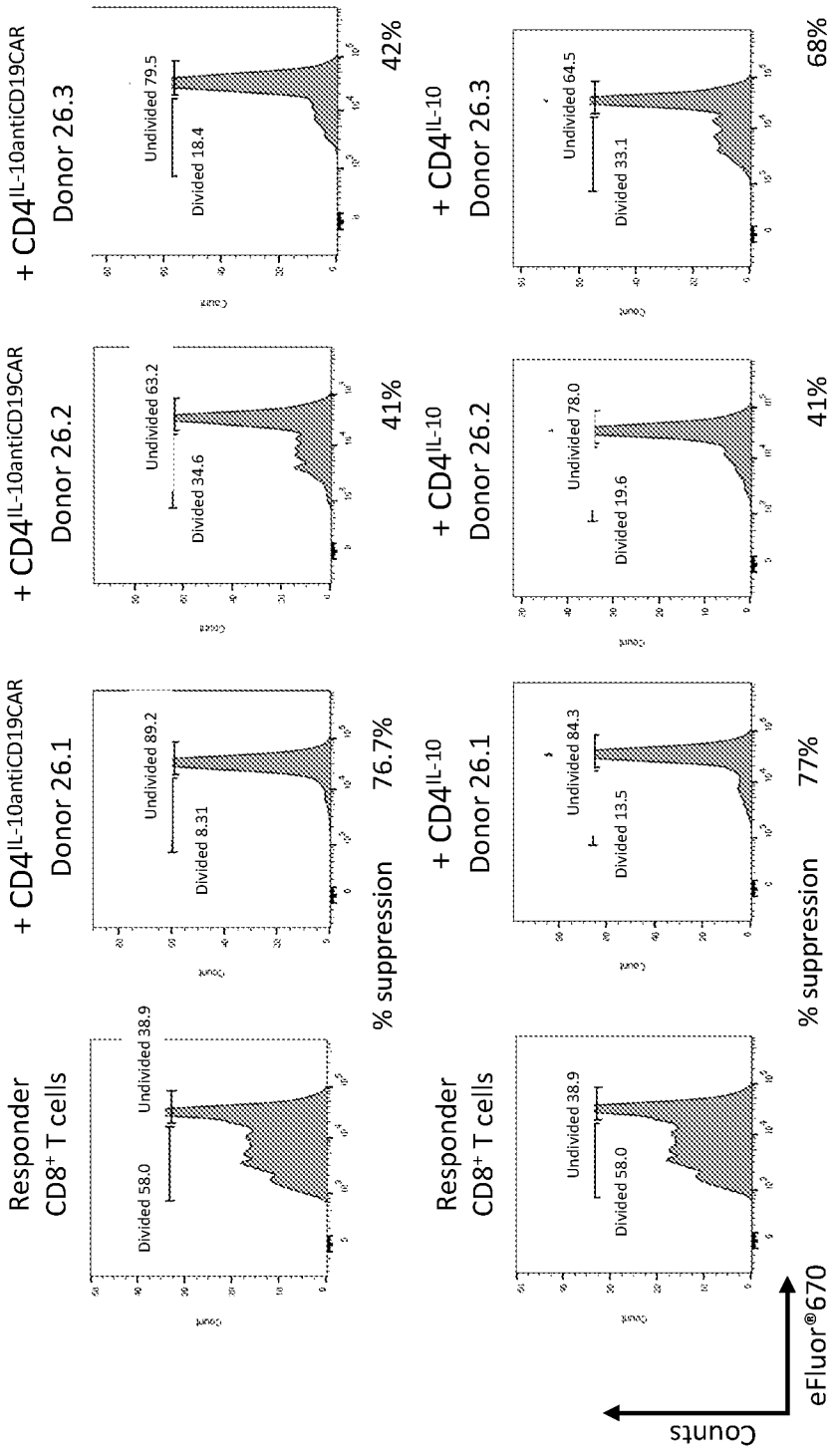

Responder CD8+ T cells
+ CD4IL-10antiCD19CAR Donor 26.1
+ CD4IL-10antiCD19CAR Donor 26.2
+ CD4IL-10antiCD19CAR Donor 26.3
+ CD4IL-10 Donor 26.1
+ CD4IL-10 Donor 26.2
+ CD4IL-10 Donor 26.3
Undivided 38.9
Divided 58.0
Undivided 89.2
Divided 8.31
Undivided 63.2
Divided 34.6
Undivided 79.5
Divided 18.4
Undivided 38.9
Divided 58.0
Undivided 84.3
Divided 13.5
Undivided 78.0
Divided 19.6
Undivided 64.5
Divided 33.1
% suppression
76.7%
41%
42%
77%
41%
68%
Counts
eFluor®670

FIG. 13

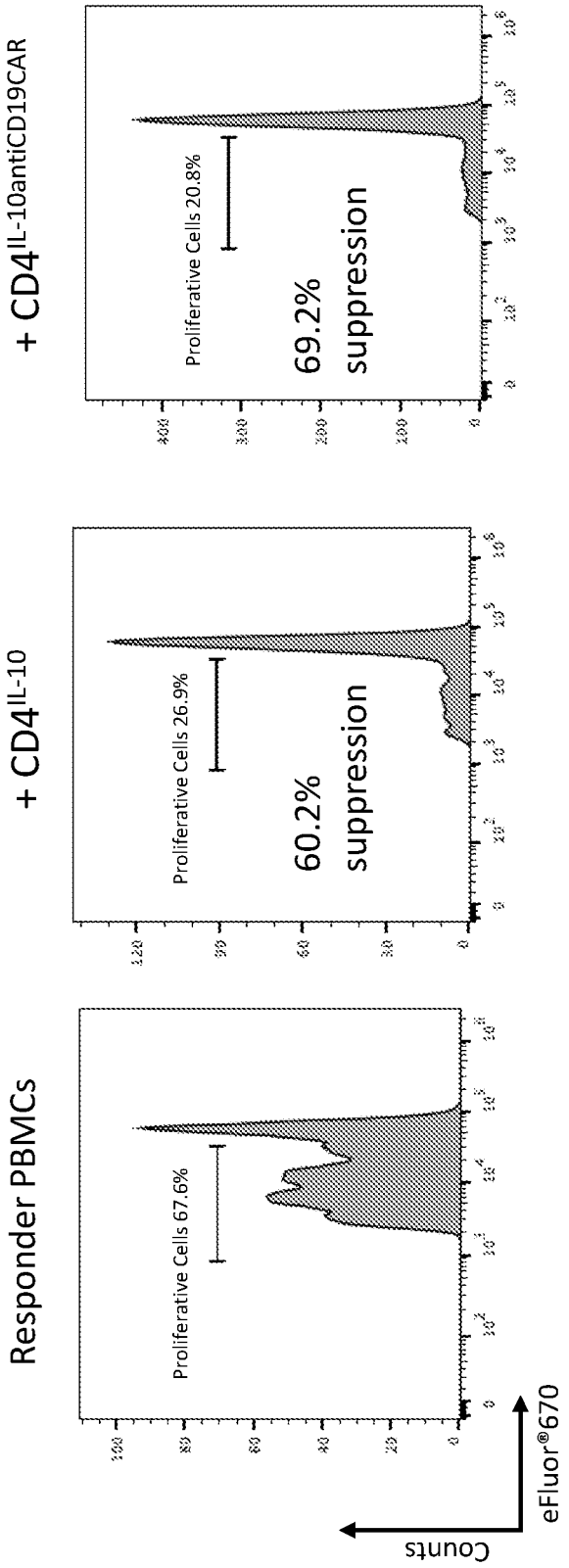

Responder PBMCs
+ CD4IL-10
+ CD4IL-10antiCD19CAR
Proliferative Cells 67.6%
Proliferative Cells 26.9%
60.2% suppression
Proliferative Cells 20.8%
69.2% suppression
Counts
eFluor®670

FIG. 14

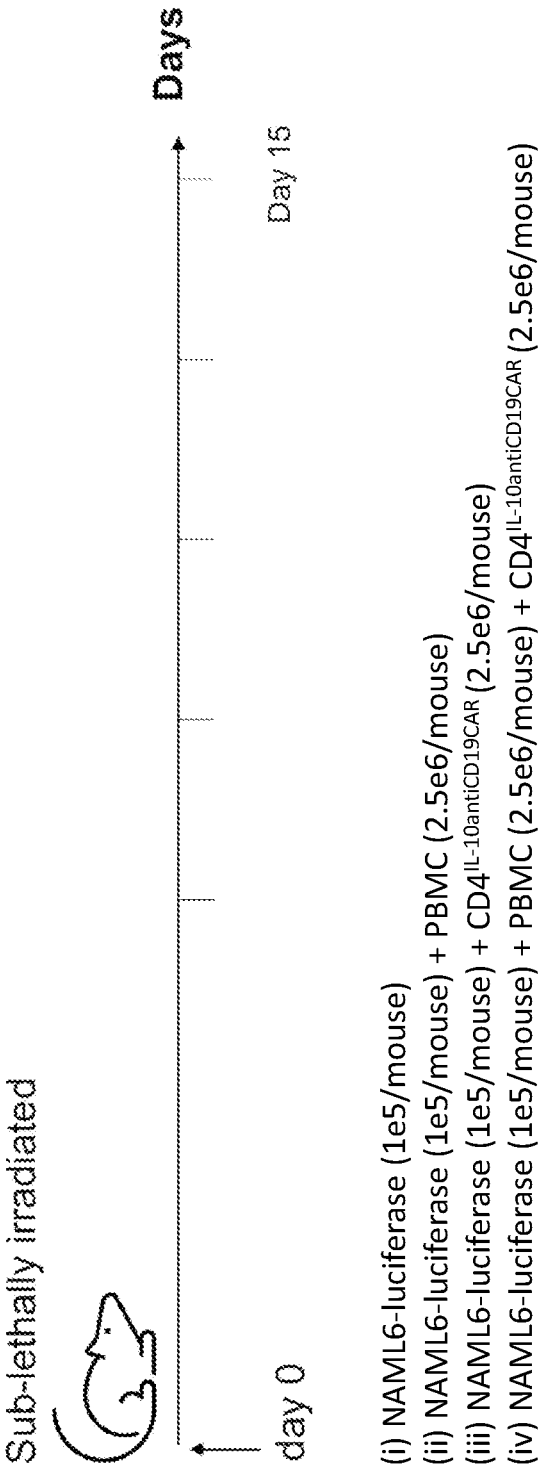

Sub-lethally irradiated
NSG
Days
day 0
Day 15
(i) NAML6-luciferase (1e5/mouse)
(ii) NAML6-luciferase (1e5/mouse) + PBMC (2.5e6/mouse)
(iii) NAML6-luciferase (1e5/mouse) + CD4IL-10antiCD19CAR (2.5e6/mouse)
(iv) NAML6-luciferase (1e5/mouse) + PBMC (2.5e6/mouse) + CD4IL-10antiCD19CAR (2.5e6/mouse)

FIG. 15

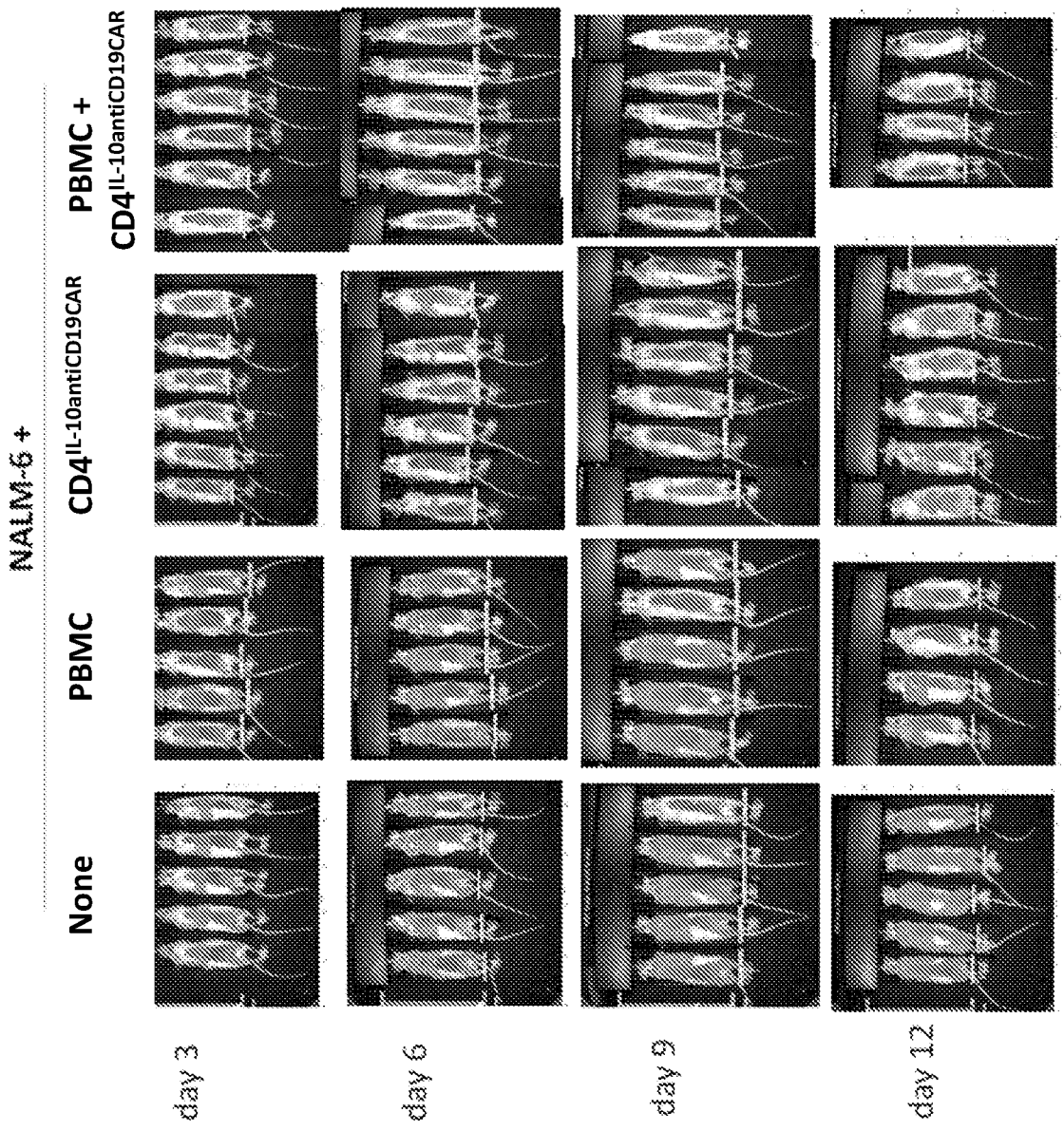

NALM-6 +
None
PBMC
CD4IL-10antiCD19CAR
PBMC + CD4IL-10antiCD19CAR
day 3
day 6
day 9
day 12

FIG. 16A

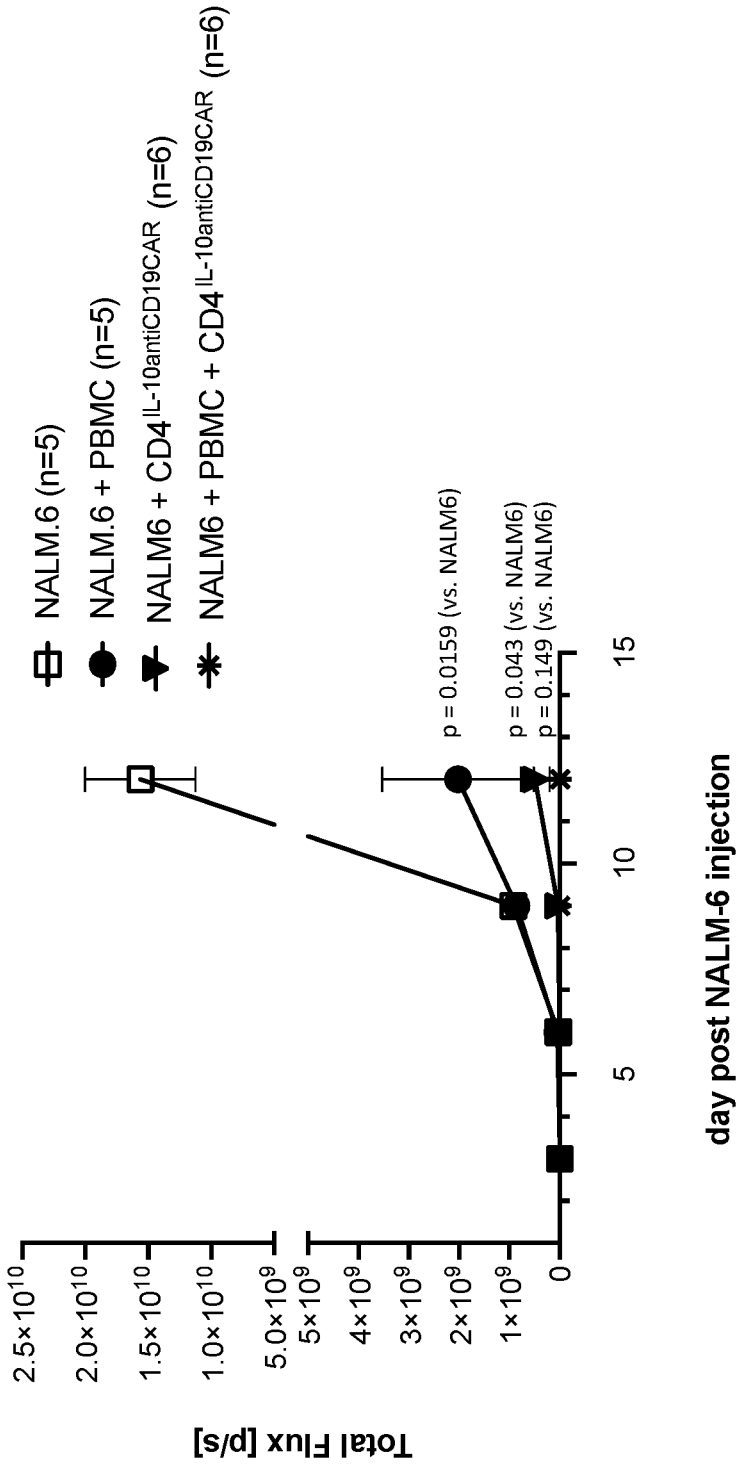

NALM.6 (n=5)
NALM.6 + PBMC (n=5)
NALM6 + CD4 IL-10antiCD19CAR (n=6)
NALM6 + PBMC + CD4 IL-10antiCD19CAR (n=6)
p = 0.0159 (vs. NALM6)
p = 0.043 (vs. NALM6)
p = 0.149 (vs. NALM6)
Total Flux [p/s]
2.5×10^10
2.0×10^10
1.5×10^10
1.0×10^10
5.0×10^9
5×10^9
4×10^9
3×10^9
2×10^9
1×10^9
0
5
10
15
day post NALM-6 injection

