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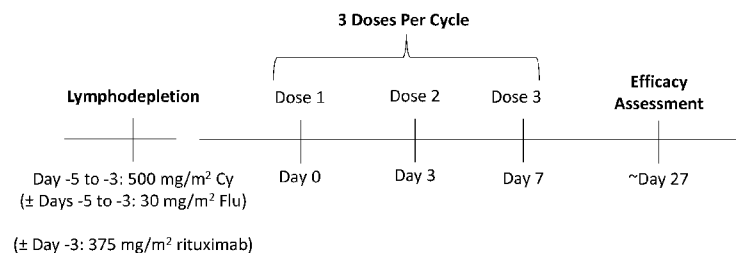
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(54) Title: METHODS FOR TREATMENT USING CD19-DIRECTED IMMUNOTHERAPIES

Figure 2A



(57) Abstract: Several embodiments disclosed herein relate to methods and compositions comprising genetically engineered cells for cancer immunotherapy. In several embodiments, the present disclosure relates to cells engineered to express chimeric antigen receptors directed to CD19, and administration of such cells in accordance with certain dosing regimens.

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- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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METHODS FOR TREATMENT USING CD19-DIRECTED IMMUNOTHERAPIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to United States Provisional Patent Application Nos. 63/523,599, filed June 27, 2023; 63/535,248 filed August 29, 2023; 63/567,795, filed March 20, 2024; and 63/574,450, filed April 4, 2024, the entire contents of each of which are incorporated by reference herein.

FIELD

[0002] Several embodiments disclosed herein relate to methods and compositions comprising genetically engineered cells for cancer immunotherapy. In several embodiments, the present disclosure relates to cells engineered to express chimeric antigen receptors directed to CD19, and administration of such cells in accordance with certain dosing regimens.

BACKGROUND

[0003] As further knowledge is gained about various cancers and what characteristics a cancerous cell has that can be used to specifically distinguish that cell from a healthy cell, therapeutics that leverage the distinct features of a cancerous cell, and dosing regimens for administration of the same, are under development. Immunotherapies that employ engineered immune cells are one approach to treating cancers.

INCORPORATION BY REFERENCE OF MATERIAL IN SEQUENCE LISTING FILE

[0004] This application incorporates by reference the material contained in the Sequence Listing XML file being submitted concurrently herewith: File name: NKT.108WO_ST26.xml; created on June 18, 2024 and is 39,496 bytes in size.

SUMMARY

[0005] Provided herein is a method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells. In some embodiments, all three doses are administered to the subject within between about 4 days and about 10 days. In some embodiments, the second dose is administered to the subject between 2-4 days after administration of the first dose to the subject; the third dose is administered to the subject between 2-4 days after administration of

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the second dose to the subject; and each of the first, second and third doses comprises at least about 2×10^9 CAR-expressing NK cells.

[0006] Also provided herein is a method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells. In some embodiments, the second dose is administered to the subject between 2-4 days after administration of the first dose to the subject; the third dose is administered to the subject between 2-4 days after administration of the second dose to the subject; and each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

[0007] Also provided herein is a method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein: all three doses are administered to the subject within between about 4 days and about 10 days; and each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

[0008] Also provided herein is a method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein: all three doses are administered to the subject within between about 4 days and about 10 days; the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle; and the lymphodepleting therapy comprises cyclophosphamide and does not comprise fludarabine. In some embodiments, the second dose is administered to the subject between 2-4 days after administration of the first dose to the subject and the third dose is administered to the subject between 2-4 days after administration of the second dose to the subject.

[0009] In some embodiments, each dose is separated by between about 24 hours and about 72 hours. In some embodiments, each dose is separated by at least about 24 hours. In some embodiments, each dose is separated by about 24 hours. In some embodiments, each dose is separated by at least about 48 hours. In some embodiments, each dose is separated by about 48 hours. In some embodiments, each dose is separated by at least about 72 hours. In some embodiments, each dose is separated by about 72 hours.

[0010] In some embodiments, the first dose is administered on about Day 0 of the dosing cycle. In some embodiments, the second dose is administered on about Day 2 of

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the dosing cycle. In some embodiments, the second dose is administered on about Day 3 of the dosing cycle. In some embodiments, the second dose is administered on about Day 4 of the dosing cycle. In some embodiments, the third dose is administered on about Day 4 of the dosing cycle. In some embodiments, the third dose is administered on about Day 5 of the dosing cycle. In some embodiments, the third dose is administered on about Day 6 of the dosing cycle. In some embodiments, the third dose is administered on about Day 7 of the dosing cycle. In some embodiments, the third dose is administered on about Day 8 of the dosing cycle.

[0011] In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 2 of the dosing cycle, and the third dose is administered on about Day 4 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 2 of the dosing cycle, and the third dose is administered on about Day 5 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 2 of the dosing cycle, and the third dose is administered on about Day 6 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 3 of the dosing cycle, and the third dose is administered on about Day 5 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 3 of the dosing cycle, and the third dose is administered on about Day 6 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 3 of the dosing cycle, and the third dose is administered on about Day 7 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 4 of the dosing cycle, and the third dose is administered on about Day 6 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 4 of the dosing cycle, and the third dose is administered on about Day 7 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 4 of the dosing cycle, and the third dose is administered on about Day 8 of the dosing cycle.

[0012] In some embodiments, each of the first, second and third doses comprises about 1×10^9 CAR-expressing NK cells. In some embodiments, each of the first, second and third doses comprises about 1.5×10^9 CAR-expressing NK cells. In some embodiments, each of the first, second and third doses comprises about 2×10^9 CAR-expressing NK cells. In some embodiments, each of the first, second and third doses comprises about 2.5×10^9 CAR-expressing NK cells.

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[0013] In some embodiments, the subject is administered a lymphodepleting therapy prior to initiation of the dosing cycle. In some embodiments, the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle. In some embodiments, the first dosing cycle is followed by an additional dosing cycle. In some embodiments, if the subject exhibits a clinical response following the first dosing cycle, the method comprises an additional dosing cycle to deepen or consolidate the response. In some embodiments, if the subject exhibits a partial response (PR) following the first dosing cycle, the method comprises an additional dosing cycle to deepen the response. In some embodiments, if the subject exhibits a clinical response following the first dosing cycle, the method comprises an additional dosing cycle as consolidation treatment. In some embodiments, if the subject exhibits a complete response (CR) following the first dosing cycle, the method comprises an additional dosing cycle as consolidation treatment. In some embodiments, if the subject exhibits a clinical response following a dosing cycle and subsequently exhibits disease progression, the method comprises an additional dosing cycle as retreatment. In some embodiments, if the subject exhibits a PR following a dosing cycle and subsequently exhibits disease progression, the method comprises an additional dosing cycle as retreatment. In some embodiments, if the subject exhibits a CR following a dosing cycle and subsequently exhibits disease progression, the method comprises an additional dosing cycle as retreatment. In some embodiments, the method comprises between one dosing cycle and five dosing cycles. In some embodiments, the subject is administered a lymphodepleting therapy prior to each dosing cycle. In some embodiments, each dosing cycle is between about 14 days and about 35 days. In some embodiments, each dosing cycle is about 28 days. In some embodiments, if the subject exhibits a partial response to the treatment, the dosing regimen comprises an additional dosing cycle. In some embodiments, if the subject exhibits a complete response to the treatment, the dosing regimen comprises an additional dosing cycle. In some embodiments, if the subject exhibits an initial clinical response to the treatment and subsequent disease progression, the dosing regimen comprises an additional dosing cycle.

[0014] In some embodiments, the lymphodepleting therapy comprises cyclophosphamide, optionally wherein a dose of cyclophosphamide is between about 300 mg/m² and about 1000 mg/m². In some embodiments, the lymphodepleting therapy comprises cyclophosphamide. In some embodiments a dose of cyclophosphamide is between about 300 mg/m² and about 1000 mg/m². In some embodiments, the lymphodepleting therapy comprises a single dose of cyclophosphamide. In some embodiments, the lymphodepleting therapy consists of a single dose of cyclophosphamide. In some embodiments, the single dose of cyclophosphamide is administered to the subject about 3 days prior to administration of the dosing cycle. In some embodiments, the single dose of cyclophosphamide is about 1000

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mg/m². In some embodiments, the lymphodepleting therapy comprises three doses of cyclophosphamide. In some embodiments, each dose of cyclophosphamide is about 500 mg/m². In some embodiments, a dose of cyclophosphamide is administered to the subject on each of about 5 days prior, 4 days prior, and 3 days prior to administration of the first dose of the dosing cycle. In some embodiments, the lymphodepleting therapy does not comprise fludarabine. In some embodiments, the lymphodepleting therapy comprises cyclophosphamide and does not comprise fludarabine. In some embodiments, the lymphodepleting therapy consists of cyclophosphamide. In some embodiments, if the subject has a cytopenia, the lymphodepleting therapy does not comprise fludarabine.

[0015] In some embodiments, the lymphodepleting therapy comprises fludarabine. In some embodiments, a dose of fludarabine is between about 20 mg/m² and about 40 mg/m². In some embodiments, the lymphodepleting therapy comprises three doses of fludarabine. In some embodiments, each dose of fludarabine is about 30 mg/m². In some embodiments, a dose of fludarabine is administered to the subject on each of about 5 days prior, 4 days prior, and 3 days prior to administration of the first dose of the dosing cycle.

[0016] In some embodiments, the method further comprises administration of a therapeutic agent that targets CD20. In some embodiments, the subject is administered a therapeutic agent that targets CD20. In some embodiments, the therapeutic agent is an anti-CD20 monoclonal antibody. In some embodiments, the anti-CD20 antibody is rituximab. In some embodiments, the therapeutic agent that targets CD20 is administered in an amount between about 150 mg/m² and about 500 mg/m². In some embodiments, the therapeutic agent that targets CD20 is administered in an amount of about 375 mg/m². In some embodiments, the therapeutic agent is administered to the subject at least one time and the at least one time is at least 2 days prior to administration of the first dose of the dosing cycle. In some embodiments, the therapeutic agent is administered to the subject one time 3 days prior to administration of the first dose of the dosing cycle.

[0017] In some embodiments, the first dose of genetically engineered NK cells is administered to the subject about 2 to 5 days after administration of the lymphodepleting therapy has concluded. In some embodiments, the first dose of the genetically engineered NK cells is administered to the subject about 2 days after administration of the lymphodepleting therapy has concluded. In some embodiments, the first dose of the genetically engineered NK cells is administered to the subject about 3 days after administration of the lymphodepleting therapy has concluded. In some embodiments, the first dose of the genetically engineered NK cells is administered to the subject about 4 days after administration of the lymphodepleting therapy has concluded. In some embodiments, the first dose of the genetically engineered NK cells is administered to the subject about 5 days after administration of the lymphodepleting therapy has concluded.