FIG. 16B

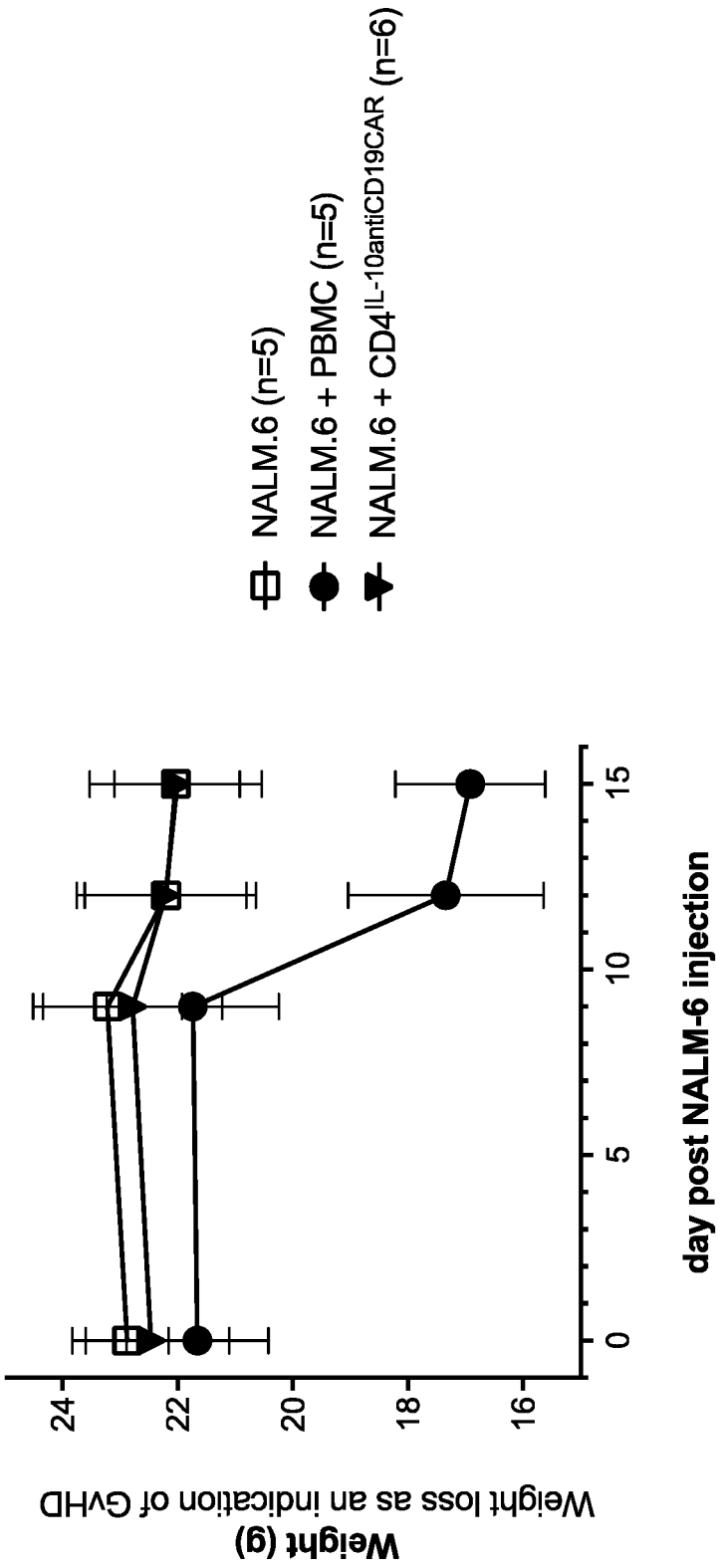

NALM.6 (n=5)
NALM.6 + PBMC (n=5)
NALM.6 + CD4IL-10antiCD19CAR (n=6)
Weight (g)
Weight loss as an indication of GvHD
24
22
20
18
16
0
5
10
15
day post NALM-6 injection

FIG. 17

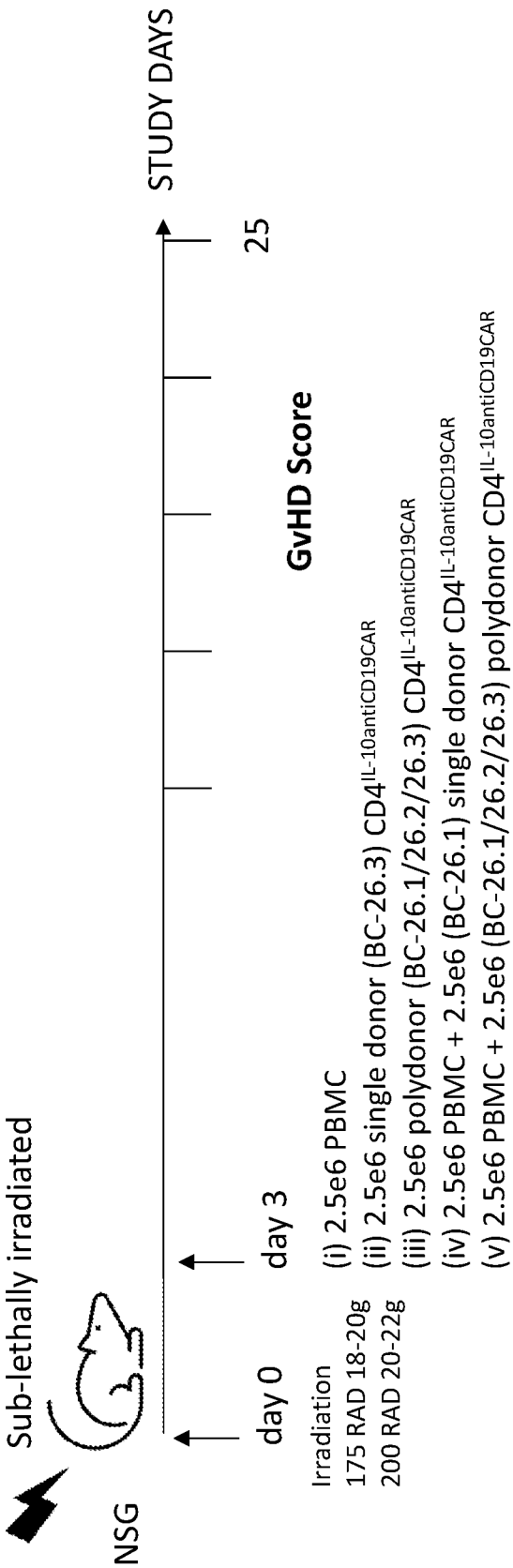

Sub-lethally irradiated
NSG
STUDY DAYS
day 0
day 3
25
Irradiation
175 RAD 18-20g
200 RAD 20-22g
GvHD Score
(i) 2.5e6 PBMC
(ii) 2.5e6 single donor (BC-26.3) CD4IL-10antiCD19CAR
(iii) 2.5e6 polydonor (BC-26.1/26.2/26.3) CD4IL-10antiCD19CAR
(iv) 2.5e6 PBMC + 2.5e6 (BC-26.1) single donor CD4IL-10antiCD19CAR
(v) 2.5e6 PBMC + 2.5e6 (BC-26.1/26.2/26.3) polydonor CD4IL-10antiCD19CAR

FIG. 18A

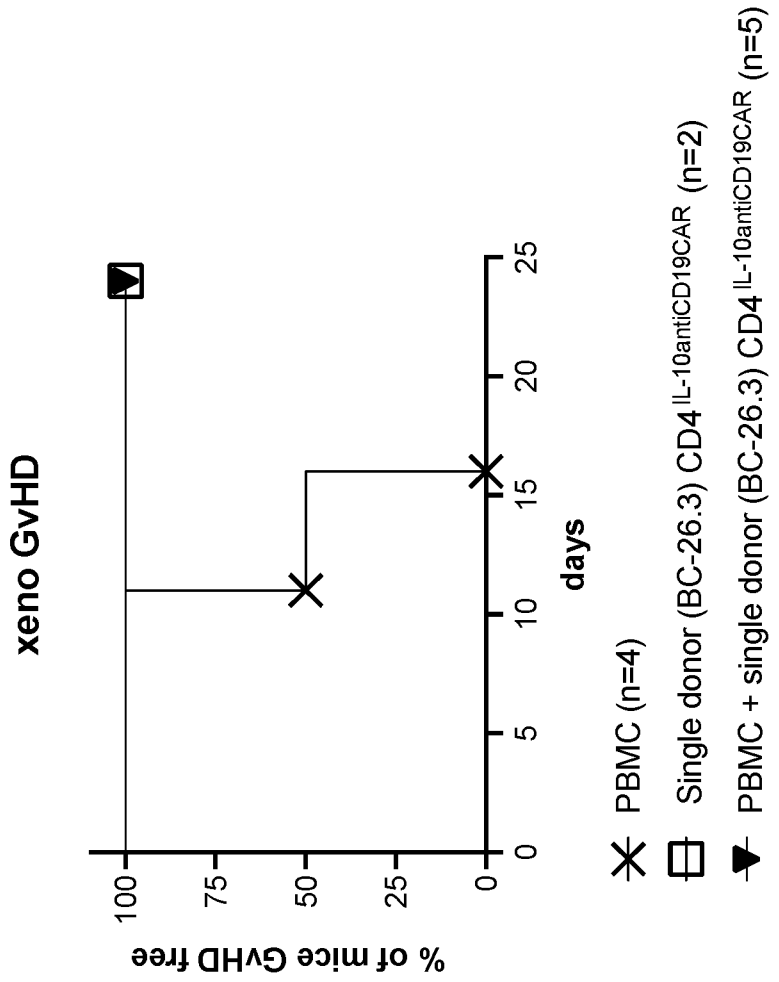

xeno GvHD
% of mice GvHD free
100
75
50
25
0
0
5
10
15
20
25
days
PBMC (n=4)
Single donor (BC-26.3) CD4 IL-10antiCD19CAR (n=2)
PBMC + single donor (BC-26.3) CD4 IL-10antiCD19CAR (n=5)

FIG. 18B

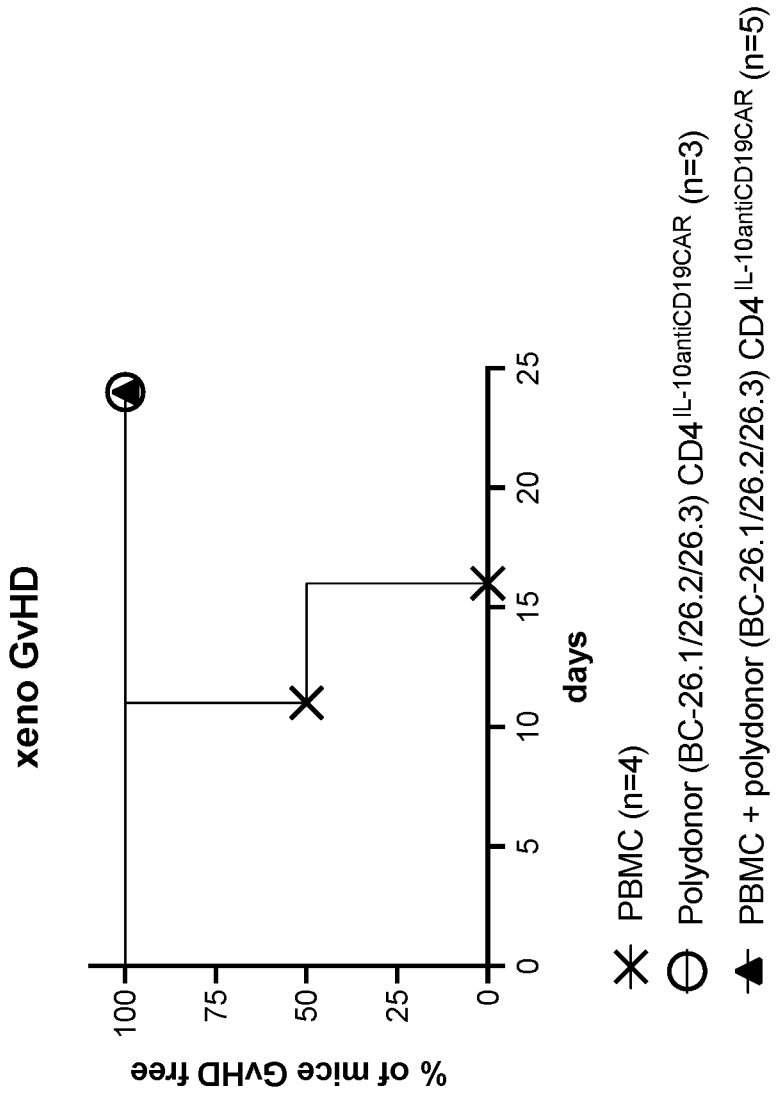

xeno GvHD
% of mice GvHD free
100
75
50
25
0
0
5
10
15
20
25
days
PBMC (n=4)
Polydonor (BC-26.1/26.2/26.3) CD4 IL-10antiCD19CAR (n=3)
PBMC + polydonor (BC-26.1/26.2/26.3) CD4 IL-10antiCD19CAR (n=5)

FIG. 18C

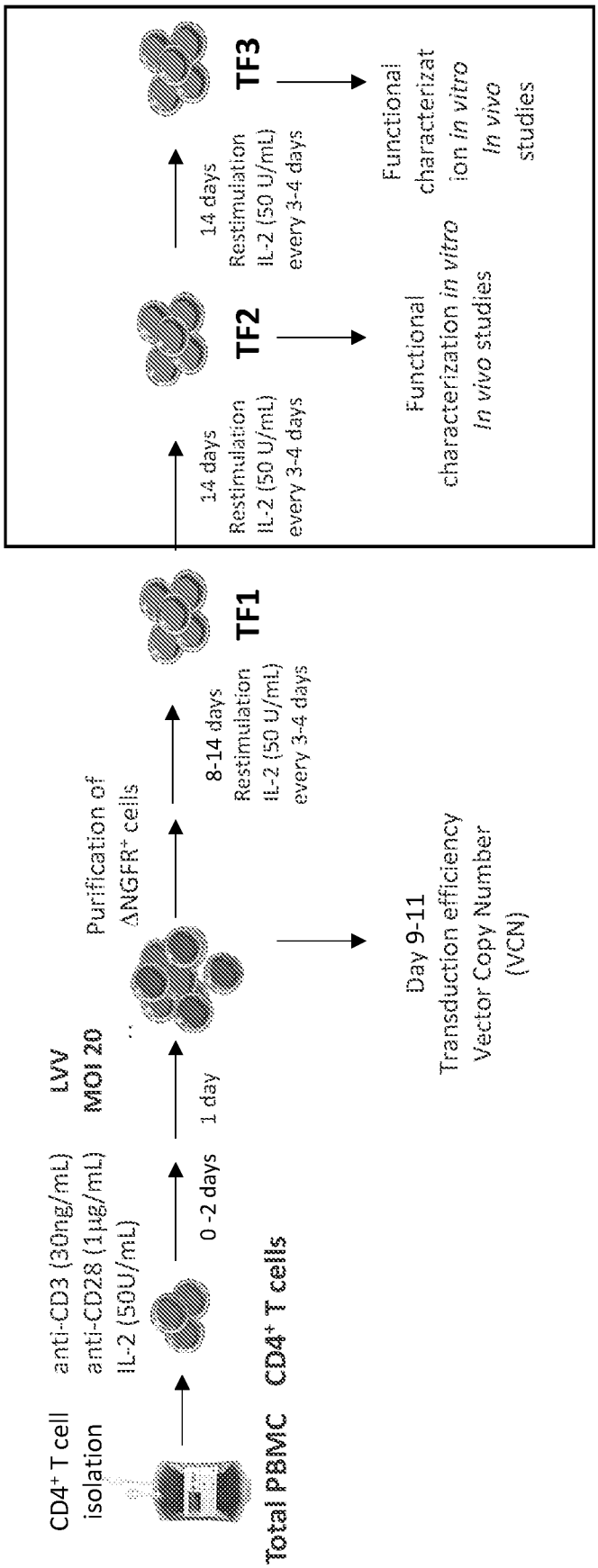

CD4+ T cell isolation
Total PBMC
anti-CD3 (30ng/mL)
anti-CD28 (1µg/mL)
IL-2 (50U/mL)
CD4+ T cells
0 -2 days
1 day
LVV
MOI 20
Purification of ΔNGFR+ cells
8-14 days
Restimulation
IL-2 (50 U/mL)
every 3-4 days
TF1
Day 9-11
Transduction efficiency
Vector Copy Number
(VCN)
14 days
Restimulation
IL-2 (50 U/mL)
every 3-4 days
TF2
Functional characterization in vitro
In vivo studies
14 days
Restimulation
IL-2 (50 U/mL)
every 3-4 days
TF3
Functional characterization in vitro
In vivo studies

FIG. 19

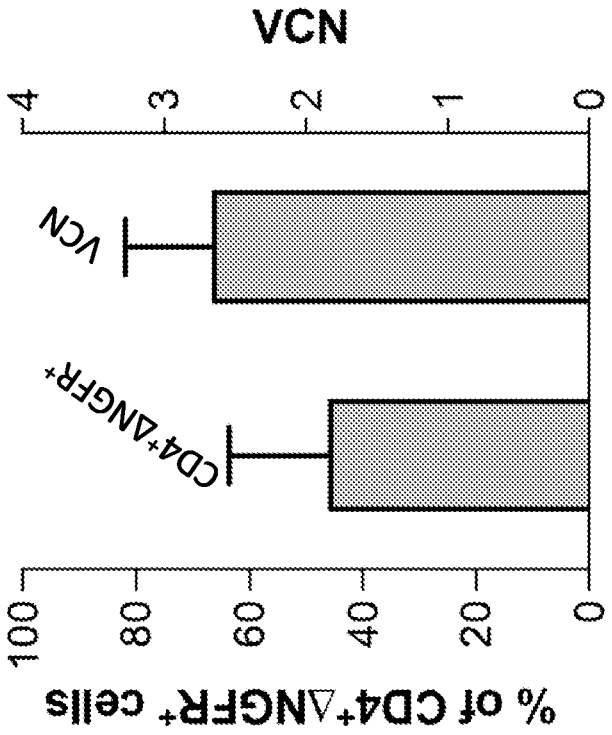


FIG. 20A

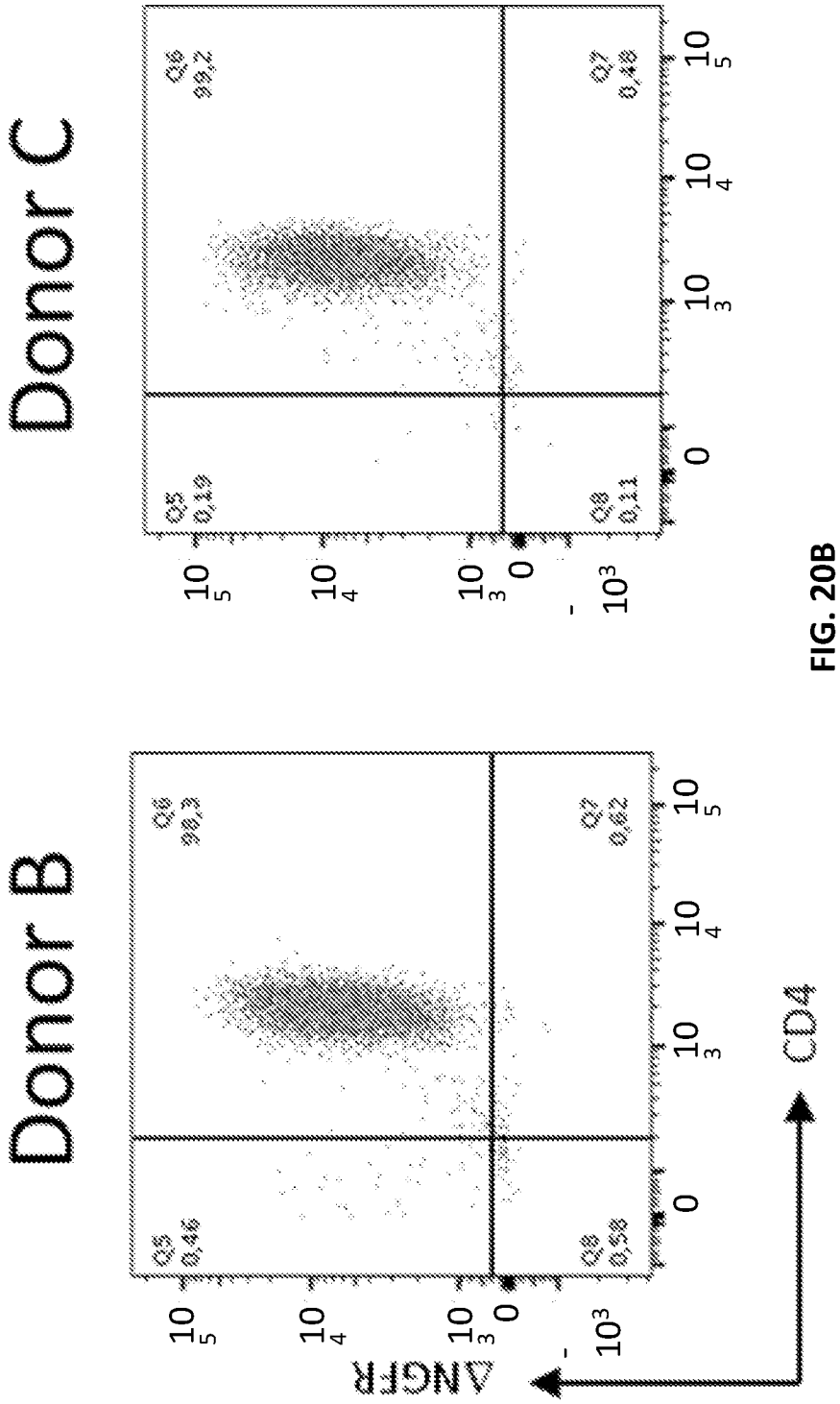

Donor C
Donor B
Q6
99,2
Q7
0,48
Q5
0,19
Q8
0,11
Q6
98,3
Q7
0,62
Q5
0,46
Q8
0,58
10 5
10 4
10 3
0
- 10³
ANGFR
CD4