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[0018] Also provided herein is a method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein: the second dose is administered to the subject about 3 days after administration of the first dose to the subject, the third dose is administered to the subject about 4 days after administration of the second dose to the subject; each of the first, second and third doses comprises between about 1.0×10^9 CAR-expressing NK cells and about 2×10^9 CAR-expressing NK cells; the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle; and the lymphodepleting therapy consists of a dose of about 500 mg/m² of cyclophosphamide on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle.

[0019] Also provided herein is a method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein: the second dose is administered to the subject about 3 days after administration of the first dose to the subject, the third dose is administered to the subject about 4 days after administration of the second dose to the subject; each of the first, second and third doses comprises about 1.5×10^9 CAR-expressing NK cells, the subject is administered a lymphodepleting therapy comprising (i) a dose of about 500 mg/m² of cyclophosphamide on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle and (ii) a dose of about 30 mg/m² of fludarabine on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle; and the subject is administered a dose of about 375 mg/m² of rituximab about 3 days prior to administration of the first dose of the dosing cycle.

[0020] Also provided herein is a method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein: the second dose is administered to the subject about 3 days after administration of the first dose to the subject, the third dose is administered to the subject about 4 days after administration of the second dose to the subject; each of the first, second and third doses comprises about 2×10^9 CAR-expressing NK cells or about 2.5×10^9 CAR-expressing NK cells, the subject is administered a lymphodepleting therapy comprising (i) a dose of about 500 mg/m² of cyclophosphamide on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle and (ii) a dose of about 30 mg/m² of

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fludarabine on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle; and the subject is administered a dose of about 375 mg/m² of rituximab about 3 days prior to administration of the first dose of the dosing cycle.

[0021] Also provided herein is a method of preparing a subject having a cancer for treatment with natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, the method comprising administering a lymphodepleting therapy to the subject prior to administration of a first dosing cycle of the genetically engineered NK cells to the subject, wherein: the first dosing cycle comprises a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells; each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells; and all three doses are administered to the subject within between about 4 days and about 10 days. In some embodiments, all three doses are administered to the subject within about 10 days. In some embodiments, the lymphodepleting therapy comprises cyclophosphamide and does not comprise fludarabine. In some embodiments, the lymphodepleting therapy consists of cyclophosphamide.

[0022] Also provided herein is a method of preparing a subject having a cancer for treatment with a composition comprising natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds to CD19, the method comprising administering a lymphodepleting therapy to the subject prior to administration of the composition to the subject, wherein the lymphodepleting therapy consists of cyclophosphamide. In some embodiments, the composition is administered in a dosing cycle comprising a first dose of the CAR-expressing NK cells, a second dose of the CAR-expressing NK cells, and a third dose of the CAR-expressing NK cells.

[0023] In some embodiments, each dose is separated by between about 24 hours and about 72 hours. In some embodiments, each dose is separated by at least about 24 hours. In some embodiments, each dose is separated by about 24 hours. In some embodiments, each dose is separated by at least about 48 hours. In some embodiments, each dose is separated by about 48 hours. In some embodiments, each dose is separated by at least about 72 hours. In some embodiments, each dose is separated by about 72 hours.

[0024] In some embodiments, the first dose is administered on about Day 0 of the dosing cycle. In some embodiments, the second dose is administered on about Day 2 of the dosing cycle. In some embodiments, the second dose is administered on about Day 3 of the dosing cycle. In some embodiments, the second dose is administered on about Day 4 of the dosing cycle. In some embodiments, the third dose is administered on about Day 4 of the dosing cycle. In some embodiments, the third dose is administered on about Day 5 of the dosing cycle. In some embodiments, the third dose is administered on about Day 6 of the

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dosing cycle. In some embodiments, the third dose is administered on about Day 7 of the dosing cycle. In some embodiments, the third dose is administered on about Day 8 of the dosing cycle.

[0025] In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 2 of the dosing cycle, and the third dose is administered on about Day 4 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 2 of the dosing cycle, and the third dose is administered on about Day 5 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 2 of the dosing cycle, and the third dose is administered on about Day 6 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 3 of the dosing cycle, and the third dose is administered on about Day 5 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 3 of the dosing cycle, and the third dose is administered on about Day 6 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 3 of the dosing cycle, and the third dose is administered on about Day 7 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 4 of the dosing cycle, and the third dose is administered on about Day 6 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 4 of the dosing cycle, and the third dose is administered on about Day 7 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 4 of the dosing cycle, and the third dose is administered on about Day 8 of the dosing cycle.

[0026] In some embodiments, the cancer is a CD19-expressing cancer. In some embodiments, the cancer is a blood cancer. In some embodiments, the cancer is a leukemia or a lymphoma. In some embodiments, the cancer is a B cell cancer. In some embodiments, the cancer is a Non-Hodgkin lymphoma (NHL). In some embodiments, wherein the cancer is a large B-cell lymphoma (LBCL), optionally an aggressive LBCL. In some embodiments, the cancer is diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), marginal zone lymphoma (MZL), mantle cell lymphoma (MCL), Waldenström macroglobulinemia (WM), or B-cell acute lymphoblastic leukemia (B-ALL). In some embodiments, the cancer is diffuse large B-cell lymphoma (DLBCL). In some embodiments, the cancer is follicular lymphoma (FL). In some embodiments, the cancer is marginal zone lymphoma (MZL). In some embodiments, the cancer is mantle cell lymphoma (MCL). In some

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embodiments, the cancer is Waldenström macroglobulinemia (WM). In some embodiments, the cancer is or B-cell acute lymphoblastic leukemia (B-ALL). In some embodiments, the cancer is a chronic lymphocytic leukemia (CLL) or a small lymphocytic lymphoma (SLL). In some embodiments, the cancer is a chronic lymphocytic leukemia (CLL). In some embodiments, the cancer is a small lymphocytic lymphoma (SLL). In some embodiments, the cancer is a relapsed/refractory (R/R) cancer.

[0027] In some embodiments, the subject has less than or equal to 5% peripheral blasts. In some embodiments the subject has no evidence of extramedullary disease. In some embodiments, the subject has received at least 1 but not more than 7 lines of previous therapy, optionally wherein the subject has received at least 1 but not more than 4 lines of previous therapy. In some embodiments, the subject has received at least one line of previous therapy. In some embodiments, the subject has received at least two lines of previous therapy. In some embodiments, the subject has received at least three lines of previous therapy. In some embodiments, a line of previous therapy comprises an anti-CD20 monoclonal antibody and a cytotoxic chemotherapy. In some embodiments, the cytotoxic therapy is anthracycline. In some embodiments, a line of previous therapy comprises chimeric antigen receptor-expressing T (CAR T) cells. In some embodiments, a line of previous therapy comprises autologous anti-CD19 CAR T cells. In some embodiments, a line of previous therapy does not comprise CAR T cells. In some embodiments, a line of previous therapy does not comprise autologous anti-CD19 CAR T cells. In some embodiments, a line of previous therapy comprises an inhibitor of Bruton's tyrosine kinase (BTKi). In some embodiments, the BTKi is ibrutinib. In some embodiments, a line of previous therapy comprises an inhibitor of Bcl-2. In some embodiments, the Bcl-2 inhibitor is venetoclax.

[0028] In some embodiments, the CAR comprises: (a) an antigen-binding moiety that targets CD19; (b) a transmembrane domain; and (c) an intracellular signaling domain comprising an OX40 domain and a CD3zeta domain. In some embodiments, the antigen-binding moiety comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 24, 25, and 26, respectively; and the VL comprises a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 27, 28, and 29, respectively. In some embodiments, the antigen-binding moiety comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 30, 31, and 32, respectively; and the VL comprises a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 33, 34, and 29, respectively. In some embodiments, the antigen-binding moiety comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises the

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amino acid sequence set forth in SEQ ID NO: 35 and/or the VL comprises the amino acid sequence set forth in SEQ ID NO:36. In some embodiments, the VH comprises the amino acid sequence set forth in SEQ ID NO: 35. In some embodiments, the VL comprises the amino acid sequence set forth in SEQ ID NO:36. In some embodiments, the VH comprises the amino acid sequence set forth in SEQ ID NO: 35, and the VL comprises the amino acid sequence set forth in SEQ ID NO:36. In some embodiments, the antigen-binding moiety is an scFv comprising the amino acid sequence of SEQ ID NO:37.

[0029] In some embodiments, the genetically engineered NK cells are also engineered to express membrane-bound interleukin 15 (mbIL15). In some embodiments, the mbIL15 has at least 95% sequence identity to SEQ ID NO: 23 or 40. In some embodiments, the mbIL15 has at least 95% sequence identity to SEQ ID NO: 23. In some embodiments, the mbIL15 has at least 95% sequence identity to SEQ ID NO: 40. In some embodiments, the CAR and the mbIL15 are bicistronically encoded by the same nucleic acid molecule. In some embodiments, the nucleic acid sequences encoding the CAR and the mbIL15 are separated by a nucleic acid sequence encoding a T2A peptide. In some embodiments, the T2A peptide comprises the amino acid sequence set forth in SEQ ID NO:20.

[0030] In some embodiments, the NK cells genetically engineered to express a CAR are also genetically edited. In some embodiments, the NK cells are genetically edited to increase IL15 signaling. In some embodiments, the methods comprise genetically editing the NK cells to increase IL15 signaling. In some embodiments, the NK cells are genetically edited to reduce expression of the CISH gene. In some embodiments, the methods comprise genetically editing the NK cells to reduce expression of the CISH gene. In some embodiments, the NK cells are genetically edited to reduce expression of the Cis protein. In some embodiments, the methods comprise genetically editing the NK cells to reduce expression of the Cis protein. In some embodiments, the NK cells comprise a disruption in one or both alleles of the CISH gene. In some embodiments, the NK cells comprise a disruption in one allele of the CISH gene. In some embodiments, the NK cells comprise a disruption in both alleles of the CISH gene.

[0031] In some embodiments, the dosing cycle does not result in cytokine release syndrome, immune effector cell-associated neurotoxicity syndrome (ICANS)/neurotoxicity, and/or graft versus host disease. In some embodiments, the engineered NK cells are allogeneic with respect to the subject.

[0032] In some embodiments, one dose of each dosing cycle is administered to the subject on an outpatient basis. In some embodiments, at least one dose of each dosing cycle is administered to the subject on an outpatient basis. In some embodiments, each dose of each dosing cycle is administered to the subject on an outpatient basis. In some embodiments, a dosing cycle is administered to the subject on an outpatient basis. In some

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embodiments, at least one dosing cycle is administered to the subject on an outpatient basis. In some embodiments, each dosing cycle is administered to the subject on an outpatient basis.

[0033] In some embodiments, among subjects treated according to the method, the overall response rate (ORR) is at least about 50%, at least about 60%, at least about 70%, or at least about 80%. In some embodiments, at least about 50%, at least about 60%, at least about 70%, or at least about 80% of subjects treated according to the method exhibit a complete response (CR).

[0034] Also provided herein is use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein: the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19; and the NK cells are for administration as a first dosing cycle comprising a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells. In some embodiments, the second dose is for administration to the subject between 2-4 days after administration of the first dose to the subject; the third dose is for administration to the subject between 2-4 days after administration of the second dose to the subject; and each of the first, second and third doses comprises at least about 2×10^9 CAR-expressing NK cells.

[0035] Also provided herein is use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein: the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19; the NK cells are for administration as a first dosing cycle comprising a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein all three doses are administered to the subject within between about 4 days and about 10 days; and each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

[0036] Also provide herein is use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein: the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19; the NK cells are for administration as a first dosing cycle comprising a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein all three doses are administered to the subject within between about 4 days and about 10 days; and the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle, wherein the lymphodepleting therapy comprises cyclophosphamide and does not comprise fludarabine.

[0037] In some embodiments, the second dose is for administration to the subject between 2-4 days after administration of the first dose to the subject. In some embodiments, the third dose is for administration to the subject between 2-4 days after

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administration of the second dose to the subject. In some embodiments, the second dose is for administration to the subject between 2-4 days after administration of the first dose to the subject and the third dose is for administration to the subject between 2-4 days after administration of the second dose to the subject.

[0038] Also provided herein is use of a lymphodepleting therapy for preparing a subject having a cancer for treatment with a composition comprising natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds to CD19, wherein the lymphodepleting therapy consists of cyclophosphamide.

[0039] Also provided herein is use of a lymphodepleting therapy in the manufacture of a medication for preparing a subject having a cancer for treatment with natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, wherein the lymphodepleting therapy is for administration to the subject prior to administration of a first dosing cycle of the genetically engineered NK cells to the subject, wherein: the first dosing cycle comprises a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells; each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells; and all three doses are for administration to the subject within between about 4 days and about 10 days.

[0040] In some embodiments, the composition is administered in a dosing cycle. In some embodiments, the composition is administered in a dosing cycle comprising a first dose of the CAR-expressing NK cells, a second dose of the CAR-expressing NK cells, and a third dose of the CAR-expressing NK cells.

[0041] Also provided herein is use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein: the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19; and the NK cells are for administration as a first dosing cycle comprising a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells. In some embodiments, the second dose is for administration to the subject between 2-4 days after administration of the first dose to the subject; the third dose is for administration to the subject between 2-4 days after administration of the second dose to the subject; and each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

[0042] In some embodiments, each of the first, second and third doses comprises about 1×10^9 CAR-expressing NK cells. In some embodiments, each of the first, second and third doses comprises about 1.5×10^9 CAR-expressing NK cells. In some embodiments, each of the first, second and third doses comprises about 2×10^9 CAR-

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expressing NK cells. In some embodiments, each of the first, second and third doses comprises about 2.5×10^9 CAR-expressing NK cells.

[0043] Also provided herein is use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein the NK cells are for administration as a first dosing cycle comprising a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein: the second dose is for administration to the subject between 2-4 days after administration of the first dose to the subject, the third dose is for administration to the subject between 2-4 days after administration of the second dose to the subject, and each of the first, second and third doses comprises between about 1.0×10^9 CAR-expressing NK cells and about 2×10^9 CAR-expressing NK cells; the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle; and the lymphodepleting therapy consists of a dose of about 500 mg/m² of cyclophosphamide on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle.

[0044] In some embodiments, the second dose is for administration between about 24 hours and about 72 hours after administration of the first dose, and the third dose is for administration between about 24 hours and about 72 hours after administration of the second dose.

[0045] In some embodiments, the second dose is for administration at least about 24 hours after administration of the first dose, and the third dose is for administration at least about 24 hours after administration of the second dose. In some embodiments, the second is for administration about 24 hours after administration of the first dose, and the third dose is for administration about 24 hours after administration of the second dose. In some embodiments, the second dose is for administration at least about 48 hours after administration of the first dose, and the third dose is for administration at least about 48 hours after administration of the second dose. In some embodiments, the second is for administration about 48 hours after administration of the first dose, and the third dose is for administration about 48 hours after administration of the second dose. In some embodiments, the second dose is for administration at least about 72 hours after administration of the first dose, and the third dose is for administration at least about 72 hours after administration of the second dose. In some embodiments, the second is for administration about 72 hours after administration of the first dose, and the third dose is for administration about 72 hours after administration of the second dose.

[0046] In some embodiments, the first dose is for administration on about Day 0 of the dosing cycle. In some embodiments, the second dose is for administration on about Day 2 of the dosing cycle. In some embodiments, the second dose is for administration on about Day 3 of the dosing cycle. In some embodiments, the second dose is for administration

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on about Day 4 of the dosing cycle. In some embodiments, the third dose is for administration on about Day 4 of the dosing cycle. In some embodiments, the third dose is for administration on about Day 5 of the dosing cycle. In some embodiments, the third dose is for administration on about Day 6 of the dosing cycle. In some embodiments, the third dose is for administration on about Day 7 of the dosing cycle. In some embodiments, the third dose is for administration on about Day 8 of the dosing cycle.

[0047] In some embodiments, the first dose is for administration on about Day 0 of the dosing cycle, the second dose is for administration on about Day 2 of the dosing cycle, and the third dose is for administration on about Day 4 of the dosing cycle. In some embodiments, the first dose is for administration on about Day 0 of the dosing cycle, the second dose is for administration on about Day 2 of the dosing cycle, and the third dose is for administration on about Day 5 of the dosing cycle. In some embodiments, the first dose is for administration on about Day 0 of the dosing cycle, the second dose is for administration on about Day 2 of the dosing cycle, and the third dose is for administration on about Day 6 of the dosing cycle. In some embodiments, the first dose is for administration on about Day 0 of the dosing cycle, the second dose is for administration on about Day 3 of the dosing cycle, and the third dose is for administration on about Day 5 of the dosing cycle. In some embodiments, the first dose is for administration on about Day 0 of the dosing cycle, the second dose is for administration on about Day 3 of the dosing cycle, and the third dose is for administration on about Day 6 of the dosing cycle. In some embodiments, the first dose is for administration on about Day 0 of the dosing cycle, the second dose is for administration on about Day 3 of the dosing cycle, and the third dose is for administration on about Day 7 of the dosing cycle. In some embodiments, the first dose is for administration on about Day 0 of the dosing cycle, the second dose is for administration on about Day 4 of the dosing cycle, and the third dose is for administration on about Day 6 of the dosing cycle. In some embodiments, the first dose is for administration on about Day 0 of the dosing cycle, the second dose is for administration on about Day 4 of the dosing cycle, and the third dose is for administration on about Day 7 of the dosing cycle. In some embodiments, the first dose is for administration on about Day 0 of the dosing cycle, the second dose is for administration on about Day 4 of the dosing cycle, and the third dose is for administration on about Day 8 of the dosing cycle.

[0048] Also provided herein is use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein: the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19; the NK cells are for administration as a first dosing cycle comprising a first dose of the generally engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein: the second dose is for administration to the subject about 3 days after administration of the first dose to the subject, the third dose is for administration to

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the subject about 4 days after administration of the second dose to the subject; each of the first, second and third doses comprises about 2×10^9 CAR-expressing NK cells or about 2.5×10^9 CAR-expressing NK cells; the subject was administered a lymphodepleting therapy comprising (i) a dose of about 500 mg/m² of cyclophosphamide on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle and (ii) a dose of about 30 mg/m² of fludarabine on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle; and the subject was administered a dose of about 375 mg/m² of rituximab about 3 days prior to administration of the first dose of the dosing cycle.

[0049] Also provided herein is use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein: the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19; the NK cells are for administration as a first dosing cycle comprising a first dose of the generally engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein: the second dose is for administration to the subject about 3 days after administration of the first dose to the subject, the third dose is for administration to the subject about 4 days after administration of the second dose to the subject; each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells; the subject was administered a lymphodepleting therapy comprising (i) a dose of about 500 mg/m² of cyclophosphamide on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle and (ii) a dose of about 30 mg/m² of fludarabine on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle; and the subject was administered a dose of about 375 mg/m² of rituximab about 3 days prior to administration of the first dose of the dosing cycle.

[0050] In some embodiments, each of the first, second and third doses comprises about 1×10^9 CAR-expressing NK cells. In some embodiments, each of the first, second and third doses comprises about 1.5×10^9 CAR-expressing NK cells. In some embodiments, each of the first, second and third doses comprises about 2×10^9 CAR-expressing NK cells. In some embodiments, each of the first, second and third doses comprises about 2.5×10^9 CAR-expressing NK cells.

[0051] In some embodiments, the CAR-expressing NK cells also express a membrane-bound interleukin-15 (mblIL15).

BRIEF DESCRIPTION OF THE DRAWINGS

[0052] Figure 1 depicts non-limiting schematics of CD19-directed chimeric antigen receptors (CARs).

[0053] Figures 2A-2B depict non-limiting schematics of a dosing cycle for treating a CD19-related disease (e.g., cancer) with CD19 CAR-expressing NK cells.

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[0054] Figure 3 shows the concentration of CD19 CAR-expressing NK cells in two subjects with CD19+ B cell malignancies who were administered a lymphodepleting therapy of cyclophosphamide and fludarabine (cy/flu) prior to a first dosing cycle and a lymphodepleting therapy of cyclophosphamide only (cy) prior to a second dosing cycle.

DETAILED DESCRIPTION

[0055] Some embodiments of the methods and compositions provided herein relate to engineered immune cells (e.g., natural killer cells) and use of the same for immunotherapy (e.g., cancer immunotherapy). In several embodiments, the immune cells are engineered to express a chimeric antigen receptor (CAR) that targets CD19. In several embodiments, the methods are for treatment of a cancer, such as a hematologic malignancy.

[0056] All publications, including patent documents, scientific articles and databases, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference. If a definition set forth herein is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth herein prevails over the definition that is incorporated herein by reference.

[0057] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

I. Cell Therapy and Engineering Cells

[0058] In some embodiments, genetically engineered immune cells (e.g., NK cells) for use in accord with the provided methods includes administering engineered NK cells expressing a recombinant receptor (e.g. CAR) designed to recognize and/or specifically bind to an antigen associated with a disease such as cancer. In particular embodiments, the antigen that is bound or recognized by the CAR is CD19. In some embodiments, binding to the antigen results in a response, such as an immune response against such antigen. In some embodiments, binding to the antigen results in the reduction or depletion of cells expressing the antigen (e.g., B cells expressing CD19, or a subset thereof). In some embodiments, the genetically engineered cells contain or are engineered to contain the CAR. The CAR generally includes an extracellular antigen-binding domain specific to the antigen (e.g., CD19), which is linked to one or more intracellular signaling components, in some aspects via linkers and/or transmembrane domain(s). In some aspects, the genetically engineered cells are provided as pharmaceutical compositions and formulations suitable for administration to a subjects, such as for cell therapy. Also provided are therapeutic methods for administering the cells and compositions to subjects, uses of the compositions for treatment of subjects, and uses of the compositions in the manufacture of medicaments for treating subjects.