FIG. 20B

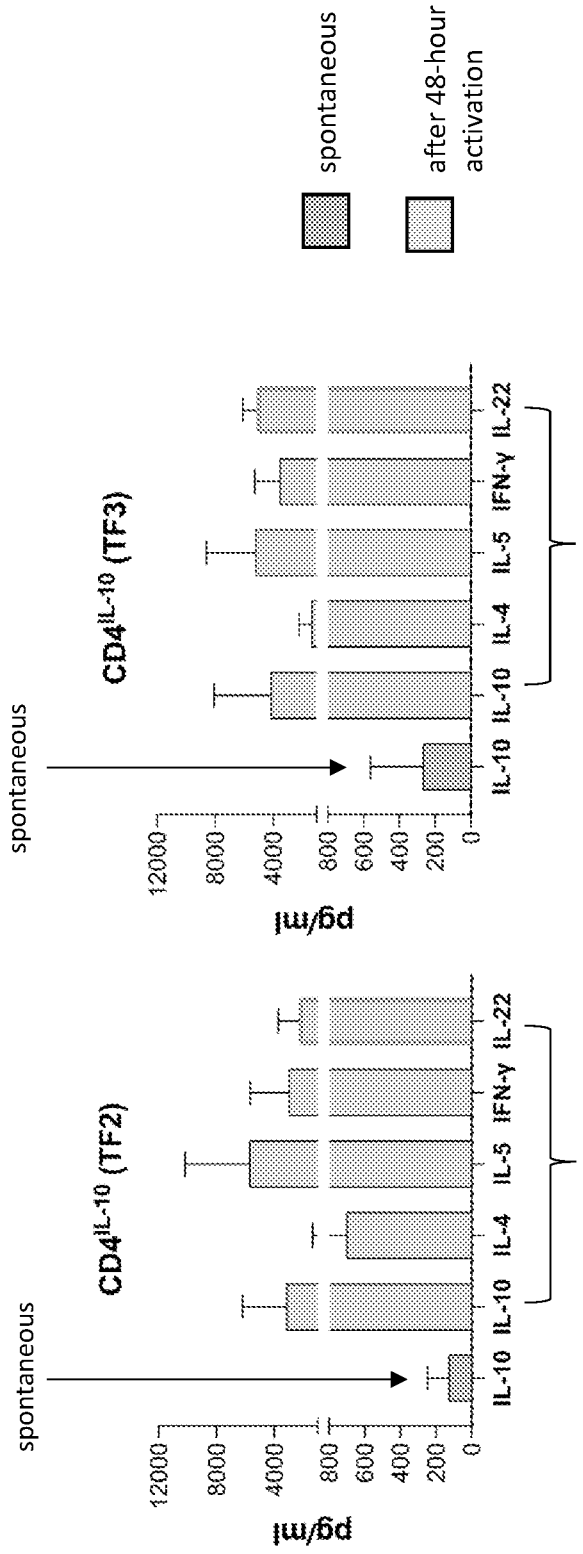

spontaneous
after 48-hour activation
CD4IL-10 (TF2)
CD4IL-10 (TF3)
pg/ml
12000
8000
4000
800
600
400
200
0
IL-10
IL-10
IL-4
IL-5
IFN-γ
IL-22

FIG. 21

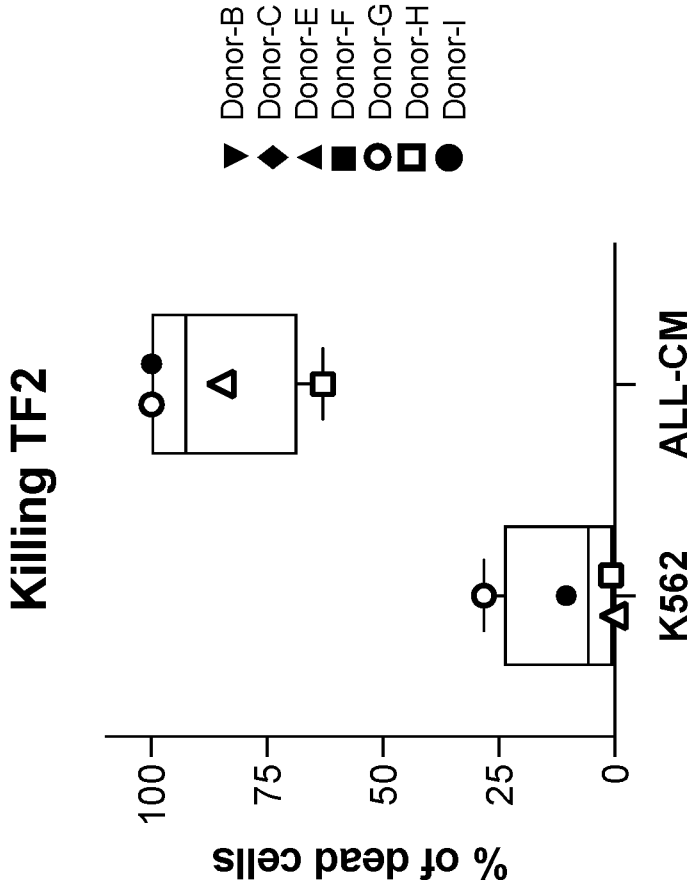


FIG. 22B

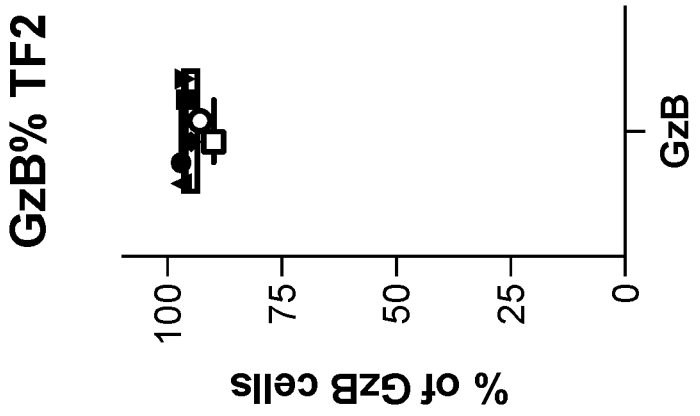


FIG. 22A

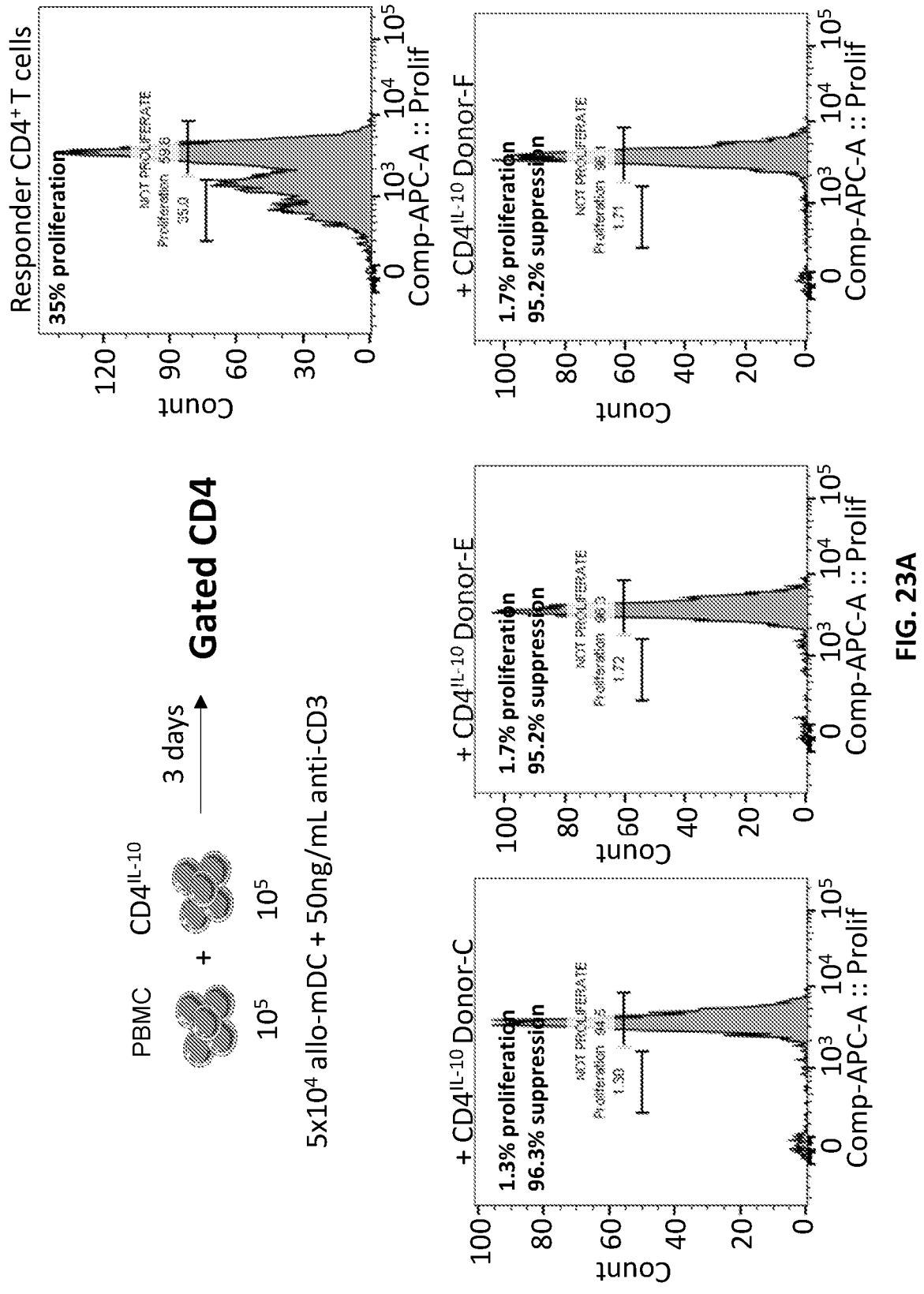

PBMC
CD4IL-10
+
10^5
10^5
5x10^4 allo-mDC + 50ng/mL anti-CD3
3 days
Gated CD4
Responder CD4+ T cells
35% proliferation
+ CD4IL-10 Donor-C
1.3% proliferation
96.3% suppression
+ CD4IL-10 Donor-E
1.7% proliferation
95.2% suppression
+ CD4IL-10 Donor-F
1.7% proliferation
95.2% suppression
Count
Comp-APC-A :: Prolif

FIG. 23A

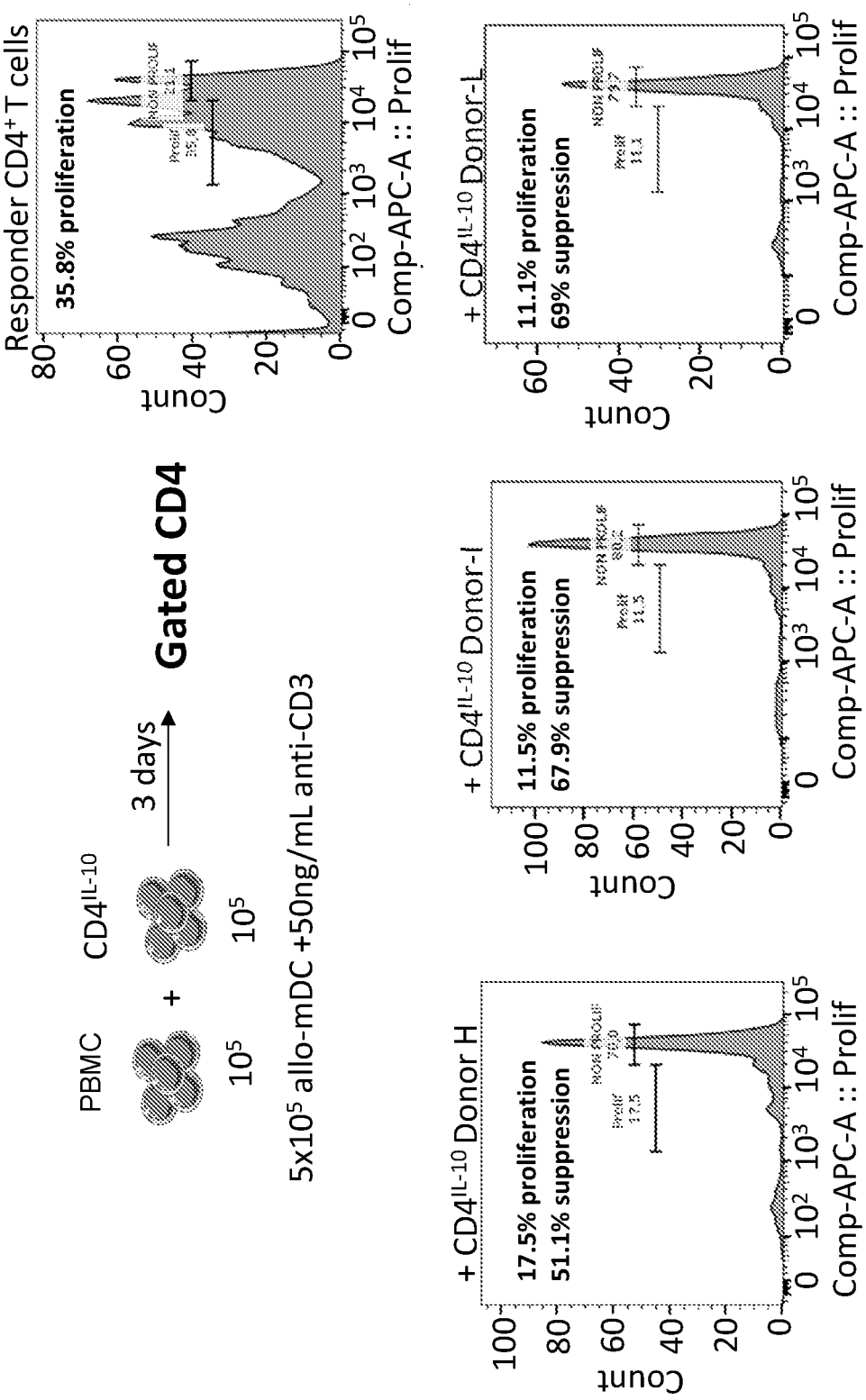

PBMC
CD4IL-10
+
3 days
Gated CD4
10^5
10^5
5x10^5 allo-mDC +50ng/mL anti-CD3
Responder CD4+ T cells
35.8% proliferation
+ CD4IL-10 Donor-L
11.1% proliferation
69% suppression
+ CD4IL-10 Donor-I
11.5% proliferation
67.9% suppression
+ CD4IL-10 Donor H
17.5% proliferation
51.1% suppression
Count
Comp-APC-A :: Prolif

FIG. 23B

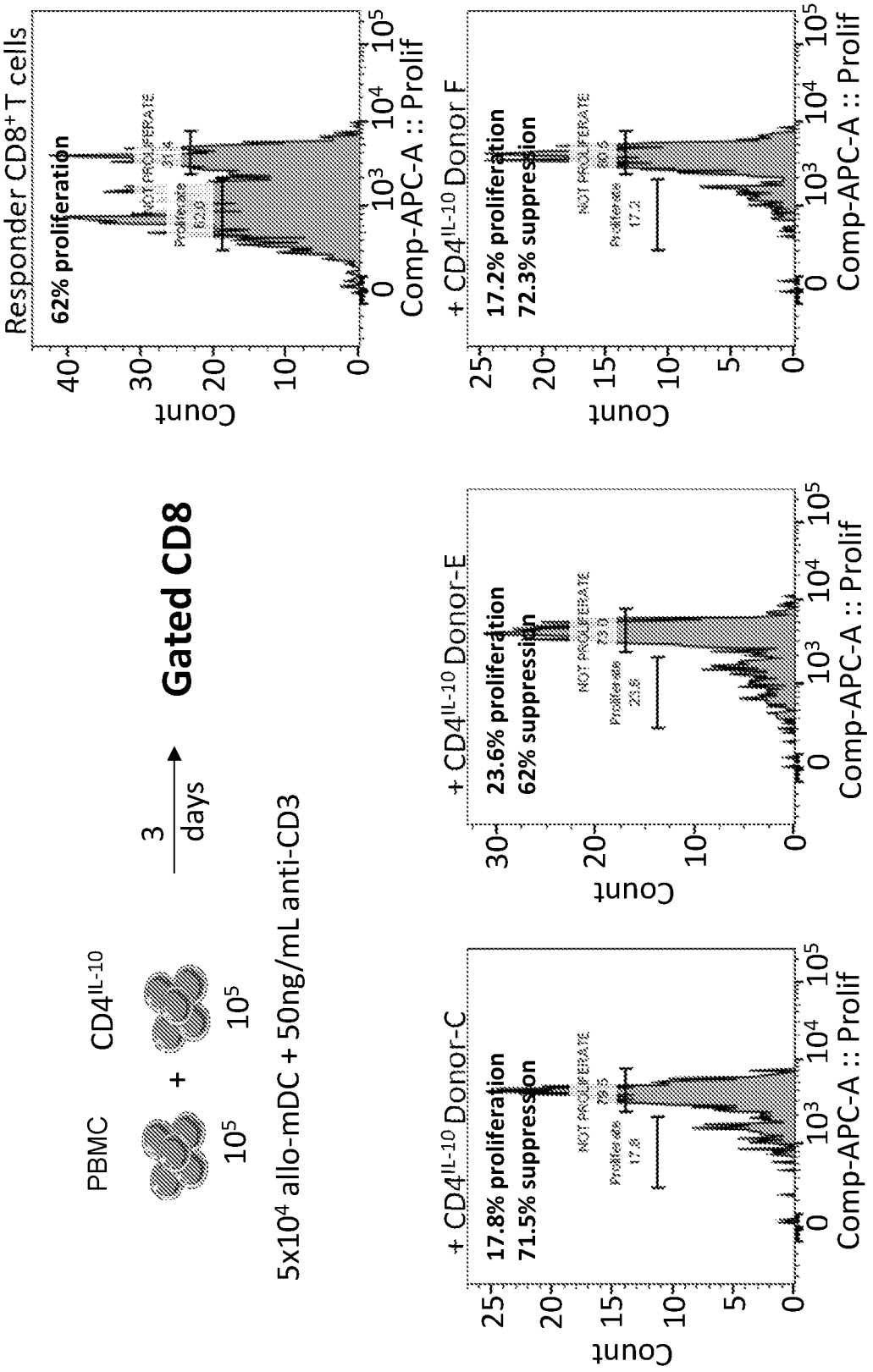

PBMC
CD4IL-10
+
10^5
10^5
5x10^4 allo-mDC + 50ng/mL anti-CD3
3
days
Gated CD8
Responder CD8+ T cells
62% proliferation
NOT PROLIFERATE
21.4
Proliferate
62.0
Count
Comp-APC-A :: Prolif
+ CD4IL-10 Donor F
17.2% proliferation
72.3% suppression
NOT PROLIFERATE
80.6
Proliferate
17.2
+ CD4IL-10 Donor-E
23.6% proliferation
62% suppression
NOT PROLIFERATE
76.0
Proliferate
23.8
+ CD4IL-10 Donor-C
17.8% proliferation
71.5% suppression
NOT PROLIFERATE
78.5
Proliferate
17.8