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A. Chimeric Antigen Receptors

[0059] Among the provided CARs, e.g., CD19-directed CARs, are CARs that specifically bind to CD19, such as receptors comprising an anti-CD19 antibody, e.g., antibody fragment. Also provided are immune cells (e.g., NK cells) expressing the recombinant receptors and uses thereof in treatment of diseases and conditions, such as cancers (e.g., CD19-expressing cancers). The CARs generally include an extracellular antigen-binding domain that includes an anti-CD19 antibody. Such CARs include antibodies (including antigen-binding fragments thereof) that specifically bind to CD19 proteins, such as human CD19 protein (e.g., SEQ ID NO:39). In some embodiments, the antibodies include those that are multi-domain antibodies, such as those containing VH and VL domains. In some embodiments, the antibodies include a variable heavy chain and a variable light chain, such as scFvs. Among the provided anti-CD19 antibodies are human and humanized antibodies.

[0060] The term “antibody” herein is used in the broadest sense and includes polyclonal and monoclonal antibodies, including intact antibodies and functional (antigen-binding) antibody fragments, including fragment antigen binding (Fab) fragments, F(ab')₂ fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, variable heavy chain (VH) regions capable of specifically binding the antigen, single chain antibody fragments, including single chain variable fragments (scFv), and single domain antibody (e.g., sdAb, sdFv, nanobody) fragments. The term encompasses genetically engineered and/or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugate antibodies, multispecific, e.g., bispecific, antibodies, diabodies, triabodies, and tetrabodies, tandem di-scFv, tandem tri-scFv. Unless otherwise stated, the term “antibody” should be understood to encompass functional antibody fragments thereof. The term also encompasses intact or full-length antibodies, including antibodies of any class or sub-class, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD.

[0061] The terms “complementarity determining region,” and “CDR,” synonymous with “hypervariable region” or “HVR,” are known in the art to refer to non-contiguous sequences of amino acids within antibody variable regions, which confer antigen specificity and/or binding affinity. In general, there are three CDRs in each heavy chain variable region (CDR-H1, CDR-H2, CDR-H3) and three CDRs in each light chain variable region (CDR-L1, CDR-L2, CDR-L3). “Framework regions” and “FR” are known in the art to refer to the non-CDR portions of the variable regions of the heavy and light chains. In general, there are four FRs in each full-length heavy chain variable region (FR-H1, FR-H2, FR-H3, and FR-H4), and four FRs in each full-length light chain variable region (FR-L1, FR-L2, FR-L3, and FR-L4).

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[0062] The precise amino acid sequence boundaries of a given CDR or FR can be readily determined using any of a number of well-known schemes, including the Kabat numbering scheme (Sequences of Proteins of Immunological Interest, 1987 and 1991, NIH, Bethesda, MD), the Chothia numbering scheme (Chothia & Lesk, 1987, J. Mol. Biol. 196:901-917; Chothia et al., 1989, Nature 342:878-883), the Contact numbering scheme (MacCallum et al., J. Mol. Biol. 262:732-745 (1996), "Antibody-antigen interactions: Contact analysis and binding site topography," J. Mol. Biol. 262, 732-745), the AbM numbering scheme (Martin et al., Proc. Natl. Acad. Sci., 86:9268-9272; 1989), the IMGT numbering scheme (the international ImMunoGeneTics information system; Lefranc et al, Dev. Comp. Immunol. 29:185-203; 2005), and the Aho numbering scheme (Honegger and Pluckthun, J. Mol. Biol. 309(3):657-670; 2001).

[0063] The boundaries of a given CDR or FR may vary depending on the scheme used for identification. For example, the Kabat scheme is based structural alignments, while the Chothia scheme is based on structural information. Numbering for both the Kabat and Chothia schemes is based upon the most common antibody region sequence lengths, with insertions accommodated by insertion letters, for example, "30a," and deletions appearing in some antibodies. The two schemes place certain insertions and deletions ("indels") at different positions, resulting in differential numbering. The Contact scheme is based on analysis of complex crystal structures and is similar in many respects to the Chothia numbering scheme.

[0064] Table 1, below, lists non-limiting position boundaries of CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 as identified by Kabat, Chothia, and Contact schemes, respectively. For CDR-H1, residue numbering is listed using both the Kabat and Chothia numbering schemes. FRs are located between CDRs, for example, with FR-L1 located between CDR-L1 and CDR-L2, and so forth. It is noted that because the shown Kabat numbering scheme places insertions at H35A and H35B, the end of the Chothia CDR-H1 loop when numbered using the shown Kabat numbering convention varies between H32 and H34, depending on the length of the loop.

Table 1. Boundaries of CDRs according to various numbering schemes				
CDR	Kabat	Chothia	AbM	Contact
CDR-L1	L24 - - L34	L24 - - L34	L24 - - L34	L30 - - L36
CDR-L2	L50 - - L56	L50 - - L56	L50 - - L56	L46 - - L55
CDR-L3	L89 - - L97	L89 - - L97	L89 - - L97	L89 - - L96
CDR-H1 (Kabat numbering ¹)	H31 - - H35B	H26 - - H32.34	H26 - - H35B	H30 - - H35B
CDR-H1 (Chothia numbering ²)	H31 - - H35	H26 - - H32	H26 - - H35	H30 - - H35

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CDR-H2	H50 - - H65	H52 - - H56	H50 - - H58	H47 - - H58
CDR-H3	H95 - - H102	H95 - - H102	H95 - - H102	H93 - - H101

¹ Kabat et al., "Sequences of Proteins of Immunological Interest," (1991) 5th Ed. Public Health

Service, National Institutes of Health, Bethesda, MD.

² Al-Lazikani et al., J. Mol. Biol. (1997) 273(4):927-48.

[0065] Thus, unless otherwise specified, a "CDR" or "complementary determining region," or individual specified CDRs (e.g., "CDR-H1, CDR-H2), of a given antibody or region thereof, such as a variable region thereof, should be understood to encompass a (or the specific) complementary determining region as defined by any of the aforementioned schemes. For example, where it is stated that a particular CDR (e.g., a CDR-H3) contains the amino acid sequence of a corresponding CDR in a given VH or VL amino acid sequence, it is understood that such a CDR has a sequence of the corresponding CDR (e.g., CDR-H3) within the variable region, as defined by any of the aforementioned schemes. In some embodiments, specific CDR sequences are specified.

[0066] Likewise, unless otherwise specified, a FR or individual specified FR(s) (e.g., FR-H1, FR-H2), of a given antibody or region thereof, such as a variable region thereof, should be understood to encompass a (or the specific) framework region as defined by any of the known schemes. In some instances, the scheme for identification of a particular CDR, FR, or FRs or CDRs is specified, such as the CDR as defined by the IMGT, Kabat, Chothia, or Contact method. In other cases, the particular amino acid sequence of a CDR or FR is given.

[0067] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three CDRs. A single VH or VL domain may be sufficient to confer antigen-binding specificity.

[0068] Among the provided antibodies are antibody fragments. An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; variable heavy chain (VH) regions, single-chain antibody molecules such as scFvs and single-domain VH single antibodies; and multispecific antibodies formed from antibody fragments. In particular embodiments, the antibodies are single-chain antibody fragments comprising a variable heavy chain region and a variable light chain region, such as scFvs.

[0069] In some embodiments, the antibodies are recombinantly-produced fragments, such as fragments comprising arrangements that do not occur naturally, such as those with two or more antibody regions or chains joined by synthetic linkers, e.g., peptide

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linkers, and/or that are may not be produced by enzyme digestion of a naturally-occurring intact antibody. In some aspects, the antibody fragments are scFvs.

[0070] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human or humanized single-domain antibody.

[0071] A “humanized” antibody is an antibody in which all or substantially all CDR amino acid residues are derived from non-human CDRs and all or substantially all FR amino acid residues are derived from human FRs. A humanized antibody optionally may include at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of a non-human antibody, refers to a variant of the non-human antibody that has undergone humanization, typically to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the CDR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[0072] Among the provided anti-CD19 antibodies are human antibodies. A “human antibody” is an antibody with an amino acid sequence corresponding to that of an antibody produced by a human or a human cell, or non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences, including human antibody libraries. The term excludes humanized forms of non-human antibodies comprising non-human antigen-binding regions, such as those in which all or substantially all CDRs are non-human. The term includes antigen-binding fragments of human antibodies.

[0073] Among the provided antibodies are monoclonal antibodies, including monoclonal antibody fragments. The term “monoclonal antibody” as used herein refers to an antibody obtained from or within a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical, except for possible variants containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different epitopes, each monoclonal antibody of a monoclonal antibody preparation is directed against a single epitope on an antigen. The term is not to be construed as requiring production of the antibody by any particular method. A monoclonal antibody may be made by a variety of techniques, including but not limited to generation from a hybridoma, recombinant DNA methods, phage-display and other antibody display methods.

[0074] The terms “polypeptide” and “protein” are used interchangeably to refer to a polymer of amino acid residues and are not limited to a minimum length. Polypeptides,

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including the provided antibodies and antibody chains and other peptides, e.g., linkers and CD19-binding peptides, may include amino acid residues including natural and/or non-natural amino acid residues. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. In some aspects, the polypeptides may contain modifications with respect to a native or natural sequence, as long as the protein maintains the desired activity.

[0075] The antigen-binding domain may be or comprise any antibody (e.g., anti-CD19 antibody) as described herein.

[0076] In some embodiments, the extracellular antigen-binding domain comprises a heavy chain variable region (VH) having a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 24, 25, and 26, respectively. In some embodiments, the extracellular antigen-binding domain comprises a light chain variable region (VL) having a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 27, 28, and 29, respectively. In some embodiments, the extracellular antigen-binding domain comprises a VH having a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 24, 25, and 26, respectively; and a VL having a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 27, 28, and 29, respectively.

[0077] In some embodiments, the extracellular antigen-binding domain comprises a heavy chain variable region (VH) having a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 30, 31, 32, respectively. In some embodiments, the extracellular antigen-binding domain comprises a light chain variable region (VL) having a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 33, 34, and 29, respectively. In some embodiments, the extracellular antigen-binding domain comprises a VH having a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 30, 31, and 32, respectively; and a VL having a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 33, 34, and 29, respectively.

[0078] In some embodiments, the VH comprises an amino acid sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the amino acid sequence set forth in SEQ ID NO:35. In some embodiments, the VH comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence set forth in SEQ ID NO:35. In some embodiments, the VH comprises an amino acid sequence having at least about 85% sequence identity to the amino acid sequence set forth in SEQ ID NO:35. In some embodiments, the VH comprises an amino acid sequence having at least about 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:35. In some

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embodiments, the VH comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO:35. In some embodiments, the VH comprises the amino acid sequence set forth in SEQ ID NO:35. In some embodiments, the VL comprises an amino acid sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the amino acid sequence set forth in SEQ ID NO:36. In some embodiments, the VL comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence set forth in SEQ ID NO:36. In some embodiments, the VL comprises an amino acid sequence having at least about 85% sequence identity to the amino acid sequence set forth in SEQ ID NO:36. In some embodiments, the VL comprises an amino acid sequence having at least about 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:36. In some embodiments, the VL comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO:36. In some embodiments, the VL comprises the amino acid sequence set forth in SEQ ID NO:36. In some embodiments, the VH comprises an amino acid sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the amino acid sequence set forth in SEQ ID NO:35; and the VL comprises an amino acid sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the amino acid sequence set forth in SEQ ID NO:36. In some embodiments, the VH comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence set forth in SEQ ID NO:35; and the VL comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence set forth in SEQ ID NO:36. In some embodiments, the VH comprises an amino acid sequence having at least about 85% sequence identity to the amino acid sequence set forth in SEQ ID NO:35; and the VL comprises an amino acid sequence having at least about 85% sequence identity to the amino acid sequence set forth in SEQ ID NO:36. In some embodiments, the VH comprises an amino acid sequence having at least about 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:35; and the VL comprises an amino acid sequence having at least about 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:36. In some embodiments, the VH comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO:35; and the VL comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO:36. In some embodiments, the VH comprises the amino acid sequence set forth in SEQ ID NO:35, and the VL comprises the amino acid sequence set forth in SEQ ID NO:36.

[0079] In some embodiments, the antigen-binding domain is an scFv comprising a VH and a VL joined by a linker (e.g., a linker comprising any of SEQ ID NOS:1-

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3). In some embodiments the linker comprises the amino acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3. In some embodiments, the extracellular antigen-binding domain is an scFv comprising the linker set forth in SEQ ID NO:1. In some embodiments, the antigen-binding domain is an scFv comprising an amino acid sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the amino acid sequence set forth in SEQ ID NO:37. In some embodiments, the antigen-binding domain is an scFv comprising an amino acid sequence having at least about 80% sequence identity to the amino acid sequence set forth in SEQ ID NO:37. In some embodiments, the antigen-binding domain is an scFv comprising an amino acid sequence having at least about 85% sequence identity to the amino acid sequence set forth in SEQ ID NO:37. In some embodiments, the antigen-binding domain is an scFv comprising an amino acid sequence having at least about 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:37. In some embodiments, the antigen-binding domain is an scFv comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO:37. In some embodiments, the antigen-binding domain is an scFv comprising the amino acid sequence set forth in SEQ ID NO:37. In some embodiments, the extracellular antigen-binding domain is an scFv comprising the linker set forth in SEQ ID NO:2. In some embodiments, the extracellular antigen-binding domain is an scFv comprising the linker set forth in SEQ ID NO:3.

[0080] Additional CD19-binding domains are known and described in the art, including any of those as described in PCT Application Nos. PCT/US2015/024671, PCT/US2018/029107, PCT/US2020/020824, PCT/US2020/033559, PCT/IB2021/060213, and PCT/CN2021/106892, each of which is incorporated herein in its entirety by reference.

[0081] Provided herein are recombinant receptors (e.g., CARs) comprising any of the CD19 antibodies or binding domains described herein. The extracellular antigen-binding domain generally is linked to an intracellular signaling domain comprising intracellular signaling components, such as signaling components that mimic activation through an antigen receptor complex, such as a TCR complex, in the case of a CAR. In some embodiments, the extracellular antigen-binding domain of a CAR is linked to an intracellular signaling domain by a transmembrane domain. Thus, in some embodiments, the CD19-binding molecule (e.g., antibody) is linked to a transmembrane and intracellular signaling domain. In some embodiments, a CAR comprises an extracellular antigen-binding domain that binds to CD19, a transmembrane domain, and an intracellular signaling domain comprising a co-stimulatory signal region and a primary signaling domain (e.g., CD3zeta).

[0082] In some embodiments, the transmembrane domain is fused to the extracellular domain. The transmembrane domain in some embodiments is derived either from a natural or from a synthetic source. Where the source is natural, the domain in some aspects

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is derived from any membrane-bound or transmembrane protein. Transmembrane regions include those derived from (e.g., comprising at least the transmembrane region(s) of) CD3, CD4, CD5, CD8, CD9, CD 16, CD22, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD154, or a combination thereof. Alternatively, the transmembrane domain in some embodiments is synthetic.

[0083] In several embodiments, the transmembrane domain comprises at least a portion of CD8, a transmembrane glycoprotein normally expressed on both T cells and NK cells. In several embodiments, the transmembrane domain comprises CD8alpha (CD8a). In several embodiments, the transmembrane domain comprises a CD8 (e.g., CD8a) hinge and a CD8 (e.g., CD8a) transmembrane region.

[0084] In several embodiments, the transmembrane domain comprises a hinge, e.g. a CD8a hinge. In several embodiments, the sequence encoding the CD8a hinge is truncated or modified. In some embodiments, the CD8a hinge is encoded by a nucleic acid sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the nucleic acid sequence of SEQ ID NO:5. In several embodiments, the CD8a hinge comprises the nucleic acid sequence of SEQ ID NO:5. In several embodiments, the CD8a hinge is truncated or modified. In some embodiments, the CD8a hinge has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the amino acid sequence of SEQ ID NO:6. In several embodiments, the hinge of CD8a comprises the amino acid sequence of SEQ ID NO:6.

[0085] In several embodiments, the transmembrane domain comprises a CD8a transmembrane region. In several embodiments, the CD8a transmembrane region is truncated or modified. In some embodiments, the CD8a transmembrane region is encoded by a nucleic acid sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the sequence of SEQ ID NO:7. In several embodiments, the CD8a transmembrane region is encoded by a nucleic acid sequence of SEQ ID NO:7. In several embodiments, the CD8a transmembrane region is truncated or modified. In some embodiments, the CD8a transmembrane region has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the sequence of SEQ ID NO:8. In several embodiments, the CD8a transmembrane region comprises the amino acid sequence of SEQ ID NO:8.

[0086] Thus, in several embodiments, the CD8 transmembrane domain is truncated or modified and is encoded by a nucleic acid sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the sequence of SEQ ID NO:9. In several embodiments, the CD8 transmembrane domain is encoded by the nucleic acid sequence of SEQ ID NO:9. In some embodiments, the CD8 transmembrane domain is truncated or modified and comprises an amino acid sequence that

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has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the sequence of SEQ ID NO:10. In several embodiments, the transmembrane domain comprises the amino acid sequence of SEQ ID NO:10.

[0087] In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain or a fragment thereof. In several embodiments, the CD28 transmembrane domain is truncated or modified. In some embodiments, the CD28 transmembrane domain has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the amino acid sequence of SEQ ID NO:11. In several embodiments, the CD28 transmembrane domain comprises the amino acid sequence of SEQ ID NO:11.

[0088] The receptor, e.g., the CAR, generally includes an intracellular signaling domain comprising intracellular signaling components. In some embodiments, the receptor includes an intracellular component of a TCR complex, such as a TCR CD3 chain that mediates T-cell activation and cytotoxicity, e.g., CD3 zeta chain. Thus, in some aspects, the antigen-binding portion is linked to one or more cell signaling modules. In some embodiments, upon ligation of the CAR or other chimeric receptor, the cytoplasmic domain or intracellular signaling domain of the receptor activates at least one of the normal effector functions or responses of the immune cell, e.g., NK cell engineered to express the CAR. For example, in some contexts, the CAR induces a function of an immune cell (e.g., NK cell) such as cytolytic activity and/or secretion of cytokines or other factors. In some embodiments, a truncated portion of an intracellular signaling domain of an antigen receptor component or costimulatory molecule is used in place of an intact immunostimulatory chain, for example, if it transduces the effector function signal. In some embodiments, the intracellular signaling domain includes the cytoplasmic sequences of the T cell receptor (TCR), and in some aspects, also those of co-receptors that in the natural context act in concert with such receptors to initiate signal transduction following antigen receptor engagement.

[0089] In the context of a natural TCR, full activation generally requires not only signaling through the TCR, but also a costimulatory signal. Thus, in some embodiments, to promote full activation, a component for generating secondary or co-stimulatory signal is also included in the receptor. T cell activation is in some aspects described as being mediated by two classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling sequences), and those that act in an antigen independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences). In some aspects, the receptor includes one or both of such signaling components.

[0090] In some aspects, the receptor includes a primary cytoplasmic signaling sequence that regulates primary activation of the TCR complex. Primary cytoplasmic signaling

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sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Examples of ITAM containing primary cytoplasmic signaling sequences include those derived from TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CDS, CD22, CD79a, CD79b, and CD66d. In some embodiments, cytoplasmic signaling molecule(s) in the CAR contain(s) a cytoplasmic signaling domain, portion thereof, or sequence derived from CD3zeta.

[0091] For example, immune cells engineered according to several embodiments disclosed herein may comprise at least one subunit of the CD3 T cell receptor complex (or a fragment thereof). In several embodiments, the signaling domain comprises the CD3 zeta subunit. In several embodiments, the CD3zeta can be truncated or modified. In some embodiments, the CD3zeta is encoded by a nucleic acid sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the nucleic acid sequence of SEQ ID NO:17. In several embodiments, the CD3zeta is encoded by the nucleic acid sequence of SEQ ID NO:17. In several embodiments, the CD3zeta is truncated or modified. In some embodiments, the CD3zeta comprises an amino acid sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the amino acid sequence of SEQ ID NO:18. In several embodiments, the CD3zeta comprises the amino acid sequence of SEQ ID NO:18.

[0092] In some embodiments, the intracellular signaling domain comprises a costimulatory signaling region, such as an intracellular signaling region of CD28, 4-1BB, OX40, DAP10, ICOS, or any combination thereof. In some embodiments, the intracellular signaling domain comprises an intracellular signaling region of CD28. In some embodiments, the intracellular signaling domain comprises an intracellular signaling region of 4-1BB. In some embodiments, the intracellular signaling domain comprises an intracellular signaling region of OX40. In some embodiments, the intracellular signaling domain comprises an intracellular signaling region of DAP10. In some embodiments, the intracellular signaling domain comprises an intracellular signaling region of ICOS. In some embodiments, the intracellular signaling domain does not include DAP10 and/or DAP12. In some embodiments, the intracellular signaling domain does not include DAP10. In some embodiments, the intracellular signaling domain does not include DAP12. In some aspects, the same receptor includes both a CD3zeta and a costimulatory signaling region. Thus, in some embodiments, the intracellular signaling domain of the recombinant receptor, such as CAR, comprises a CD3zeta intracellular domain and a costimulatory signaling region.