FIG. 24A

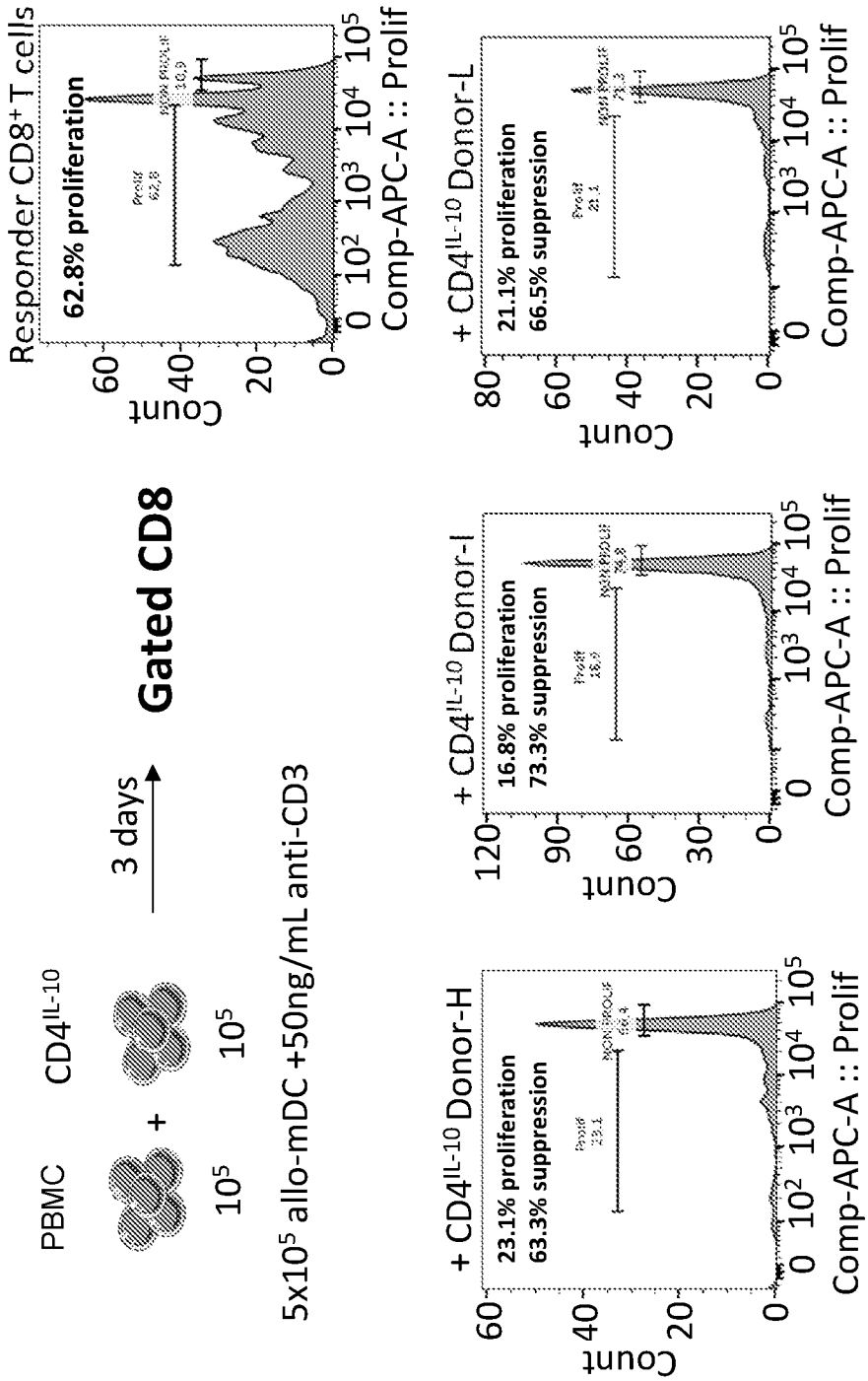

PBMC
CD4IL-10
+
10^5
10^5
5x10^5 allo-mDC +50ng/mL anti-CD3
3 days
Gated CD8
Responder CD8+ T cells
62.8% proliferation
Count
Comp-APC-A :: Prolif
+ CD4IL-10 Donor-L
21.1% proliferation
66.5% suppression
+ CD4IL-10 Donor-I
16.8% proliferation
73.3% suppression
+ CD4IL-10 Donor-H
23.1% proliferation
63.3% suppression

FIG. 24B

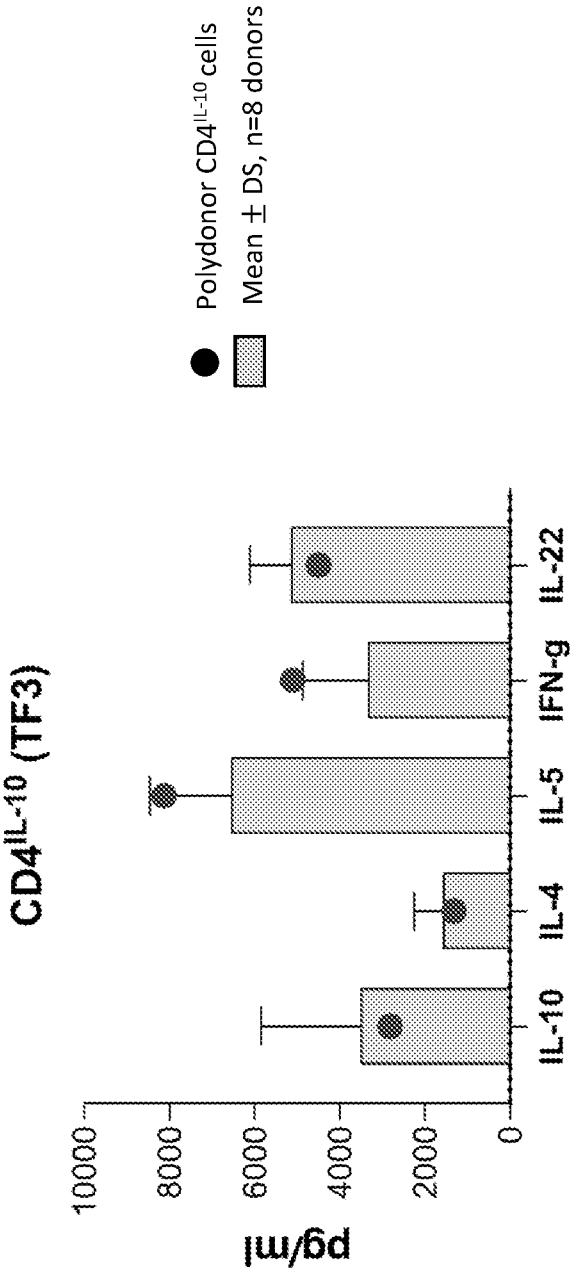

Polydonor CD4IL-10 cells
Mean ± DS, n=8 donors
CD4IL-10 (TF3)
pg/ml
10000
8000
6000
4000
2000
0
IL-10
IL-4
IL-5
IFN-g
IL-22

FIG. 25

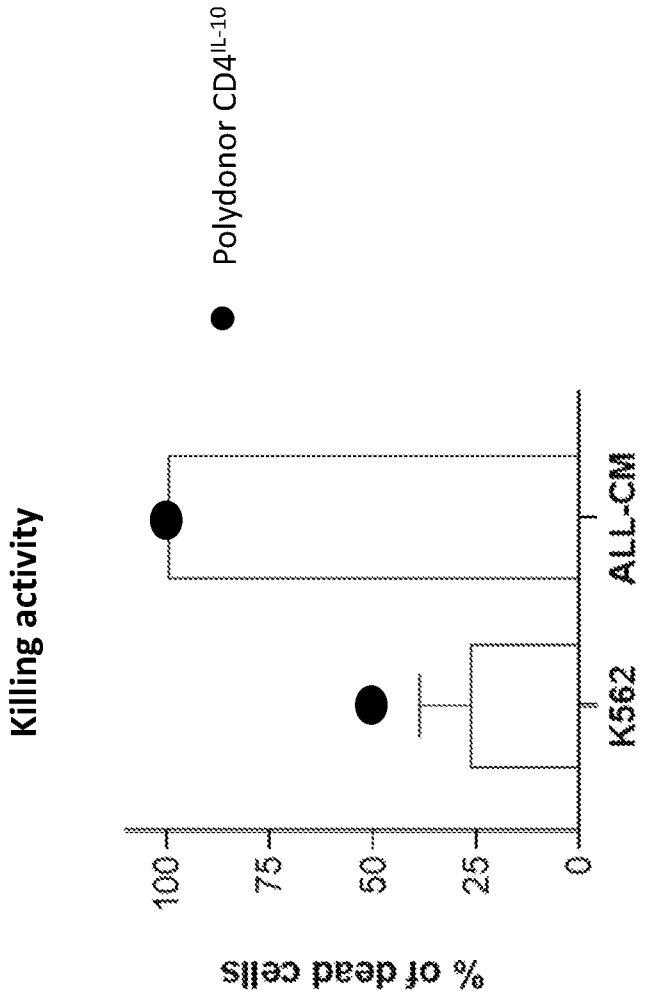


FIG. 26B

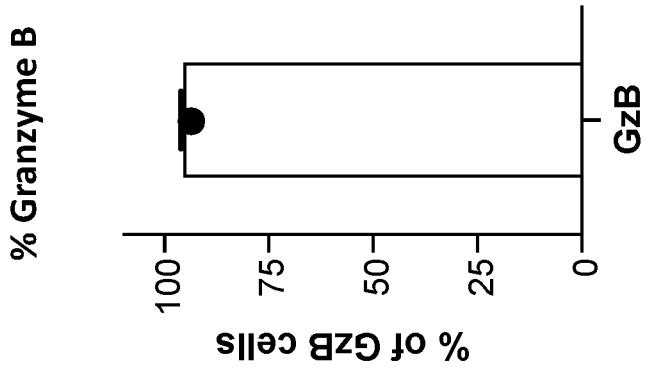


FIG. 26A

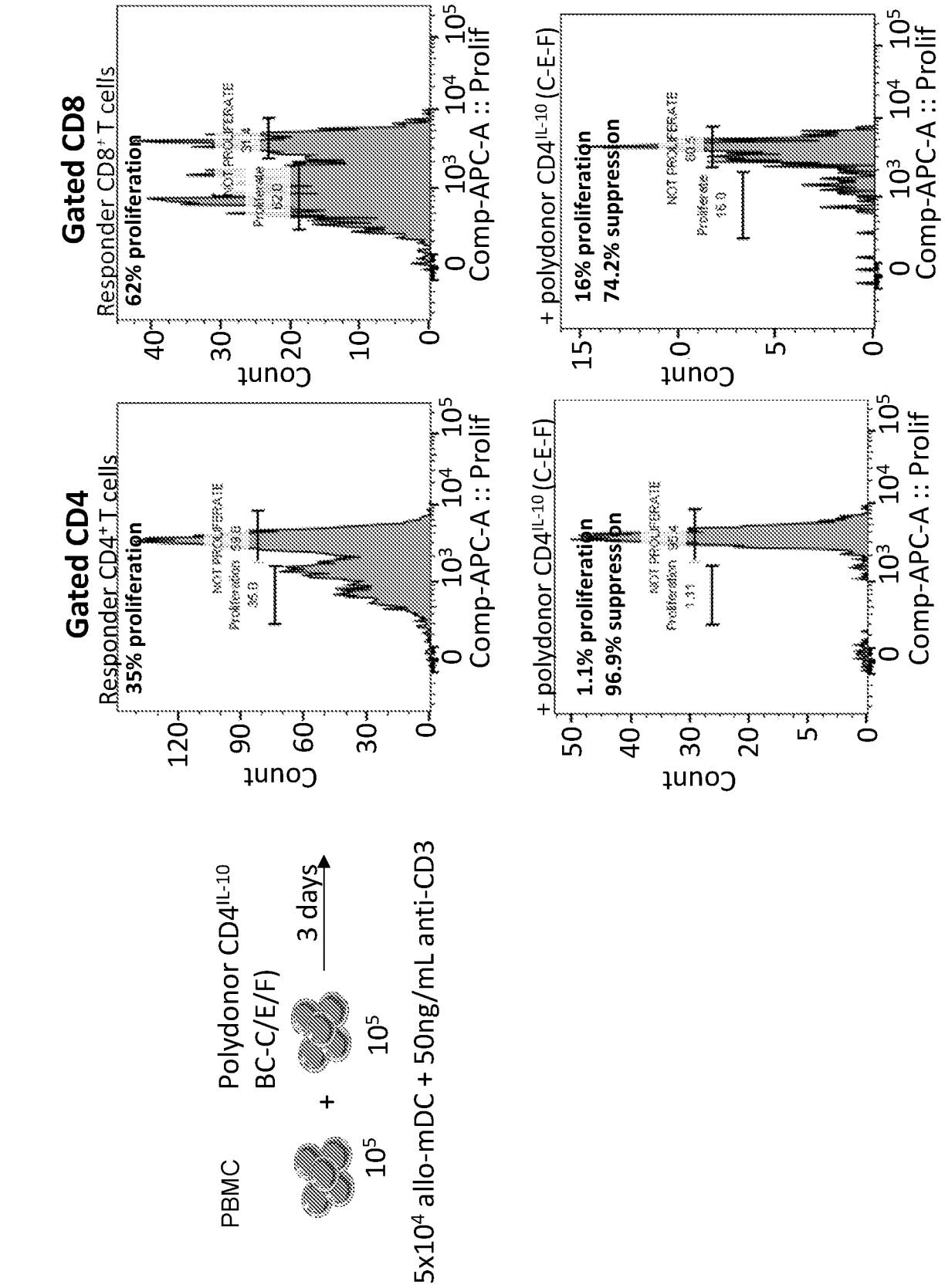

PBMC
Polydonor CD4IL-10
BC-C/E/F)
3 days
+
10^5
10^5
5x10^4 allo-mDC + 50ng/mL anti-CD3
Gated CD4
Responder CD4+ T cells
35% proliferation
Gated CD8
Responder CD8+ T cells
62% proliferation
+ polydonor CD4IL-10 (C-E-F)
1.1% proliferation
96.9% suppression
+ polydonor CD4IL-10 (C-E-F)
16% proliferation
74.2% suppression
Count
Comp-APC-A :: Prolif

FIG. 27A

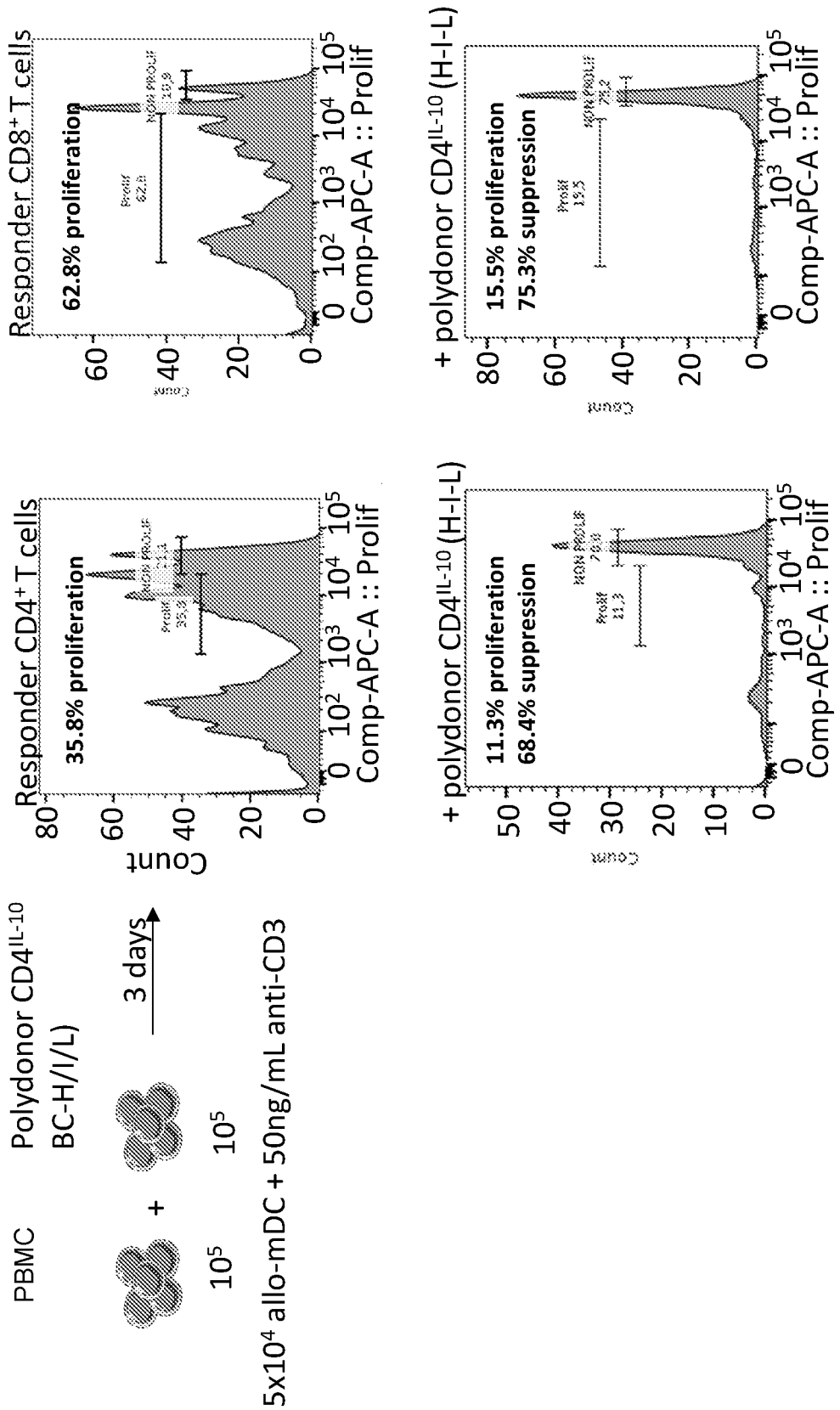

Gated CD4
Responder CD4+ T cells
35.8% proliferation
Gated CD8
Responder CD8+ T cells
62.8% proliferation
+ polydonor CD4IL-10 (H-I-L)
11.3% proliferation
68.4% suppression
+ polydonor CD4IL-10 (H-I-L)
15.5% proliferation
75.3% suppression
Count
Comp-APC-A :: Prolif
PBMC
Polydonor CD4IL-10
BC-H/I/L)
3 days
10^5
10^5
5x10^4 allo-mDC + 50ng/mL anti-CD3

FIG. 27B

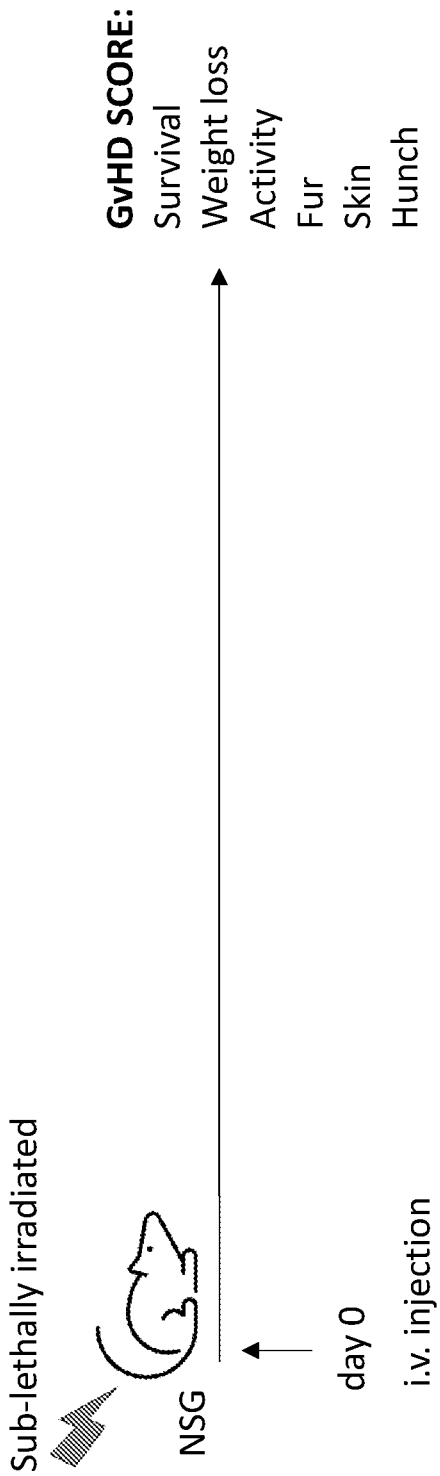

Sub-lethally irradiated
NSG
day 0
i.v. injection
5x10^6 PBMC (n=8)
5x10^6 PBMC + 5x10^6 polydonor CD4^IL-10 (BC-C/E/F) (n=8)
5x10^6 Polydonor CD4^IL-10 (BC-C/E/F) (n=8)
GvHD SCORE:
Survival
Weight loss
Activity
Fur
Skin
Hunch

FIG. 28

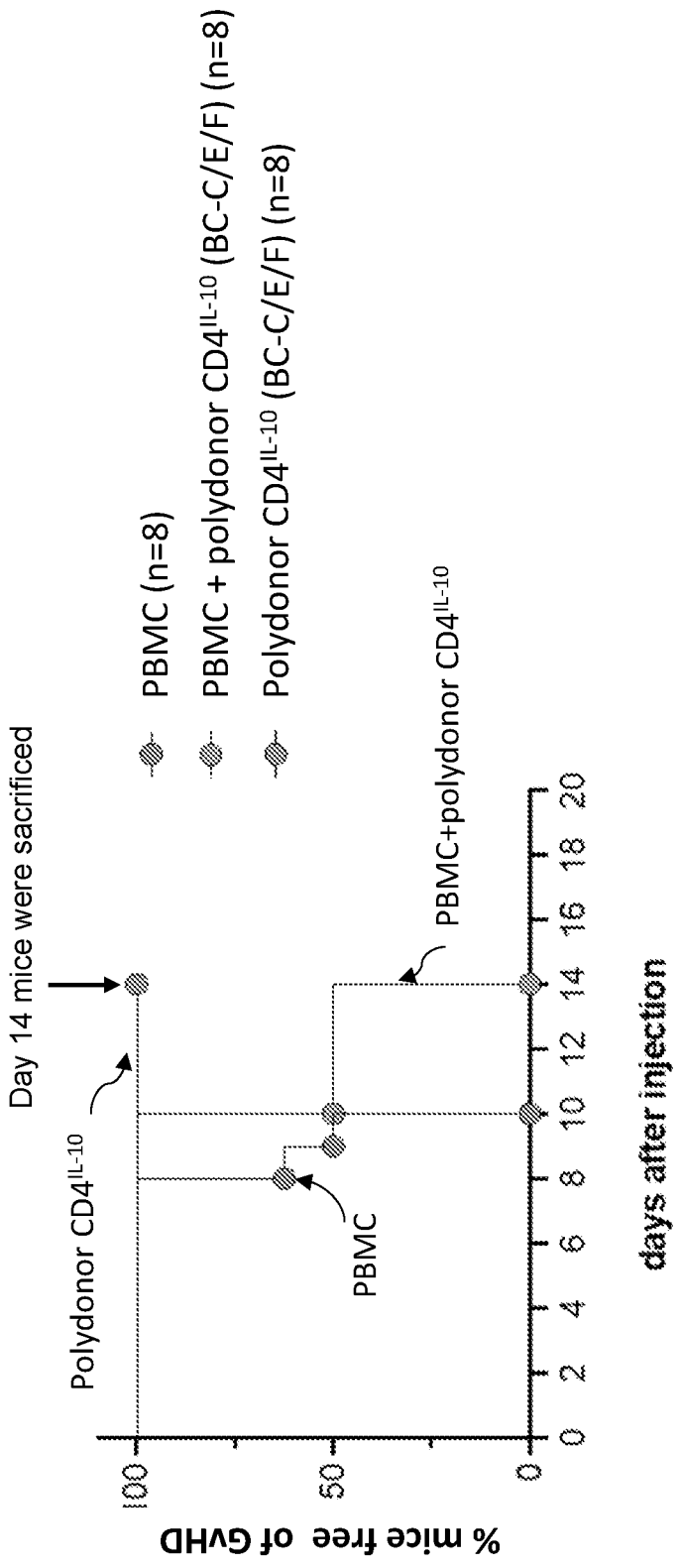

PBMC (n=8)
PBMC + polydonor CD4IL-10 (BC-C/E/F) (n=8)
Polydonor CD4IL-10 (BC-C/E/F) (n=8)
Day 14 mice were sacrificed
Polydonor CD4IL-10
PBMC
PBMC+polydonor CD4IL-10
% mice free of GvHD
100
50
0
0 2 4 6 8 10 12 14 16 18 20
days after injection

FIG. 29

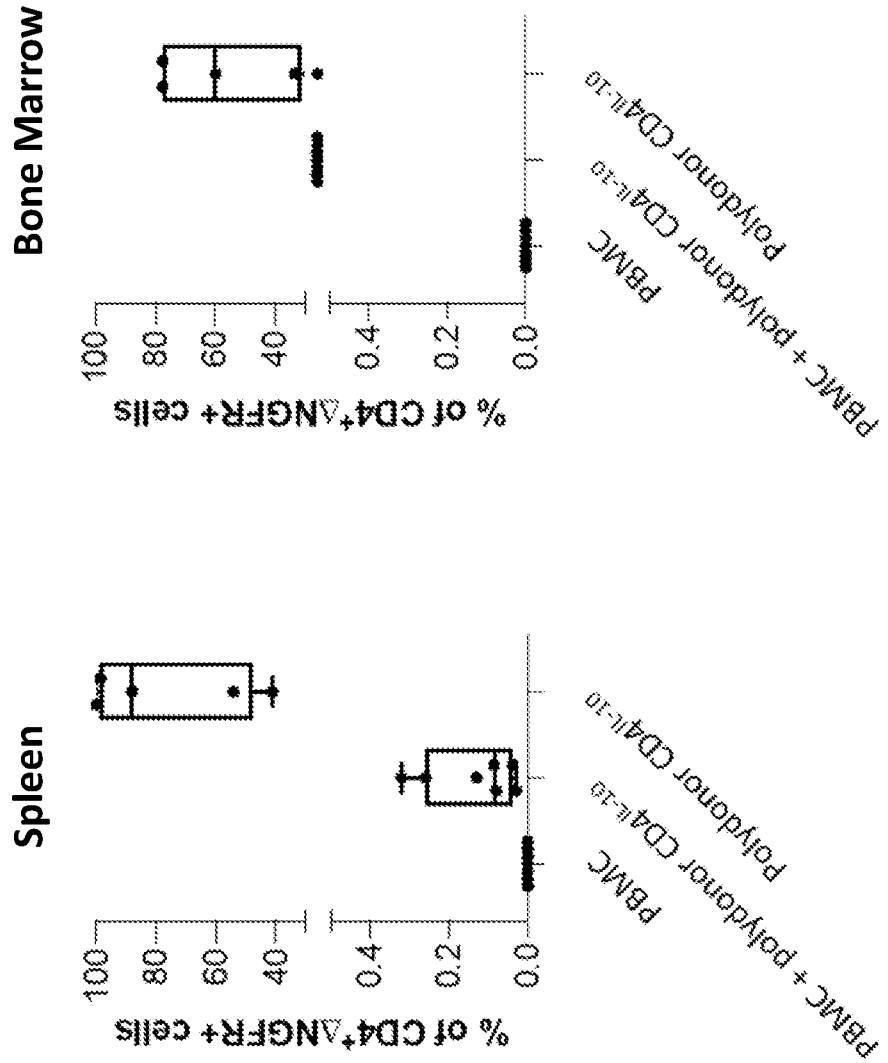

Bone Marrow
% of CD4+ΔNGFR+ cells
100
80
60
40
0.4
0.2
0.0
PBMC
PBMC + polydonor CD4IL-10
Polydonor CD4IL-10
Spleen
% of CD4+ΔNGFR+ cells
100
80
60
40
0.4
0.2
0.0
PBMC
PBMC + polydonor CD4IL-10
Polydonor CD4IL-10

FIG. 30

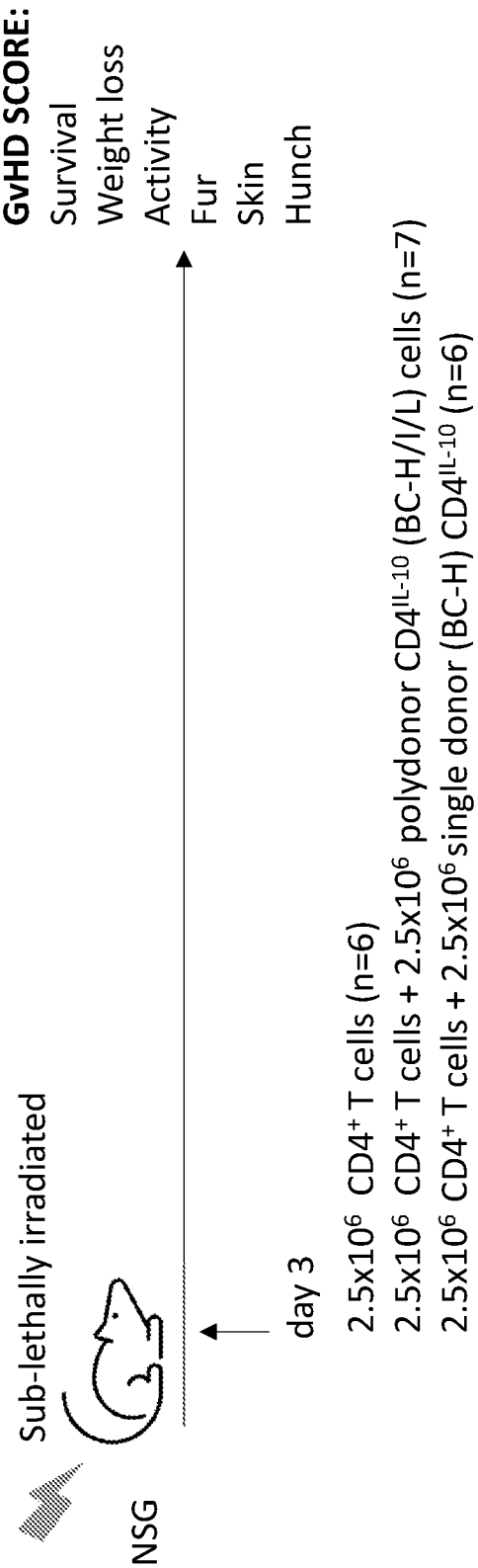

GvHD SCORE:
Survival
Weight loss
Activity
Fur
Skin
Hunch
Sub-lethally irradiated
NSG
day 3
2.5x10^6 CD4+ T cells (n=6)
2.5x10^6 CD4+ T cells + 2.5x10^6 polydonor CD4IL-10 (BC-H/I/L) cells (n=7)
2.5x10^6 CD4+ T cells + 2.5x10^6 single donor (BC-H) CD4IL-10 (n=6)

FIG. 31

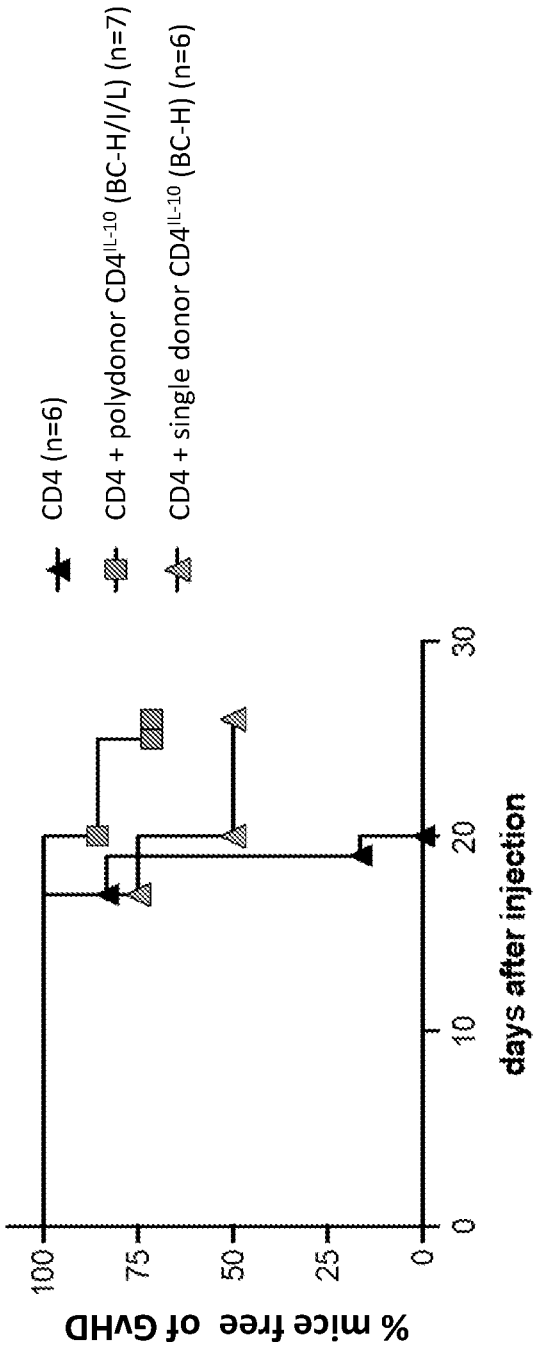

CD4 (n=6)
CD4 + polydonor CD4IL-10 (BC-H/I/L) (n=7)
CD4 + single donor CD4IL-10 (BC-H) (n=6)
% mice free of GvHD
100
75
50
25
0
0
10
20
30
days after injection

FIG. 32

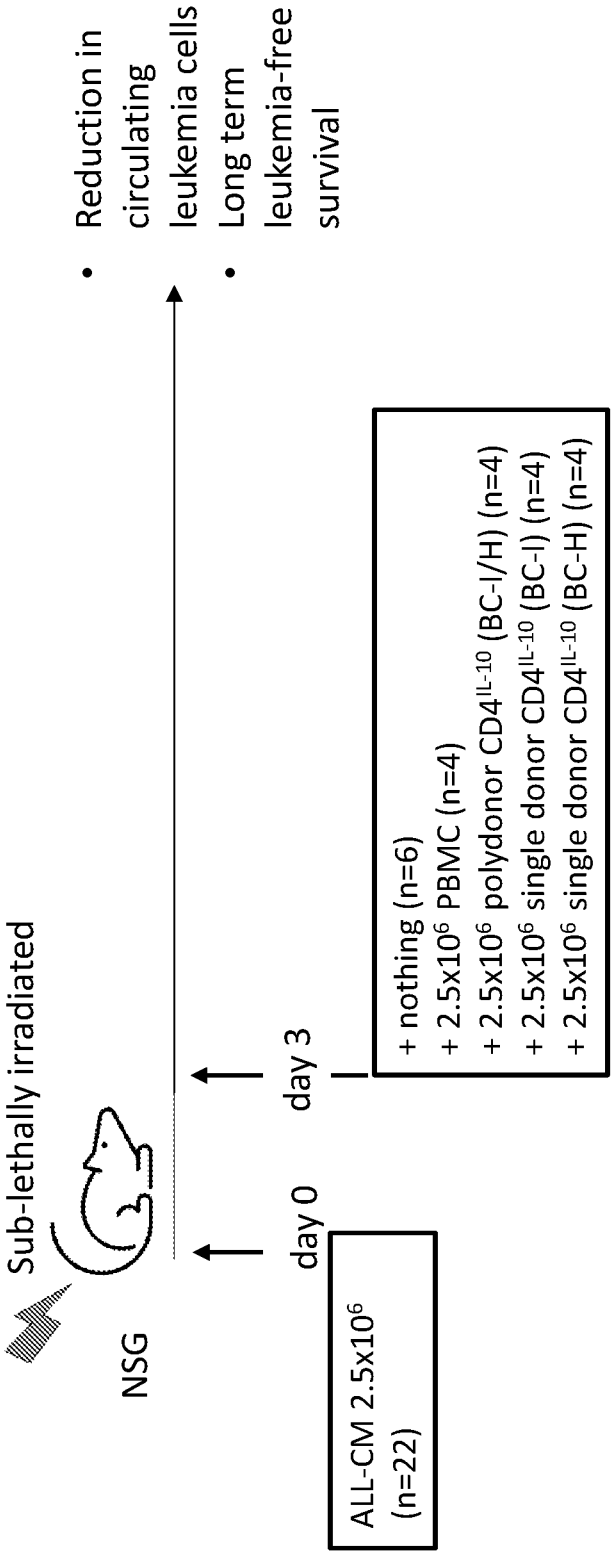

Sub-lethally irradiated
NSG
day 0
day 3
ALL-CM 2.5x10^6 (n=22)
+ nothing (n=6)
+ 2.5x10^6 PBMC (n=4)
+ 2.5x10^6 polydonor CD4^IL-10 (BC-I/H) (n=4)
+ 2.5x10^6 single donor CD4^IL-10 (BC-I) (n=4)
+ 2.5x10^6 single donor CD4^IL-10 (BC-H) (n=4)
• Reduction in circulating leukemia cells
• Long term leukemia-free survival

FIG. 33A

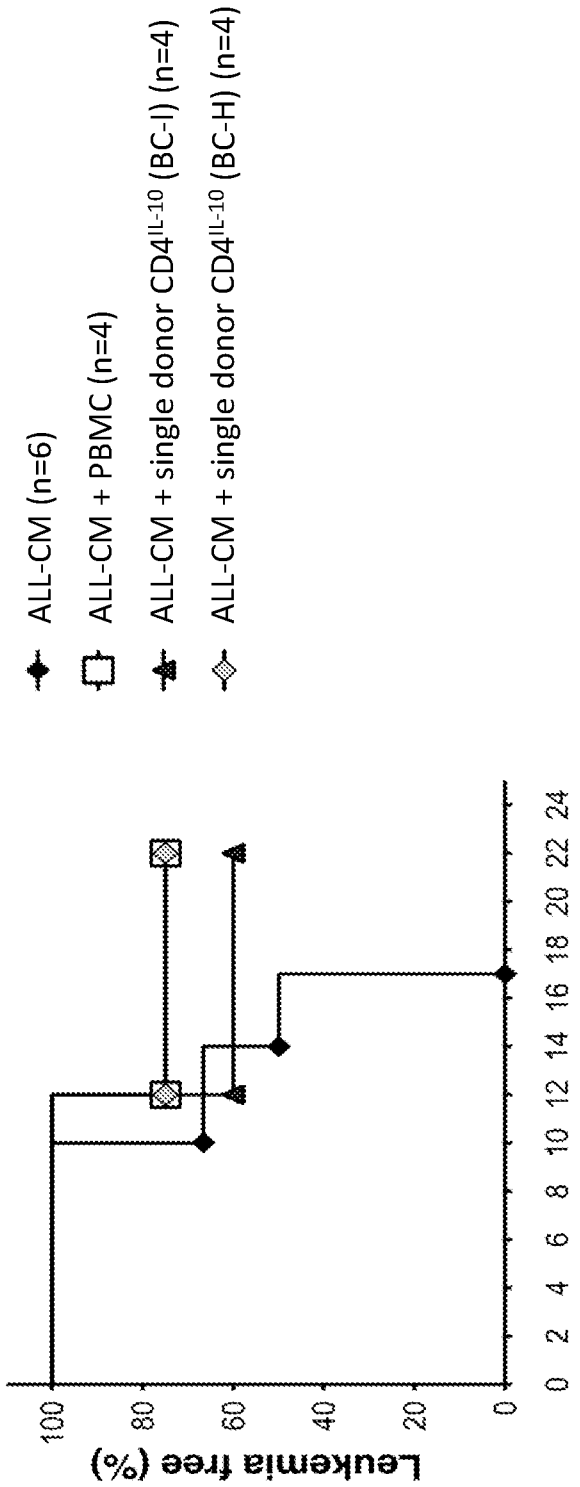

ALL-CM (n=6)
ALL-CM + PBMC (n=4)
ALL-CM + single donor CD4IL-10 (BC-I) (n=4)
ALL-CM + single donor CD4IL-10 (BC-H) (n=4)
Leukemia free (%)
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Days after ALL-CM Injection