[0093] In several embodiments, the intracellular signaling domain comprises an intracellular signaling region of OX40. In several embodiments, the OX40 intracellular signaling region is encoded by a nucleic acid sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the nucleic acid

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sequence of SEQ ID NO:13. In several embodiments, the OX40 intracellular signaling region is encoded by the nucleic acid sequence of SEQ ID NO:13. In several embodiments, the OX40 intracellular signaling region comprises an amino acid sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the amino acid sequence of SEQ ID NO:14. In several embodiments, the OX40 intracellular signaling region comprises the amino acid sequence of SEQ ID NO:14. In several embodiments, OX40 is used as the sole intracellular signaling component in the construct, however, in several embodiments, OX40 can be used with one or more other components. For example, combinations of OX40 and CD3zeta are used in some embodiments. In some embodiments, the intracellular signaling domain comprises an OX40 costimulatory signaling region linked to CD3zeta.

[0094] In some embodiments, the CAR comprises an extracellular antigen-binding domain comprising the sequence set forth in SEQ ID NO:37, a CD8alpha transmembrane domain comprising the amino acid sequence set forth in SEQ ID NO:8, an OX40 intracellular signaling region comprising the amino acid sequence set forth in SEQ ID NO:14, and a CD3zeta domain comprising the amino acid sequence set forth in SEQ ID NO:18. In some embodiments, the CAR comprises an amino acid sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the amino acid sequence set forth in SEQ ID NO:38. In some embodiments, the CAR comprises the amino acid sequence set forth in SEQ ID NO:38.

[0095] By way of further example, combinations of CD28, OX40, 4-1BB and/or CD3zeta are used in some embodiments.

[0096] In several embodiments, the intracellular signaling domain comprises an intracellular signaling region of 4-1BB. In several embodiments, the 4-1BB intracellular signaling region is encoded by a nucleic acid sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the nucleic acid sequence of SEQ ID NO:15. In several embodiments, the 4-1BB intracellular signaling region is encoded by the nucleic acid sequence of SEQ ID NO:15. In several embodiments, the 4-1BB intracellular signaling region comprises an amino acid sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the amino acid sequence of SEQ ID NO:16. In several embodiments, the 4-1BB intracellular signaling region comprises the amino acid sequence of SEQ ID NO:16. In several embodiments, 4-1BB is used as the sole intracellular signaling component in the construct, however, in several embodiments, 4-1BB can be used with one or more other components. For example, combinations of 4-1BB and CD3zeta are used in some embodiments. In some embodiments, the intracellular signaling domain comprises a 4-1BB costimulatory signaling

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region linked to CD3zeta. By way of further example, combinations of CD28, OX40, 4-1BB and/or CD3zeta are used in some embodiments.

[0097] In several embodiments, the intracellular signaling domain comprises an intracellular signaling region of CD28. In several embodiments, the CD28 intracellular signaling region comprises an amino acid sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the amino acid sequence of SEQ ID NO:12. In several embodiments, the CD28 intracellular signaling region comprises the amino acid sequence of SEQ ID NO:12. In several embodiments, CD28 is used as the sole intracellular signaling component in the construct, however, in several embodiments, CD28 can be used with one or more other components. For example, combinations of CD28 and CD3zeta are used in some embodiments. In some embodiments, the intracellular signaling domain comprises a CD28 costimulatory signaling region linked to CD3zeta. By way of further example, combinations of CD28, OX40, 4-1BB and/or CD3zeta are used in some embodiments.

[0098] Additional CD19-directed CARs are known and described in the art, including any of those as described in Kalos et al., Sci Transl Med 3:95ra73 (2011); Porter et al., NEJM 365:725-733 (2011); Grupp et al., NEJM 368: 1509-1518 (2013); and PCT Application Nos. PCT/US2015/024671, PCT/US2018/029107, PCT/US2020/020824, and PCT/CN2021/106892.

[0099] In any of the provided embodiments, the nucleic acid encoding the chimeric receptor, or a portion thereof, is codon-optimized. In some embodiments, the polynucleotides are optimized, or contain certain features designed for optimization, such as for codon usage, to reduce RNA heterogeneity and/or to modify, e.g., increase or render more consistent among cell product lots, expression, such as surface expression, of the encoded receptor. In some embodiments, polynucleotides, encoding chimeric receptors, are modified as compared to a reference polynucleotide, such as to remove cryptic or hidden splice sites, to reduce RNA heterogeneity. In some embodiments, polynucleotides, encoding chimeric receptors, are codon optimized, such as for expression in a mammalian, e.g., human, cell such as in a human T cell. In some aspects, the modified polynucleotides result in improved, e.g., increased or more uniform or more consistent level of, expression, e.g., surface expression, when expressed in a cell.

B. Engineered Cells

[00100] Also provided are methods, nucleic acids, compositions, and kits, for producing the genetically engineered immune cells (e.g., NK cells). In some aspects, the genetic engineering involves introduction of a nucleic acid encoding the genetically engineered component or other component for introduction into the cell, such as a component encoding a gene-disrupting protein or nucleic acid. Among additional nucleic acids, e.g., genes

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for introduction are those to improve the efficacy of therapy, such as by promoting viability and/or function of transferred cells; genes to provide a genetic marker for selection and/or evaluation of the cells, such as to assess in vivo survival or localization; genes to improve safety, for example, by making the cell susceptible to negative selection in vivo.

i. Vectors and Methods for Genetic Engineering

[00101] Also provided are methods, polynucleotides, compositions, and kits, for expressing the binding molecules (e.g., anti-CD19 binding molecules), including recombinant receptors (e.g., CARs) comprising the binding molecules, and for producing the genetically engineered immune cells (e.g., NK cells) expressing such binding molecules. In some embodiments, one or more binding molecules, including recombinant receptors (e.g., CARs) can be genetically engineered into cells or a plurality of cells. The genetic engineering generally involves introduction of a nucleic acid encoding the recombinant or engineered component into the cell, such as by retroviral transduction, transfection, or transformation.

[00102] Also provided are polynucleotides encoding the antibodies and chimeric antigen receptors and/or portions, e.g., chains, thereof. Among the provided polynucleotides are those encoding the anti-CD19 chimeric antigen receptors (e.g., antigen-binding fragment) described herein. Also provided are polynucleotides encoding one or more antibodies and/or portions thereof, e.g., those encoding one or more of the anti-CD19 antibodies (e.g., antigen-binding fragment) described herein and/or other antibodies and/or portions thereof, e.g., antibodies and/or portions thereof that binds other target antigens. The polynucleotides may include those encompassing natural and/or non-naturally occurring nucleotides and bases, e.g., including those with backbone modifications. The terms “nucleic acid molecule”, “nucleic acid” and “polynucleotide” may be used interchangeably, and refer to a polymer of nucleotides. Such polymers of nucleotides may contain natural and/or non-natural nucleotides, and include, but are not limited to, DNA, RNA, and PNA. “Nucleic acid sequence” refers to the linear sequence of nucleotides that comprise the nucleic acid molecule or polynucleotide. Also provided are polynucleotides that have been optimized for codon usage.

[00103] Also provided are vectors containing the polynucleotides, such as any of the polynucleotides described herein, and cells containing the vectors, e.g., for producing the antibodies or antigen-binding fragments thereof. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a retroviral vector. In some embodiments, the vector is a lentiviral vector. Also provided are methods for producing the antibodies or antigen-binding fragments thereof. The nucleic acid may encode an amino acid sequence comprising the VL region and/or an amino acid sequence comprising the VH region of the antibody (e.g., the light and/or heavy chains of the antibody). The nucleic acid may encode one or more amino acid sequence comprising the VL region and/or an amino acid sequence comprising the VH region of the antibody (e.g., the light and/or heavy chains of the antibody). In a further

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embodiment, one or more vectors (e.g., expression vectors) comprising such polynucleotides are provided. In a further embodiment, a host cell comprising such polynucleotides is provided. In another such embodiment, a host cell comprises (e.g., has been transformed with) (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL region of the antibody and an amino acid sequence comprising the VH region of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL region of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH region of the antibody. In some embodiments, a host cell comprises (e.g., has been transformed with) one or more vectors comprising one or more nucleic acid that encodes one or more an amino acid sequence comprising one or more antibodies and/or portions thereof, e.g., antigen-binding fragments thereof. In some embodiments, one or more such host cells are provided. In some embodiments, a composition containing one or more such host cells are provided. In some embodiments, the one or more host cells can express different antibodies, or the same antibody. In some embodiments, each of the host cells can express more than one antibody.

[00104] Also provided are methods of making the anti-CD19 chimeric antigen receptors. For recombinant production of the chimeric receptors, a nucleic acid sequence encoding a chimeric receptor antibody, e.g., as described herein, may be isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid sequences may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). In some embodiments, a method of making the anti-CD19 chimeric antigen receptor is provided, wherein the method comprises culturing a host cell comprising a nucleic acid sequence encoding the antibody, as provided above, under conditions suitable for expression of the receptor. In particular examples, immune cells, such as human immune cells are used to express the provided polypeptides encoding chimeric antigen receptors. In some examples, the immune cells are NK cells including primary NK cells.

[00105] In some embodiments, gene transfer is accomplished by transduction of the immune cells (e.g., activated immune cells), and expansion in culture to numbers sufficient for clinical applications. In some aspects, the cells further are engineered to promote expression of cytokines or other factors. Various methods for the introduction of genetically engineered components, e.g., antigen receptors, e.g., CARs, are well known and may be used with the provided methods and compositions. Non-limiting examples of methods include those for transfer of polynucleotides encoding the receptors, including via viral, e.g., retroviral or lentiviral, transduction, transposons, and electroporation.

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[00106] In some embodiments, recombinant polynucleotides are transferred into immune cells (e.g., NK cells) using recombinant infectious virus particles, such as, e.g., vectors derived from simian virus 40 (SV40), adenoviruses, adeno-associated virus (AAV). In some embodiments, recombinant polynucleotides are transferred into immune cells using recombinant lentiviral vectors or retroviral vectors, such as gamma-retroviral vectors. In some embodiments, the retroviral vector has a long terminal repeat sequence (LTR), e.g., a retroviral vector derived from the Moloney murine leukemia virus (MoMLV), myeloproliferative sarcoma virus (MPSV), murine embryonic stem cell virus (MESV), murine stem cell virus (MSCV), spleen focus forming virus (SFFV), or human immunodeficiency virus type 1 (HIV-1). Most retroviral vectors are derived from murine retroviruses. In some embodiments, the retroviruses include those derived from any avian or mammalian cell source. The retroviruses typically are amphotropic, meaning that they are capable of infecting host cells of several species, including humans. In one embodiment, the gene to be expressed replaces the retroviral gag, pol and/or env sequences. A number of illustrative retroviral systems have been described. Methods of lentiviral transduction are known and described in the art.