FIG. 33B

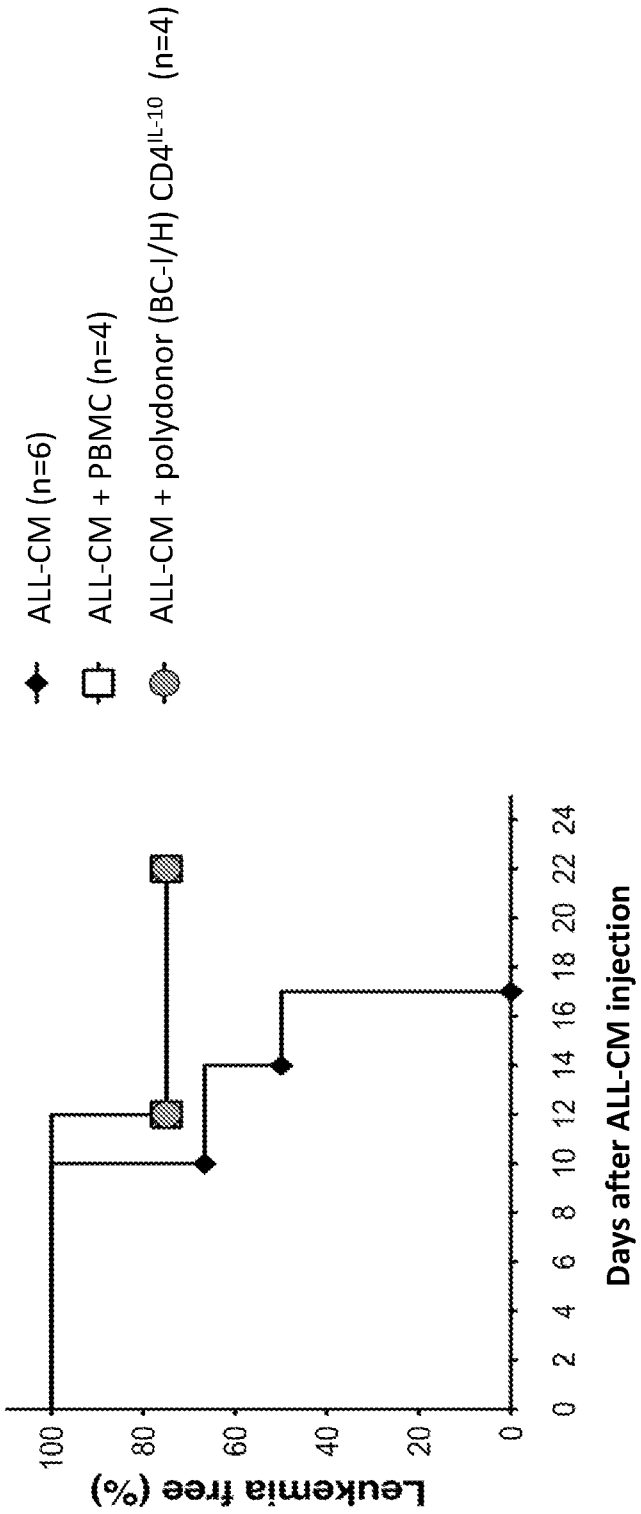

ALL-CM (n=6)
ALL-CM + PBMC (n=4)
ALL-CM + polydonor (BC-I/H) CD4IL-10 (n=4)
Leukemia free (%)
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Days after ALL-CM injection

FIG. 33C

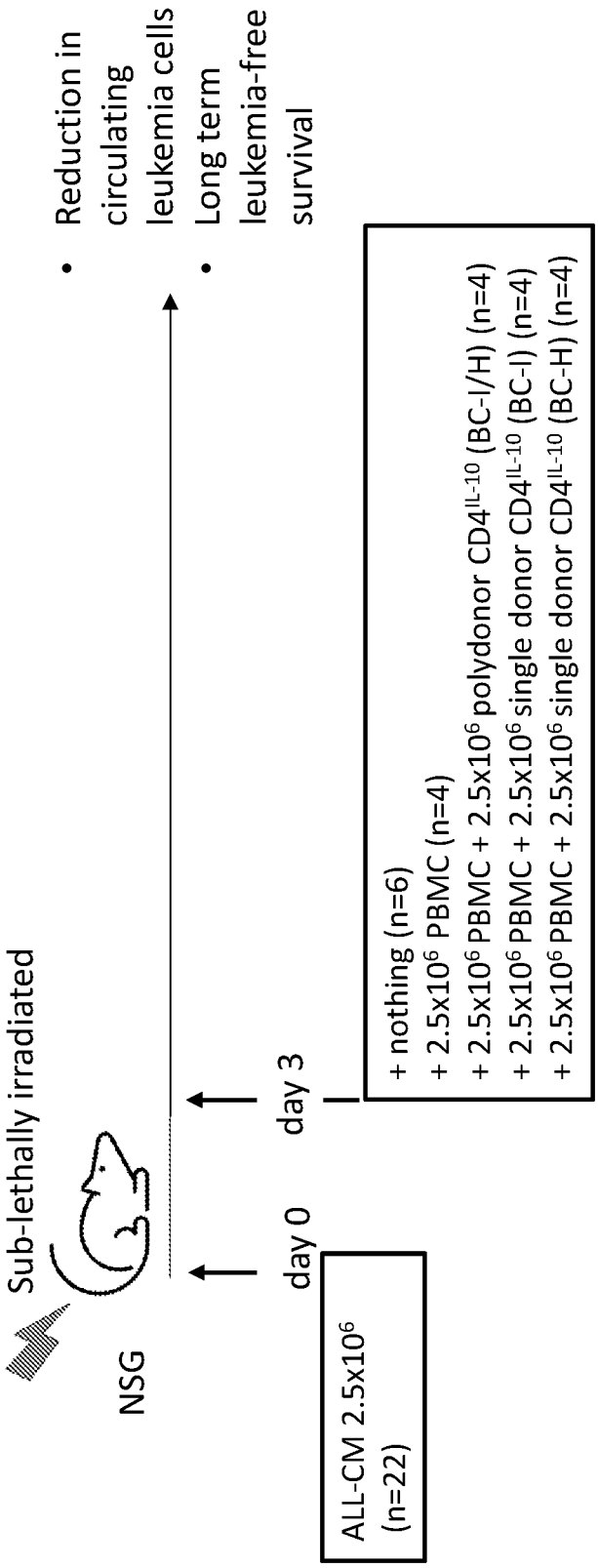

Sub-lethally irradiated
NSG
ALL-CM 2.5x10^6 (n=22)
day 0
day 3
+ nothing (n=6)
+ 2.5x10^6 PBMC (n=4)
+ 2.5x10^6 PBMC + 2.5x10^6 polydonor CD4^IL-10 (BC-I/H) (n=4)
+ 2.5x10^6 PBMC + 2.5x10^6 single donor CD4^IL-10 (BC-I) (n=4)
+ 2.5x10^6 PBMC + 2.5x10^6 single donor CD4^IL-10 (BC-H) (n=4)
• Reduction in circulating leukemia cells
• Long term leukemia-free survival

FIG. 34A

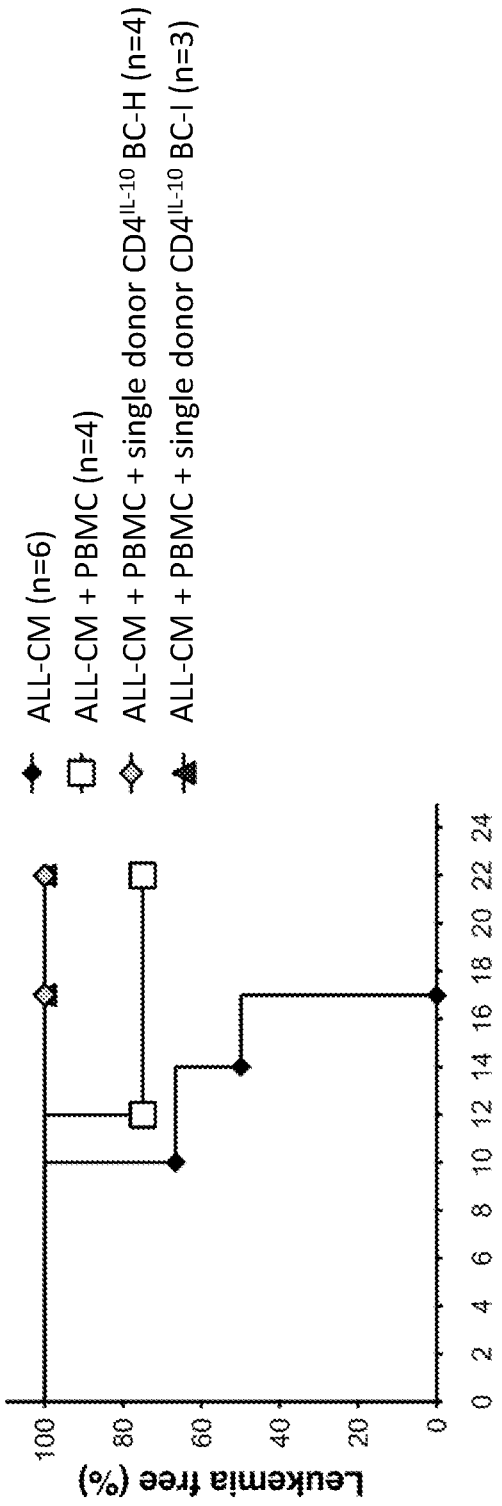

ALL-CM (n=6)
ALL-CM + PBMC (n=4)
ALL-CM + PBMC + single donor CD4IL-10 BC-H (n=4)
ALL-CM + PBMC + single donor CD4IL-10 BC-I (n=3)
Leukemia free (%)
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Days after ALL-CM injection

FIG. 34B

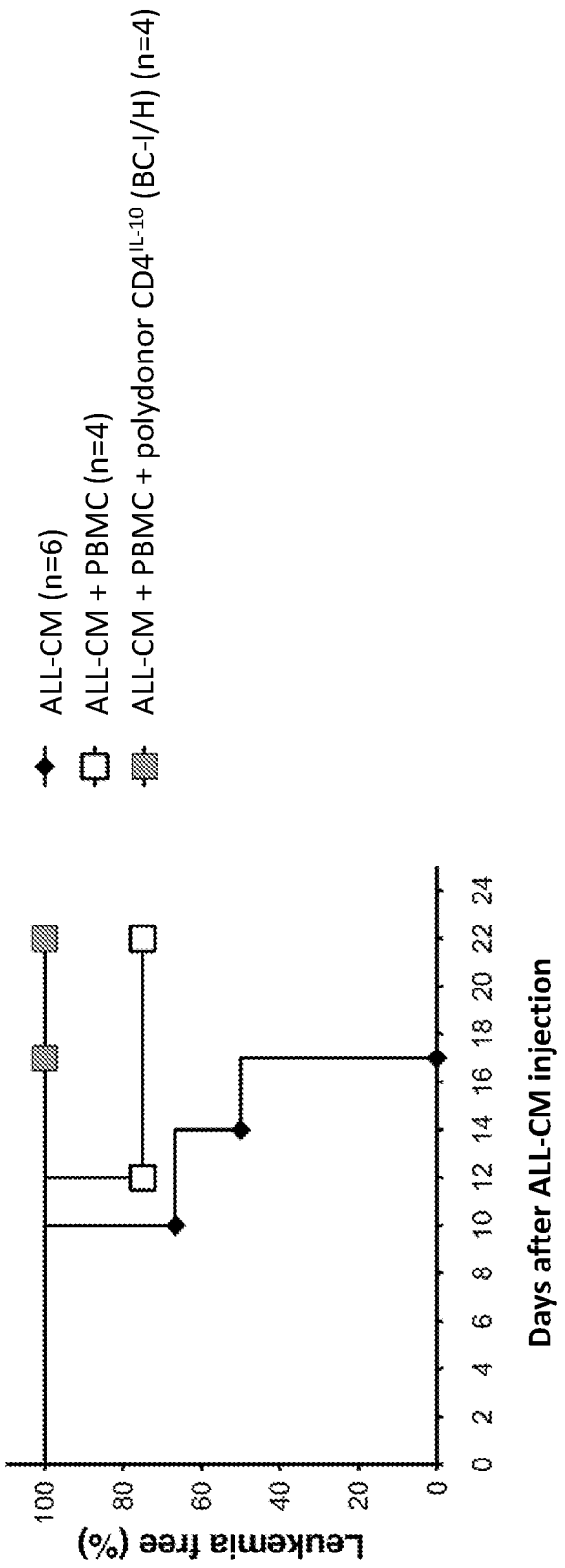

ALL-CM (n=6)
ALL-CM + PBMC (n=4)
ALL-CM + PBMC + polydonor CD4IL-10 (BC-I/H) (n=4)
Leukemia free (%)
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Days after ALL-CM injection

FIG. 34C

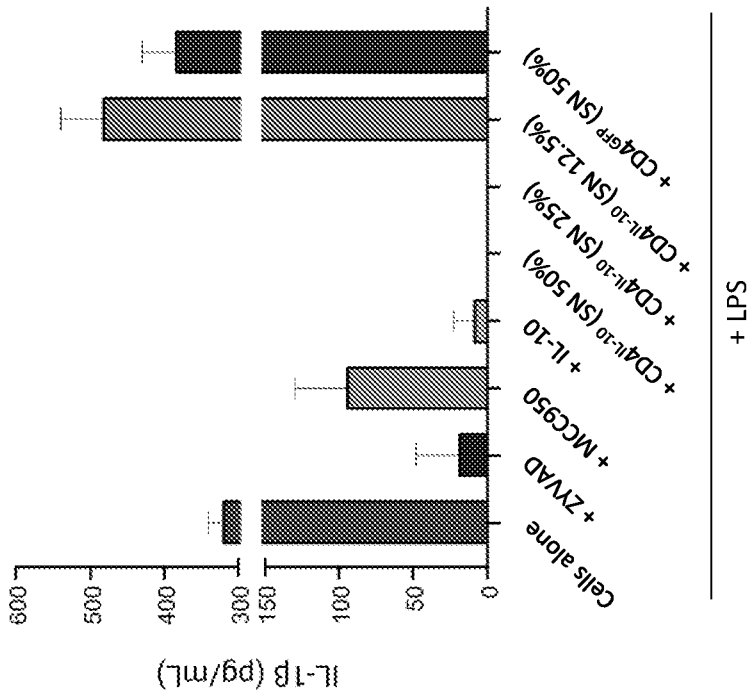

IL-1β (pg/mL)
600
500
400
300
150
100
50
0
Cells alone
+ ZVAD
+ MCC950
+ IL-10
+ CD4IL-10 (SN 50%)
+ CD4IL-10 (SN 25%)
+ CD4IL-10 (SN 12.5%)
+ CD4GFP (SN 50%)
+ LPS

FIG. 35A

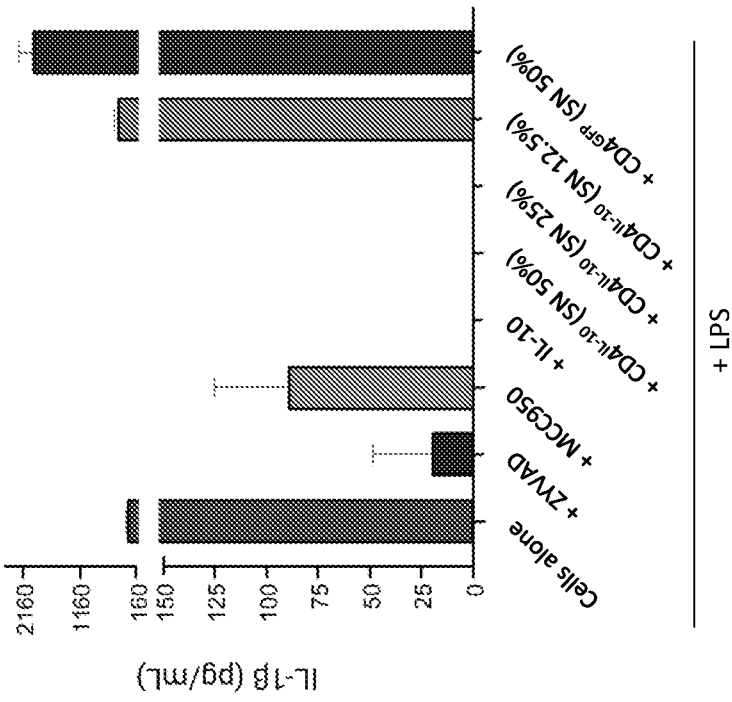

IL-1β (pg/mL)
2160
1160
160
150
125
100
75
50
25
0
Cells alone
+ ZYVAD
+ MCC950
+ IL-10
+ CD4IL-10 (SN 50%)
+ CD4IL-10 (SN 25%)
+ CD4IL-10 (SN 12.5%)
+ CD4GFP (SN 50%)
+ LPS

FIG. 35B

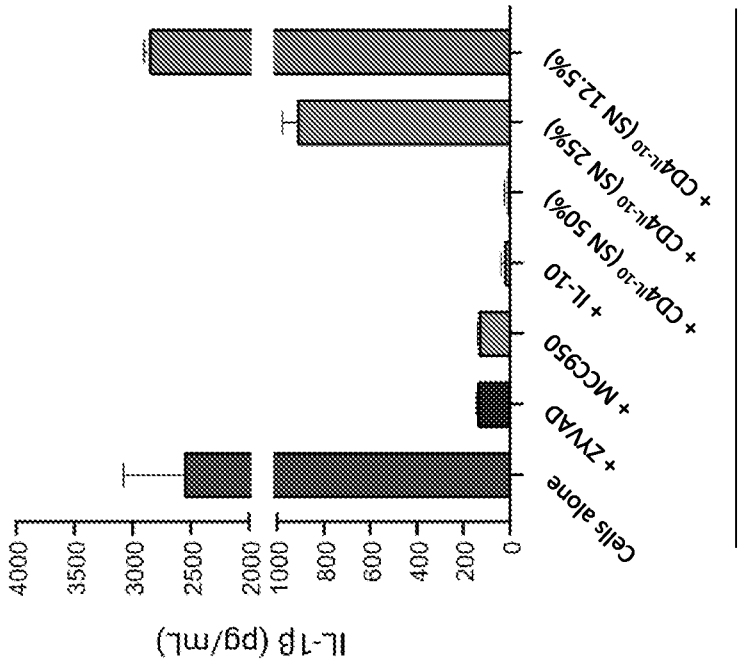

IL-1β (pg/mL)
4000
3500
3000
2500
2000
1000
800
600
400
200
0
Cells alone
+ ZVAD
+ MCC950
+ IL-10
+ CD4IL-10 (SN 50%)
+ CD4IL-10 (SN 25%)
+ CD4IL-10 (SN 12.5%)
+ LPS + NIG

FIG. 35C

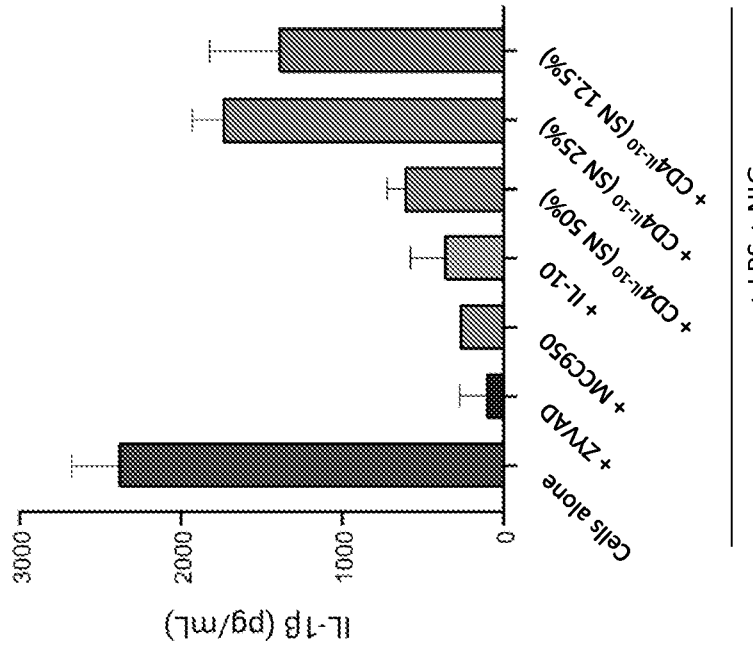

IL-1β (pg/mL)
3000
2000
1000
0
Cells alone
+ ZVAD
+ MCC950
+ IL-10
+ CD4IL-10 (SN 50%)
+ CD4IL-10 (SN 25%)
+ CD4IL-10 (SN 12.5%)
+ LPS + NIG