[00107] Among additional polynucleotides, e.g., genes for introduction are those to improve the efficacy of therapy, such as by promoting viability and/or function of transferred cells; genes to provide a genetic marker for selection and/or evaluation of the cells, such as to assess in vivo survival or localization; genes to improve safety, for example, by making the cell susceptible to negative selection in vivo.

[00108] In some cases, the polynucleotide containing nucleic acid sequences encoding the CD19-binding receptor, e.g., chimeric antigen receptor (CAR), contains a signal sequence that encodes a signal peptide. In some aspects, the signal sequence may encode a signal peptide derived from a native polypeptide. In other aspects, the signal sequence may encode a heterologous or non-native signal peptide. In some aspects, a non-limiting example of a signal peptide comprises a CD8 alpha (CD8a) signal peptide set forth in SEQ ID NO:4.

[00109] In some embodiments the vector or construct can contain promoter and/or enhancer or regulatory elements to regulate expression of the encoded recombinant receptor. In some examples the promoter and/or enhancer or regulatory elements can be condition-dependent promoters, enhancers, and/or regulatory elements. In some examples these elements drive expression of the transgene.

[00110] In some embodiments, the vector or construct can contain a single promoter that drives the expression of one or more nucleic acid molecules. In some embodiments, such nucleic acid molecules, e.g., transcripts, can be multicistronic (bicistronic or tricistronic). For example, in some embodiments, transcription units can be engineered as a bicistronic unit containing an IRES (internal ribosome entry site), which allows coexpression of gene products (e.g. encoding a chimeric receptor and membrane-bound interleukin-15) by

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a message from a single promoter. Alternatively, in some cases, a single promoter may direct expression of an RNA that contains, in a single open reading frame (ORF), two or three genes (e.g. encoding a chimeric receptor and membrane-bound interleukin-15) separated from one another by sequences encoding a self-cleavage peptide (e.g., 2A cleavage sequences) or a protease recognition site. The ORF thus encodes a single polypeptide, which, either during (in the case of T2A) or after translation, is cleaved into the individual proteins. In some cases, the peptide, such as T2A, can cause the ribosome to skip (ribosome skipping) synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream. Many 2A elements are known. Examples of 2A peptides that can be used in the methods and polynucleotides disclosed herein, without limitation, 2A peptides from the foot-and-mouth disease virus (F2A), equine rhinitis A virus (E2A), *Thossea asigna* virus (T2A, e.g. SEQ ID NO:20, encoded by SEQ ID NO:19), and porcine teschovirus-1 (P2A). In some embodiments, the one or more different or separate promoters drive the expression of a nucleic acid molecule encoding a binding molecule, e.g., recombinant receptor and a nucleic acid encoding membrane-bound interleukin-15.

[00111] Membrane-Bound Interleukin 15 (mbIL15)

[00112] In several embodiments, any of the immune cells as provided herein are engineered to express interleukin 15 (IL15). In several embodiments, any of the immune cells as provided herein are engineered to express a membrane-bound interleukin 15 (mbIL15). In such embodiments, mbIL15 expression on the immune cell (e.g., NK cell) enhances the cytotoxic effects of the engineered cell by enhancing the proliferation and/or longevity of the cells. In some embodiments, the IL15 is expressed from a separate cassette on the construct comprising any one of the CARs disclosed herein. In some embodiments, the IL15 is expressed from the same cassette as any one of the CARs disclosed herein.

[00113] In some embodiments, the chimeric receptor and IL15 are separated by a nucleic acid sequence encoding a cleavage site, for example, a proteolytic cleavage site or a T2A, P2A, E2A, or F2A self-cleaving peptide cleavage site. In some embodiments, the chimeric receptor and IL15 are separated by a T2A peptide (e.g., SEQ ID NO:20, encoded by SEQ ID NO:19). In some embodiments, the IL15 is a membrane-bound IL15 (mbIL15). In some embodiments, the mbIL15 comprises a native IL15 sequence, such as a human native IL15 sequence (e.g., SEQ ID NO:22, encoded by SEQ ID NO:21), and at least one transmembrane domain (e.g., CD8a). In several embodiments, IL15 is encoded by the nucleic acid sequence of SEQ ID NO: 21. In several embodiments, IL15 can be truncated or modified, such that it is encoded by a nucleic acid sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the nucleic acid sequence of SEQ ID NO: 21. In several embodiments, the IL15 comprises the amino acid sequence of SEQ ID NO: 22. In several embodiments, the IL15 is truncated or modified, such that it has

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at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 22.

[00114] Thus, in some embodiments, any of the CARs as described herein are encoded by the same nucleic acid sequence as a mbIL15. In some embodiments, a nucleic acid sequence encoding the CAR and a nucleic acid sequence encoding the mbIL15 are separated by a 2A (e.g., T2A, P2A, E2A, or F2A)-encoding sequence. In some embodiments, a nucleic acid sequence encoding the CAR and a nucleic acid sequence encoding the mbIL15 are separated by a T2A-encoding sequence (e.g., SEQ ID NO:19). In some embodiments, any of the engineered cells as described herein express a CD19-targeting recombinant receptor (e.g., CAR) and a mbIL15.

[00115] In some embodiments, the mbIL15 is membrane-bound by virtue of the fusion of IL15 to a transmembrane domain. Thus, in some embodiments, mbIL15 comprises a transmembrane domain. In some embodiments, the transmembrane domain comprises a CD8a transmembrane domain. In some embodiments, the transmembrane domain comprises a hinge and/or a transmembrane region. In some embodiments, the transmembrane domain comprises a hinge and a transmembrane region. In some embodiments, the hinge is a CD8a hinge sequence (e.g., SEQ ID NO:6). In some embodiments, the transmembrane region is a CD8a transmembrane region (e.g., SEQ ID NO:8). In some embodiments, the mbIL15 comprises a native IL15 sequence, such as a human native IL15 sequence, and at least one transmembrane domain (e.g., CD8a transmembrane domain). In some embodiments, the CD8a transmembrane domain comprises the sequence of SEQ ID NO:10. In several embodiments, the mbIL15 is truncated or modified such that it comprises an amino acid sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% sequence identity to the amino acid sequence of SEQ ID NO:23. In several embodiments, the mbIL15 comprises the amino acid sequence of SEQ ID NO:23. Membrane-bound IL15 sequences are described in PCT publications WO 2018/183385 and WO 2020/056045, each of which is hereby expressly incorporated by reference in its entirety.

ii. Cell Types

[00116] Some embodiments of the methods and compositions provided herein relate to a cell such as an immune cell. For example, an immune cell, such as an NK cell or a T cell, may be engineered to express a CD19-targeting CAR.

[00117] As opposed to traditional anti-cancer therapies such as surgical approaches, radiation therapy, chemotherapy, or combinations of these methods, targeted therapy is a cancer treatment that employs certain drugs that target specific genes or proteins found in cancer cells or cells supporting cancer growth, (like blood vessel cells) to reduce or arrest cancer cell growth. More recently, genetic engineering has enabled approaches to be developed that harness certain aspects of the immune system to fight cancers. In several

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embodiments, a patient's own immune cells, immune cells of a donor, or cells derived from a pluripotent cell, are modified to specifically eradicate that patient's type of cancer. Various types of immune cells can be used, such as T cells, Natural Killer (NK cells), or combinations thereof, as described in more detail below.

[00118] To facilitate cancer immunotherapies, there are also provided for herein polypeptides comprising a CAR, as well as polynucleotides and vectors encoding the same. In some aspects, the CAR comprises a target binding moiety (e.g., an extracellular antigen-binding domain) operably coupled to a cytotoxic signaling complex. For example, some embodiments include a CAR comprising an extracellular antigen-binding domain that is directed against a tumor marker, for example, CD19, to facilitate targeting of an immune cell to a CD19-expressing cancer. Also provided are engineered immune cells (e.g., NK cells) expressing such CARs. Also provided are compositions (e.g., pharmaceutical compositions) comprising engineered immune cells (e.g., NK cells) expressing such CARs. Methods of treating cancer and other uses of such cells for cancer immunotherapy are also provided for herein.

[00119] In several embodiments, cells of the immune system are engineered to have enhanced cytotoxic effects against target cells, such as tumor cells. For example, a cell of the immune system may be engineered to include a tumor-directed chimeric antigen receptor (CAR) as described herein. In several embodiments, white blood cells or leukocytes, are used, since their native function is to defend the body against growth of abnormal cells and infectious disease. There are a variety of types of white blood cells that serve specific roles in the human immune system, and are therefore a preferred starting point for the engineering of cells disclosed herein. White blood cells include granulocytes and agranulocytes (presence or absence of granules in the cytoplasm, respectively). Granulocytes include basophils, eosinophils, neutrophils, and mast cells. Agranulocytes include lymphocytes and monocytes. Cells such as those listed above or those that follow or are otherwise described herein may be engineered to express a CAR, for example by providing to the cell a nucleic acid encoding the CAR. In several embodiments, the immune cells are also engineered to express interleukin 15, such as a membrane-bound interleukin 15 (mbIL15). Thus, in several embodiments, the cells are engineered to express a CAR and IL15 (e.g., mbIL15).

a. Monocytes

[00120] In some embodiments, the immune cells comprise monocytes. Monocytes are a subtype of leukocyte. Monocytes can differentiate into macrophages and myeloid lineage dendritic cells. Monocytes are associated with the adaptive immune system and serve the main functions of phagocytosis, antigen presentation, and cytokine production.

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Phagocytosis is the process of uptake of cellular material, or entire cells, followed by digestion and destruction of the engulfed cellular material.

[00121] In some embodiments, a monocyte is positive for cell surface expression of a marker selected from among the group consisting of CCR2, CCR5, CD11c, CD14, CD16, CD62L, CD68+, CX3CR1, HLA-DR, or any combination thereof. In some embodiments, a monocyte is positive for cell surface expression of CD14. In some embodiments, a monocyte is positive for cell surface expression of CCR2. In some embodiments, a monocyte is positive for cell surface expression of CCR5. In some embodiments, a monocyte is positive for cell surface expression of CD62L.

[00122] In several embodiments, monocytes are used in connection with one or more additional engineered cells as disclosed herein. Some embodiments of the methods and compositions described herein relate to a monocyte that expresses a CAR that binds to CD19, or a nucleic acid encoding the CAR.