FIG. 35D

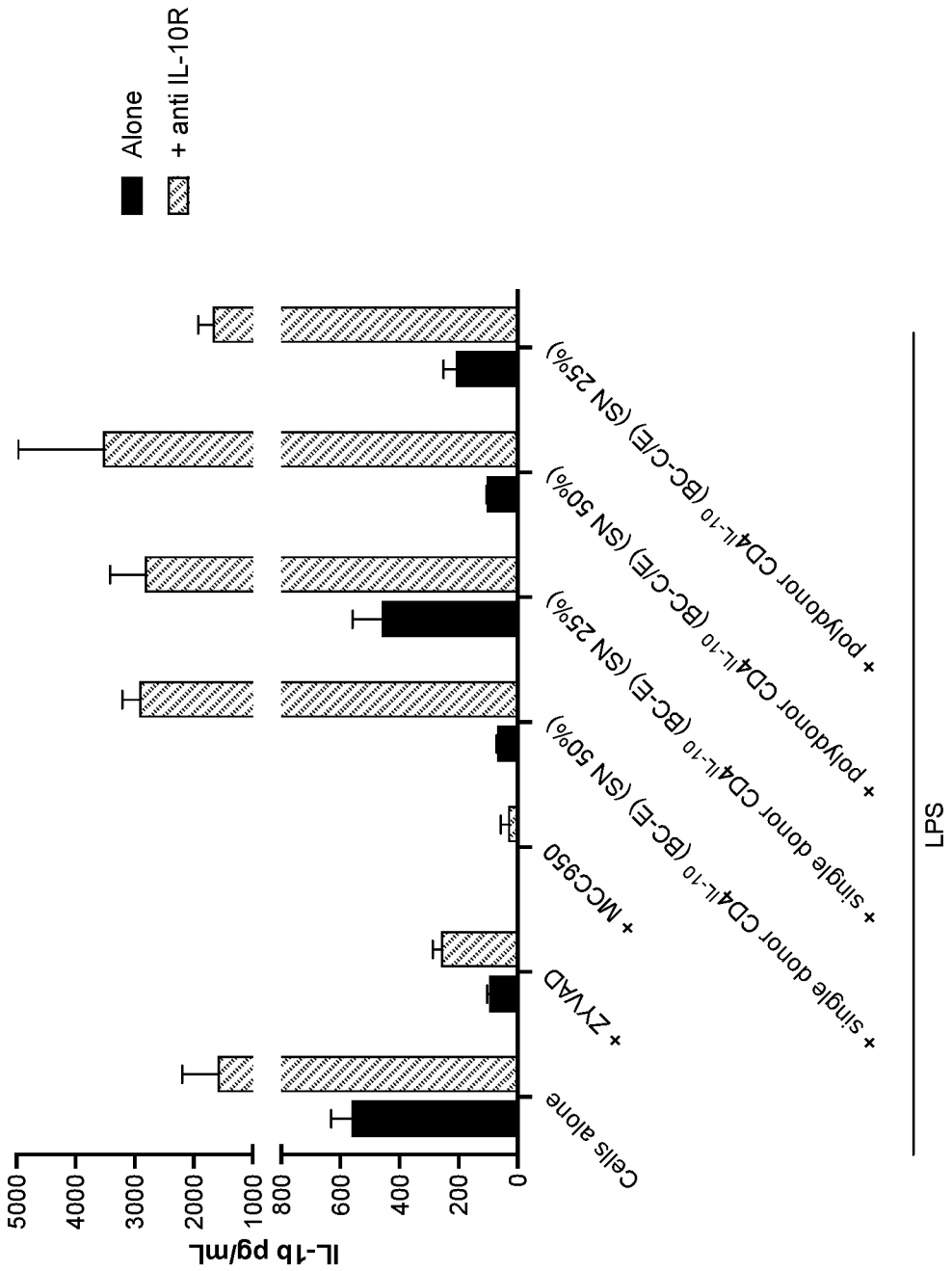

Alone
+ anti IL-10R
IL-1b pg/mL
5000
4000
3000
2000
1000
800
600
400
200
0
Cells alone
+ ZYVAD
+ MCC950
+ single donor CD4IL-10 (BC-E) (SN 50%)
+ single donor CD4IL-10 (BC-E) (SN 25%)
+ polydonor CD4IL-10 (BC-C/E) (SN 50%)
+ polydonor CD4IL-10 (BC-C/E) (SN 25%)
LPS

FIG. 35E

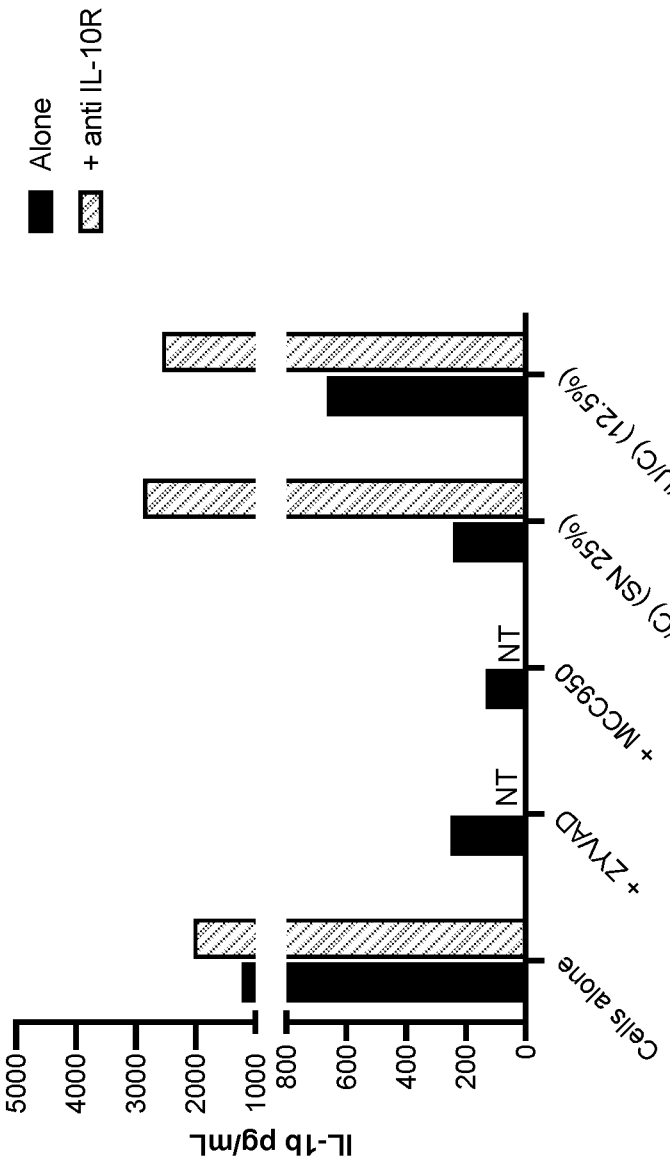

Alone
+ anti IL-10R
IL-1b pg/mL
5000
4000
3000
2000
1000
800
600
400
200
0
Cells alone
+ ZYVAD
+ MCC950
+ polydonor CD4IL-10 (BC-T/U/C) (SN 25%)
+ polydonor CD4IL-10 (BC-T/U/C) (12.5%)
NT
NT
LPS

FIG. 35F

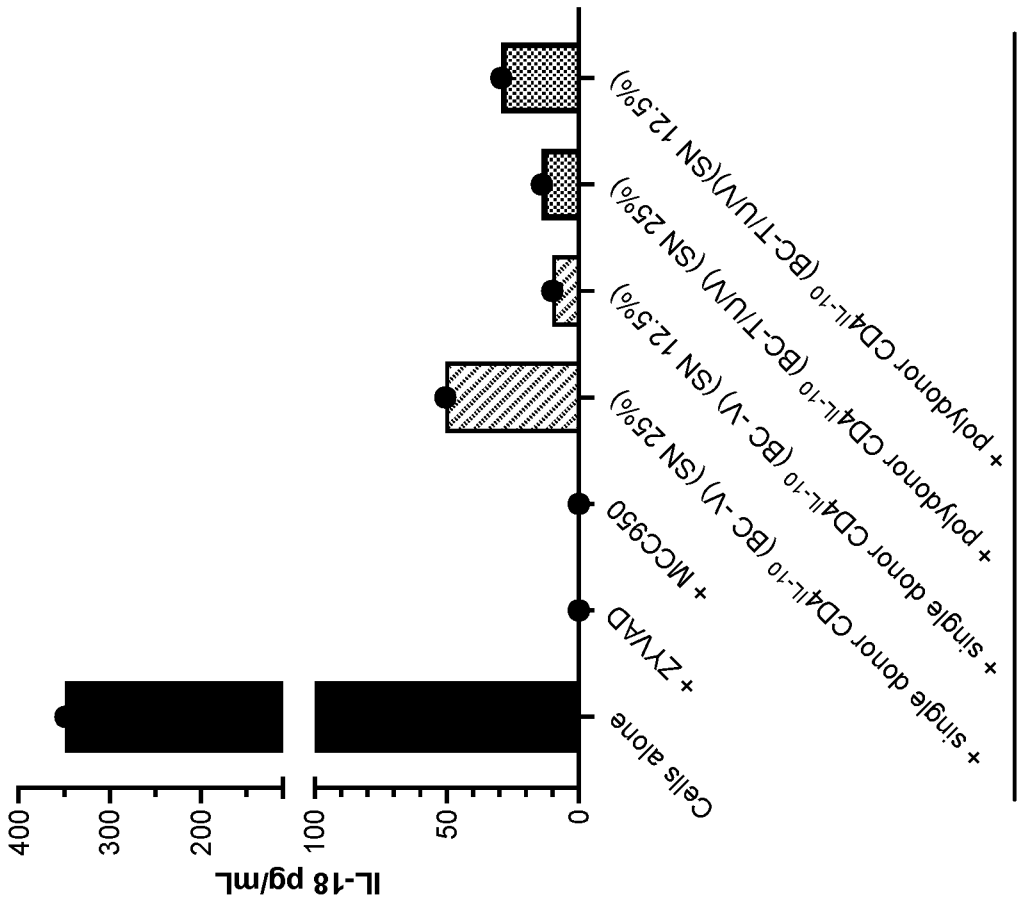

IL-18 pg/mL
400
300
200
100
50
0
Cells alone
+ ZYVAD
+ MCC950
+ single donor CD4IL-10 (BC -V) (SN 25%)
+ single donor CD4IL-10 (BC -V) (SN 12.5%)
+ polydonor CD4IL-10 (BC-T/U/V) (SN 25%)
+ polydonor CD4IL-10 (BC-T/U/V)(SN 12.5%)
LPS + NIG

FIG. 35G

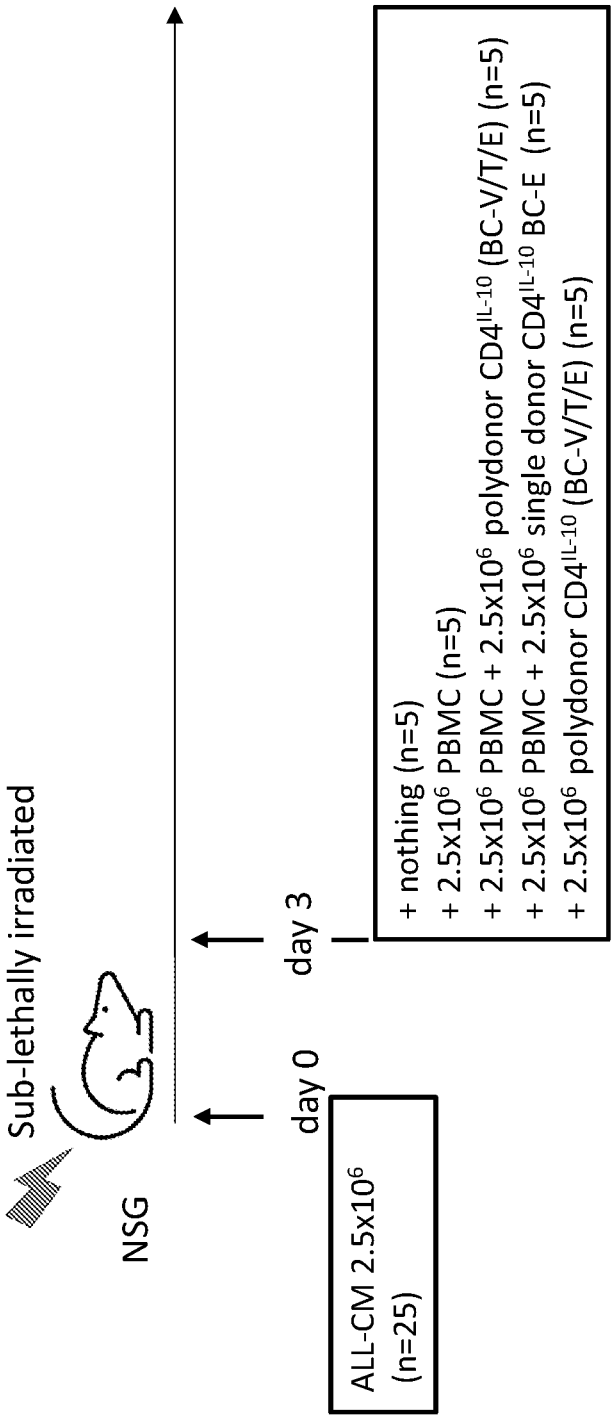

Sub-lethally irradiated
NSG
day 0
day 3
ALL-CM 2.5x10^6 (n=25)
+ nothing (n=5)
+ 2.5x10^6 PBMC (n=5)
+ 2.5x10^6 PBMC + 2.5x10^6 polydonor CD4^IL-10 (BC-V/T/E) (n=5)
+ 2.5x10^6 PBMC + 2.5x10^6 single donor CD4^IL-10 BC-E (n=5)
+ 2.5x10^6 polydonor CD4^IL-10 (BC-V/T/E) (n=5)

FIG. 36

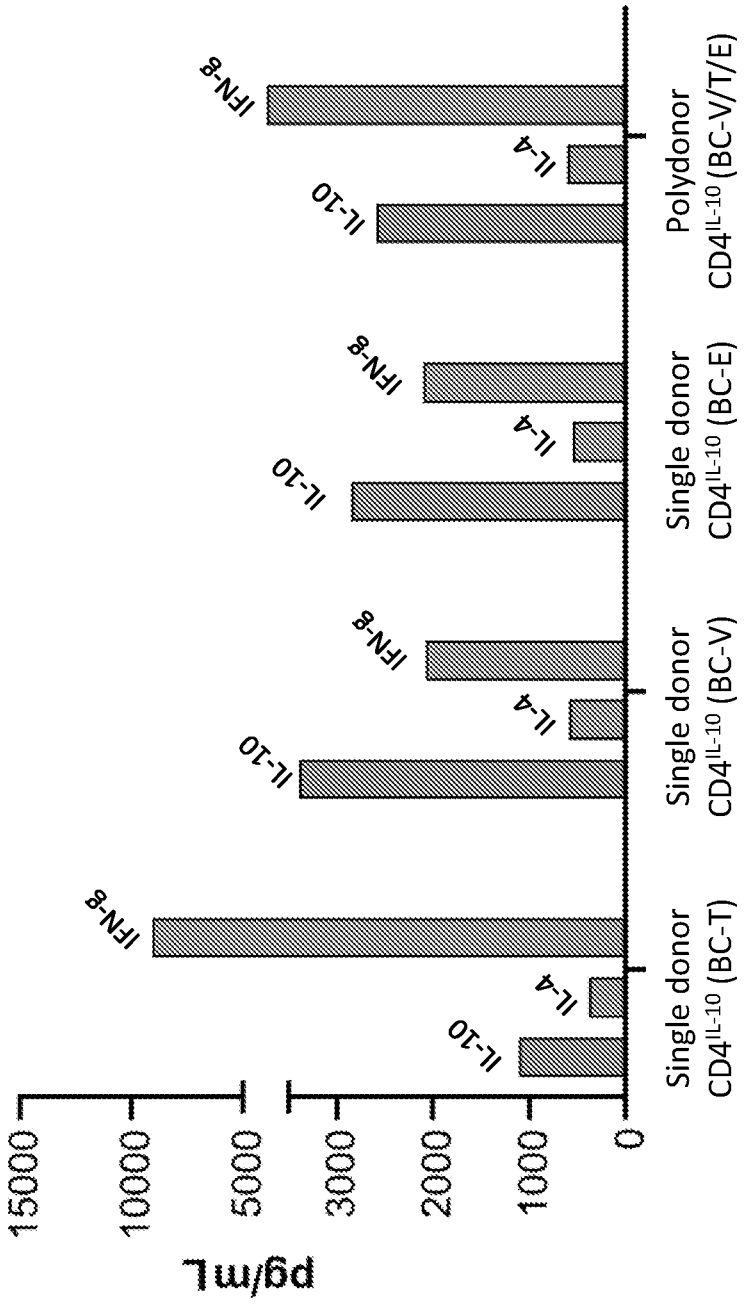

pg/mL
15000
10000
5000
3000
2000
1000
0
IFN-g
IL-4
IL-10
Single donor CD4IL-10 (BC-T)
IFN-g
IL-4
IL-10
Single donor CD4IL-10 (BC-V)
IFN-g
IL-4
IL-10
Single donor CD4IL-10 (BC-E)
IFN-g
IL-4
IL-10
Polydonor CD4IL-10 (BC-V/T/E)

FIG. 37

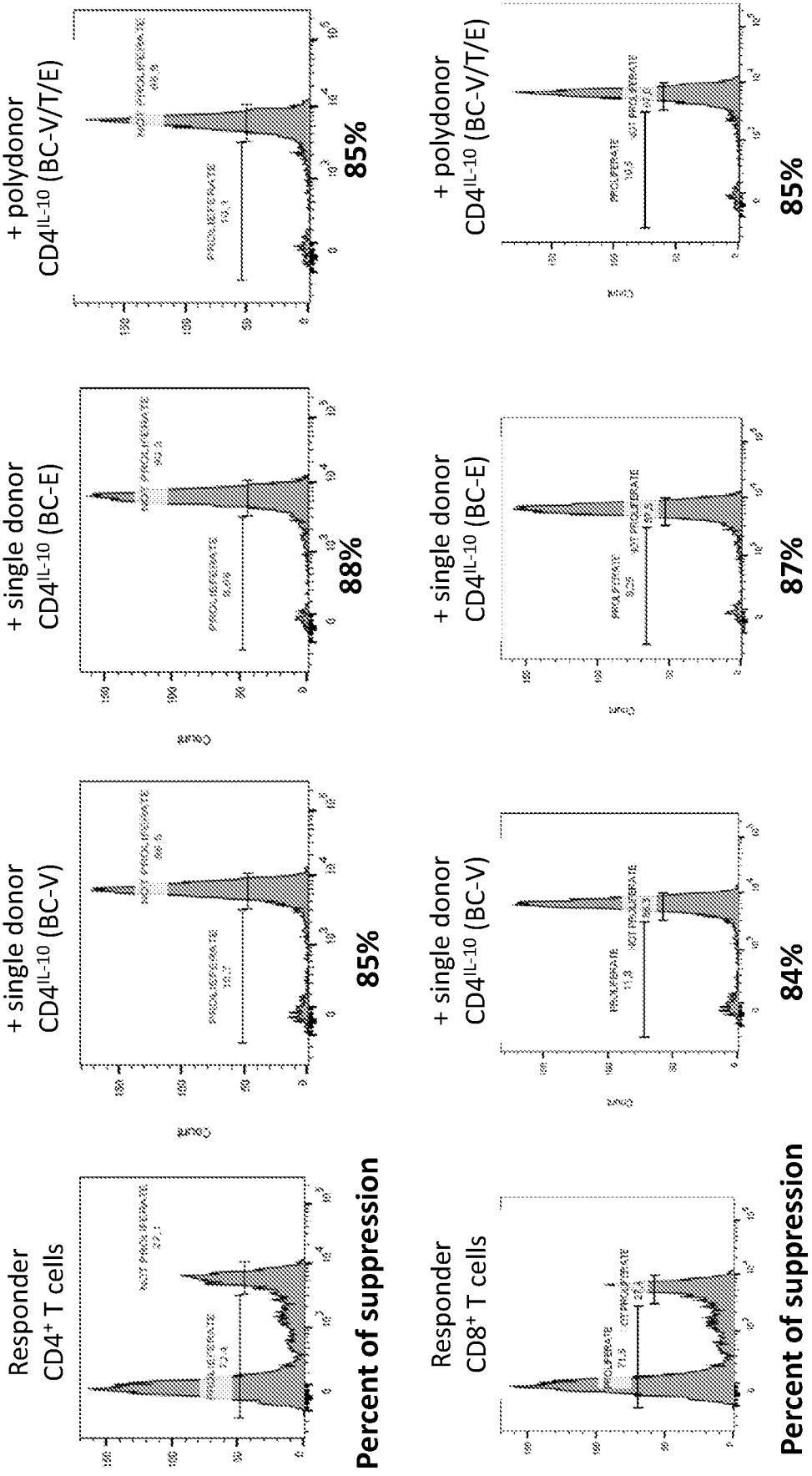

Responder CD4+ T cells
+ single donor CD4IL-10 (BC-V)
+ single donor CD4IL-10 (BC-E)
+ polydonor CD4IL-10 (BC-V/T/E)
Responder CD8+ T cells
+ single donor CD4IL-10 (BC-V)
+ single donor CD4IL-10 (BC-E)
+ polydonor CD4IL-10 (BC-V/T/E)
Count
85%
88%
85%
84%
87%
85%
Percent of suppression
Percent of suppression

FIG. 38

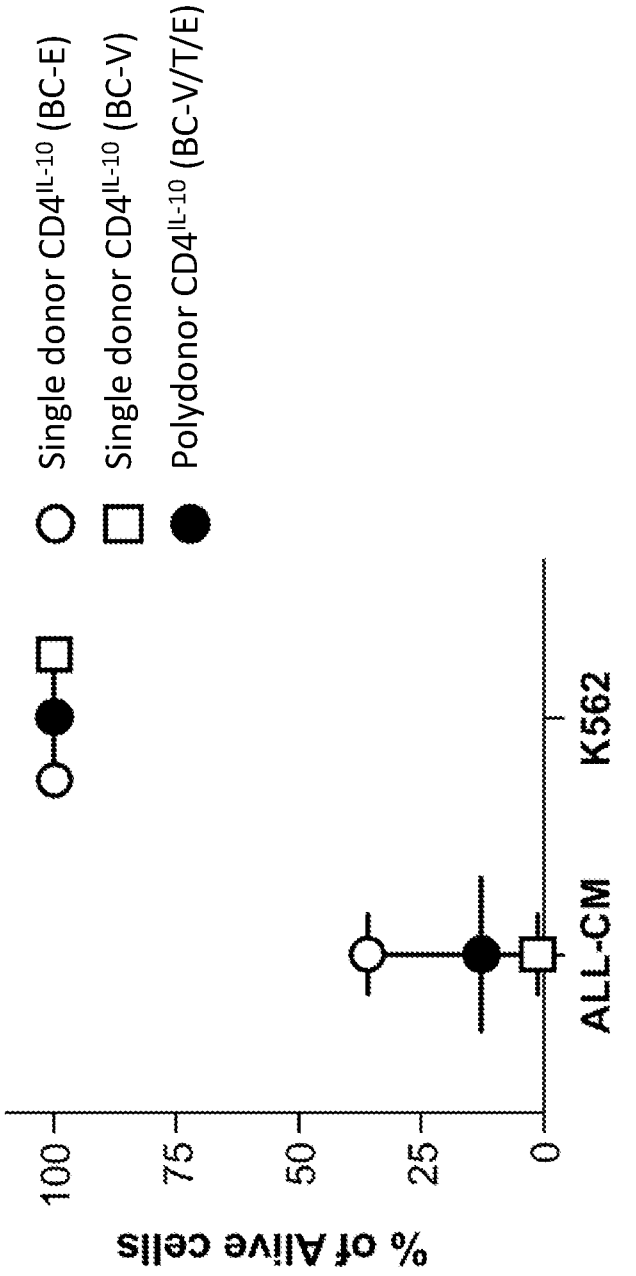

Single donor CD4IL-10 (BC-E)
Single donor CD4IL-10 (BC-V)
Polydonor CD4IL-10 (BC-V/T/E)
% of Alive cells
100
75
50
25
0
ALL-CM
K562

FIG. 39

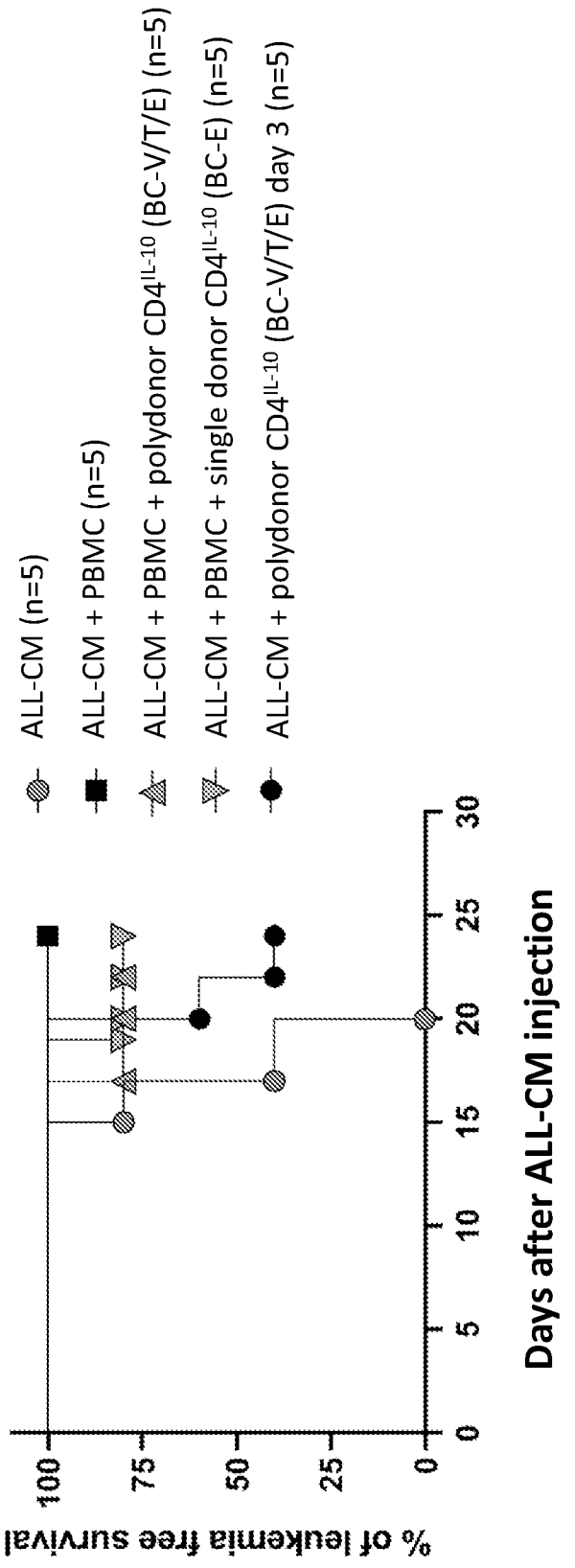

% of leukemia free survival
100
75
50
25
0
0
5
10
15
20
25
30
Days after ALL-CM injection
ALL-CM (n=5)
ALL-CM + PBMC (n=5)
ALL-CM + PBMC + polydonor CD4IL-10 (BC-V/T/E) (n=5)
ALL-CM + PBMC + single donor CD4IL-10 (BC-E) (n=5)
ALL-CM + polydonor CD4IL-10 (BC-V/T/E) day 3 (n=5)

FIG. 40

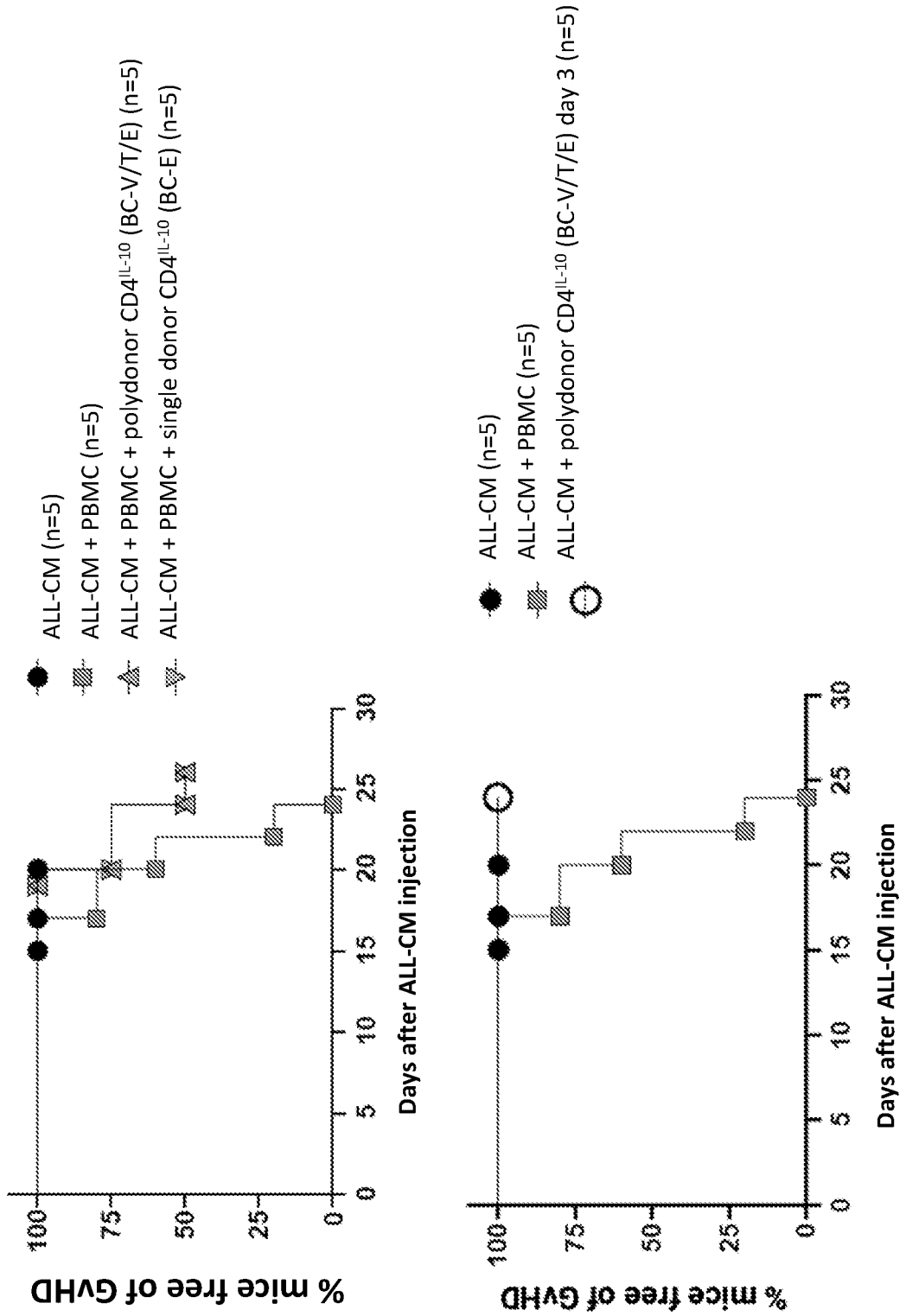

ALL-CM (n=5)
ALL-CM + PBMC (n=5)
ALL-CM + PBMC + polydonor CD4IL-10 (BC-V/T/E) (n=5)
ALL-CM + PBMC + single donor CD4IL-10 (BC-E) (n=5)
% mice free of GvHD
100
75
50
25
0
0
5
10
15
20
25
30
Days after ALL-CM injection
ALL-CM (n=5)
ALL-CM + PBMC (n=5)
ALL-CM + polydonor CD4IL-10 (BC-V/T/E) day 3 (n=5)
% mice free of GvHD
100
75
50
25
0
0
5
10
15
20
25
30
Days after ALL-CM injection

FIG. 41

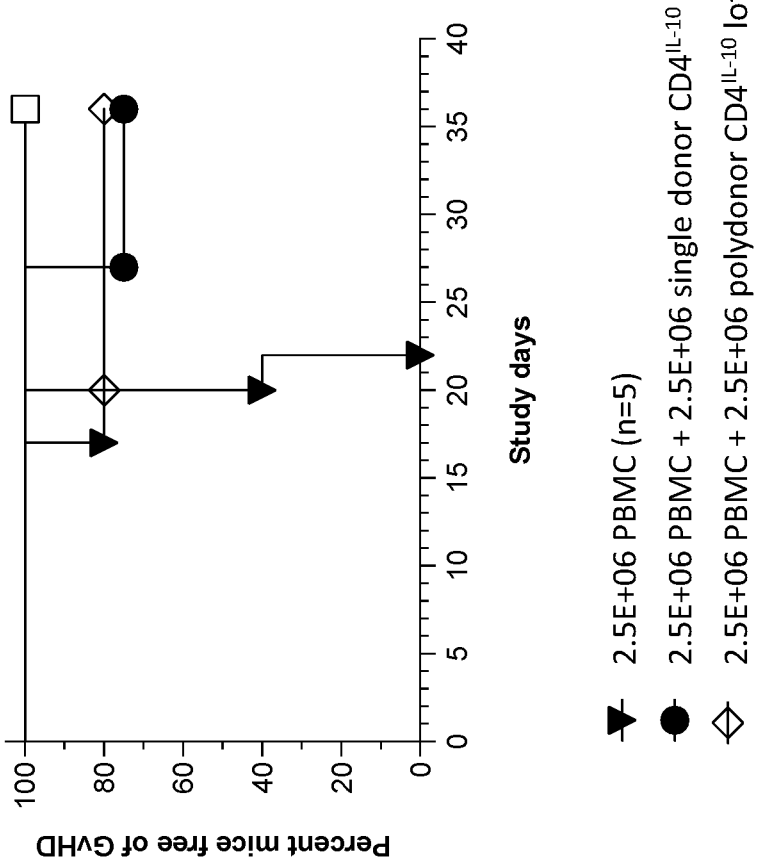

Percent mice free of GvHD
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
Study days
2.5E+06 PBMC (n=5)
2.5E+06 PBMC + 2.5E+06 single donor CD4IL-10 lot C (n=4)
2.5E+06 PBMC + 2.5E+06 polydonor CD4IL-10 lot CEFH (n=5)
2.5E+06 polydonor CD4IL-10 lot CEFH (n=3)

FIG. 42

```
(SEQ ID NO: 1) IL-10_HUMAN          1   --MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKE
(SEQ ID NO: 58)IL-10_MOUSE          1   --MPGSALLCCLLLLTGMRISRGQYSREDNNCTHFPVGQSHMLLELRTAFSQVKTFFQTKDQLDNILLTD
(SEQ ID NO: 59)IL-10_RAT            1   --MPGSALLCCLLLLAGVKTSKGHSIRGDNNCTHFPVSQTHMLRELRAAFSQVKTFFQKKDQLDNILLTD
(SEQ ID NO: 60)IL-10_MACMU          1   --MHSSALLCCLVLLTGVRASPGQGTQSENSCTRFPGNLPHMLRDLRDAFSRVKTFFQMKDQLDNILLKE
(SEQ ID NO: 61)IL-10_GORILLA        1   --MHSSALLCCLVLLTGVRASPGHGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKE
(SEQ ID NO: 62)IL-10_CYNO           1   --MHSSALLCCLVLLTGVRASPGQGTQSENSCTRFPGNLPHMLRDLRDAFSRVKTFFQMKDQLDNILLKE
(SEQ ID NO: 63)IL-10_OLIVE BABOON   1   --MHSSALLCCLVVLTGVRASPGQGTQSENSCTRFPGNLPHMLRDLRDAFSRVKTFFQMKDQLDNILLKE
(SEQ ID NO: 64)IL-10-BONOBO         1   --MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKE
(SEQ ID NO: 65)IL-10-CHIMP          1   --MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKE
(SEQ ID NO: 66)IL-10H_EBVB9         1   MERRLVVTLQCLVLL---YLAPECG--GTDQCDNFP----QMLRDLRDAFSRVKTFFQTKDEVDNLLLKE

                IL-10_HUMAN         69  SLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLKTLRLRLRRCHRFLPCENKSKA
                IL-10_MOUSE         69  SLMQDFKGYLGCQALSEMIQFYLVEVMPQAEKHGPEIKEHLNSLGEKLKTLRMRLRRCHRFLPCENKSKA
                IL-10_RAT           69  SLLQDFKGYLGCQALSEMIKFYLVEVMPQAENHGPEIKEHLNSLGEKLKTLWIQLRRCHRFLPCENKSKA
                IL-10_MACMU         69  SLLEDFKGYLGCQALSEMIQFYLEEVMPQAENHDPDIKEHVNSLGENLKTLRLRLRRCHRFLPCENKSKA
                IL-10_GORILLA       69  SLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLKTLRLRLRRCHRFLPCENKSKA
                IL-10_CYNO          69  SLLEDFKGYLGCQALSEMIQFYLEEVMPQAENHDPDIKEHVNSLGENLKTLRLRLRRCHRFLPCENKSKA
                IL-10_OLIVE BABOON  69  SLLEDFKGYLGCQALSEMIQFYLEEVMPQAENHDPDIKEHVNSLGENLKTLRLRLRRCHRFLPCENKSKA
                IL-10-BONOBO        69  SLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKVHVNSLGENLKTLRLRLRRCHRFLPCENKSKA
                IL-10-CHIMP         69  SLLEDFKGYLGCQALXEMIQFYLEEVMPQAENQDPDIKVHVNSLGENLKTLRLRLRRCHRFLPCENKSKA
                IL-10H_EBVB9        62  SLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPEAKDHVNSLGENLKTLRLRLRRCHRFLPCENKSKA

                IL-10_HUMAN         139 VEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN
                IL-10_MOUSE         139 VEQVKSDFNKLQDQGVYKAMNEFDIFINCIEAYMMIKMKS
                IL-10_RAT           139 VEQVKNDFNKLQDKGVYKAMNEFDIFINCIEAYVTLKMKN
                IL-10_MACMU         139 VEQVKNAFSKLQEKGVYKAMSEFDIFINYIEAYMTMKIQN
                IL-10_GORILLA       139 VEQVKNAFNKLQEKGVYKAMSEFDIFINYIEAYMTMKIRN
                IL-10_CYNO          139 VEQVKNAFSKLQEKGVYKAMSEFDIFINYIEAYMTMKIQN
                IL-10_OLIVE BABOON  139 VEQVKNAFSKLQEKGVYKAMSEFDIFINYIEAYMTMKIQN
                IL-10-BONOBO        139 VEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN
                IL-10-CHIMP         139 VEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN
                IL-10H_EBVB9        132 VEQIKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTIKAR-
```

FIG. 43A

Possible huIL-10 HYBRID#1 (SEQ ID NO: 67)

any combination of Change of amino acids (*)

```
                                                  *    **
MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQ%KD%%VDNLLLKESLLEDFKGY
LGCQALSEMIQFYLEEVMPQAENQDP%%AK%HVNSLGENLKTLRLRLRRCHRFLPCENKSKAVEQ%KNAFNKLQEKGI
YKAMSEFDIFINYIEAYMTMKIRN     **  *                                              *
```

Possible huIL-10 HYBRID#2 (SEQ ID NO: 68)

Preferred single aa change 110% to A10% Change of amino acids (#)

```
MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKESLLEDFKGY
LGCQALSEMIQFYLEEVMPQAENQDPD%KAHVNSLGENLKTLRLRLRRCHRFLPCENKSKAVEQVKNAFNKLQEKGI
YKAMSEFDIFINYIEAYMTMKIRN     #
```

FIG. 43B

(SEQ ID NO: 1)IL10_HUMAN 1 --MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKE
(SEQ ID NO: 66)IL10H_EBVB9 1 MERRLVQLQCLVLLKLAPEQGGL-----DQCDMFP----QMLRDLRDAFSRVKTFFQMKDEMDNLLLKE
*# *
IL10_HUMAN 69 SLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLKTLRLRLRRCHRFLPCENKSKA
IL10H_EBVB9 62 SLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDAKDHVNSLGENLKTLRLRLRRCHRFLPCENKSKA
*
IL10_HUMAN 139 VEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN
IL10H_EBVB9 132 VEQTKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTTKMR-

FIG. 43C