[00123] In some embodiments, the monocytes are engineered to express a membrane-bound interleukin 15 (mbIL15) domain. In some embodiments, the monocytes engineered to express a CAR are engineered to also express (e.g., bicistronically express) a membrane-bound interleukin 15 (mbIL15) domain. Thus, in some embodiments, the monocytes are engineered to bicistronically express the CAR and mbIL15.

[00124] In some embodiments, the monocytes are allogeneic cells. In some embodiments, the monocytes are obtained from a donor who does not have a cancer.

[00125] In some embodiments, the monocytes are autologous cells.

b. Lymphocytes

[00126] In some embodiments, the immune cells comprise lymphocytes. Lymphocytes, the other primary sub-type of leukocyte include T cells (cell-mediated, cytotoxic adaptive immunity), natural killer cells (cell-mediated, cytotoxic innate immunity), and B cells (humoral, antibody-driven adaptive immunity). While B cells are engineered according to several embodiments, disclosed herein, several embodiments also relate to engineered T cells or engineered NK cells (mixtures of T cells and NK cells are used in some embodiments, either from the same donor, or different donors). Thus, in some embodiments, the immune cells comprise T cells. In some embodiments, the immune cells comprise NK cells. In some embodiments, the immune cells comprise T cells and NK cells. In some embodiments, the immune cells comprise B cells.

[00127] In several embodiments, lymphocytes are used in connection with one or more additional engineered cells as disclosed herein. Some embodiments of the methods and compositions described herein relate to a lymphocyte that expresses a CAR that binds to CD19, or a nucleic acid encoding the CAR.

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[00128] In some embodiments, the lymphocytes are engineered to express a membrane-bound interleukin 15 (mbIL15) domain. In some embodiments, the lymphocytes engineered to express a CAR are engineered to also express (e.g., bicistronically express) a membrane-bound interleukin 15 (mbIL15) domain. Thus, in some embodiments, lymphocytes are engineered to bicistronically express the CAR and mbIL15.

[00129] In some embodiments, the lymphocytes are allogeneic cells. In some embodiments, the lymphocytes are obtained from a donor who does not have a cancer.

[00130] In some embodiments, the lymphocytes are autologous cells.

c. T Cells

[00131] In some embodiments, the immune cells comprise T cells. T cells are distinguishable from other lymphocytes sub-types (e.g., B cells or NK cells) based on the presence of a T-cell receptor on the cell surface.

[00132] T cells can be divided into various different subtypes, including effector T cells, helper T cells, cytotoxic T cells, memory T cells, regulatory T cells, natural killer T cell, mucosal associated invariant T cells and gamma delta T cells. In some embodiments, a specific subtype of T cell is engineered. In some embodiments, a T cell is positive for cell surface expression of a marker selected from among the group consisting of CD3, CD4, and/or CD8. In some embodiments, a T cell is positive for cell surface expression of CD3. In some embodiments, a T cell is positive for cell surface expression of CD4. In some embodiments, a T cell is positive for cell surface expression of CD8.

[00133] In some embodiments, CD3+ T cells are engineered. In some embodiments, CD4+ T cells are engineered. In some embodiments, CD8+ T cells are engineered. In some embodiments, regulatory T cells are engineered. In some embodiments, gamma delta T cells are engineered. In some embodiments, a mixed pool of T cell subtypes is engineered. For example, in some embodiments, CD4+ and CD8+ T cells are engineered. In some embodiments, there is no specific selection of a type of T cells to be engineered to express the cytotoxic receptor complexes disclosed herein. In several embodiments, specific techniques, such as use of cytokine stimulation are used to enhance expansion/collection of T cells with a specific marker profile. For example, in several embodiments, activation of certain human T cells, e.g. CD4+ T cells, CD8+ T cells is achieved through use of CD3 and/or CD28 as stimulatory molecules.

[00134] In several embodiments, there is provided a method of treating or preventing a cancer, comprising administering T cells expressing a cytotoxic receptor complex as described herein. In several embodiments, the engineered T cells are autologous cells, while in some embodiments, the T cells are allogeneic cells. In some embodiments, the T cells are allogeneic cells. In some embodiments, the T cells are obtained from a donor who does not have a cancer.

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[00135] Several embodiments of the methods and compositions disclosed herein relate to T cells engineered to express a CAR that binds to CD19. In some embodiments, the T cells are engineered to express a membrane-bound interleukin 15 (mbIL15) domain. In some embodiments, the T cells engineered to express a CAR are engineered to also express (e.g., bicistronically express) a membrane-bound interleukin 15 (mbIL15) domain. Thus, in some embodiments, the T cells are engineered to bicistronically express the CAR and mbIL15.

[00136] In some embodiments, the immune cells comprise T cells and NK cells (either from the same donor or from different donors).

[00137] In some embodiments, the T cells are autologous cells.

d. Natural Killer (NK) Cells

[00138] In some embodiments, the immune cells comprise natural killer (NK) cells. In several embodiments, there is provided a method of treating or preventing a cancer, comprising administering natural killer (NK) cells expressing a CD19-targeting CAR as described herein. In several embodiments, the engineered NK cells are autologous cells, while in some embodiments, the NK cells are allogeneic cells. In some embodiments, the NK cells are autologous cells. In some embodiments, the NK cells are allogeneic. In some embodiments, the NK cells are derived from a donor who does not have a cancer.

[00139] In several embodiments, NK cells are preferred because the natural cytotoxic potential of NK cells is relatively high. In several embodiments, it is unexpectedly beneficial that the engineered cells disclosed herein can further upregulate the cytotoxic activity of NK cells, leading to an even more effective activity against target cells (e.g., tumor or other diseased cells).

[00140] In some embodiments, a NK cell is positive for cell surface expression of a marker selected from among the group consisting of CCR7, CD16, CD56, CD57, CD11, CX3CR1, a Killer Ig-like receptor (KIR), NKp30, NKp44, NKp46, or any combination thereof. In some embodiments, a NK cell is positive for cell surface expression of CD16. In some embodiments, a NK cell is positive for cell surface expression of CD56. In some embodiments, a NK cell is positive for cell surface expression of a Killer Ig-like receptor.

[00141] Some embodiments of the methods and compositions described herein relate to NK cells engineered to express a CAR that binds to CD19. In some embodiments, the NK cells are engineered to a membrane-bound interleukin 15 (mbIL15) domain. In some embodiments, the NK cells engineered to express a CAR are engineered to also express (e.g., bicistronically express) a membrane-bound interleukin 15 (mbIL15) domain. Thus, in some embodiments, the NK cells are engineered to bicistronically express the CAR and mbIL15.

[00142] In some embodiments, the NK cells are derived from cell line NK-92. NK-92 cells are derived from NK cells, but lack major inhibitory receptors displayed by normal

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NK cells, while retaining the majority of activating receptors. Some embodiments of NK-92 cells described herein related to NK-92 cell engineered to silence certain additional inhibitory receptors, for example, SMAD3, allowing for upregulation of interferon- γ (IFN γ), granzyme B, and/or perforin production. Additional information relating to the NK-92 cell line is disclosed in WO 1998/49268 and U.S. Patent Application Publication No. 2002-0068044 and incorporated in their entireties herein by reference.

[00143] In some embodiments, the NK cells are used in combination with T cells. Thus, in some embodiments, the immune cells comprise T cells and NK cells (either from the same donor or from different donors). For example, in one embodiment, primary NK cells are used in combination with primary T cells.

[00144] Hematopoietic Stem Cells (HSCs)

[00145] In some embodiments, the immune cells comprise hematopoietic stem cells (HSCs). In some embodiments, HSCs are used in the methods disclosed herein. In several embodiments, the cells are engineered to express a CAR that binds to CD19.

[00146] In some embodiments, a HSC is positive for cell surface expression of a marker selected from among the group consisting of CD34, CD59, and CD90. In some embodiments, a HSC is positive for cell surface expression of CD34. In some embodiments, a HSC is positive for cell surface expression of CD59. In some embodiments, a HSC is positive for cell surface expression of CD90.

[00147] In several embodiments allogeneic HSCs are used, while in some embodiments, autologous HSCs are used. In several embodiments, HSCs are used in combination with one or more additional engineered cell type disclosed herein. Some embodiments of the methods and compositions described herein relate to a stem cell, such as a HSC engineered to express a CAR that binds to CD19, or a nucleic acid encoding the CAR.

[00148] In some embodiments, the HSCs are engineered to express a membrane-bound interleukin 15 (mbIL15) domain. In some embodiments, the HSCs engineered to express a CAR are engineered to also express (e.g., bicistronically express) a membrane-bound interleukin 15 (mbIL15) domain. Thus, in some embodiments, HSCs are engineered to bicistronically express the CAR and mbIL15.

[00149] In some embodiments, the HSCs are allogeneic cells. In some embodiments, the HSCs are obtained from a donor who does not have a cancer.

[00150] In some embodiments, the HSCs are autologous cells.

e. Induced Pluripotent Stem Cells (iPSCs)

[00151] In some embodiments, immune cells are derived (differentiated) from pluripotent stem cells (PSCs). In some embodiments, immune cells (e.g., NK cells) derived from induced pluripotent stem cells (iPSCs) are used in the method of immunotherapy

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disclosed herein. For example, in some embodiments, NK cells are derived from iPSCs. In some embodiments, induced pluripotent stem cells (iPSCs) are used in a method disclosed herein. iPSCs are used, in several embodiments, to leverage their ability to differentiate and derive into non-pluripotent cells, including, but not limited to, CD34 cells, hemogenic endothelium cells, HSCs (hematopoietic stem and progenitor cells), hematopoietic multipotent progenitor cells, T cell progenitors, NK cell progenitors, T cells, NKT cells, NK cells, and B cells comprising one or several genetic modifications at selected sites through differentiating iPSCs or less differentiated cells comprising the same genetic modifications at the same selected sites. In several embodiments, the iPSCs are used to generate iPSC-derived NK cells.

[00152] Several embodiments of the methods and compositions disclosed herein relate to induced pluripotent stem cells engineered to express a CAR that binds to CD19. In some embodiments, the iPSCs engineered to express a CAR are engineered to also express (e.g., bicistronically express) a membrane-bound interleukin 15 (mbIL15).

[00153] In several embodiments, the engineered iPSCs are differentiated into NK, T, or other immune cells, such as for use in a composition or method provided herein. In several embodiments, the engineered iPSCs are differentiated into NK cells.

[00154] In some embodiments, the iPSCs are allogeneic cells. In some embodiments, the iPSCs are obtained from a donor who does not have a cancer.

[00155] In some embodiments, the iPSCs are autologous cells.

C. Preparation of Cells for Genetic Engineering

[00156] In some embodiments, preparation of the engineered cells includes one or more culture and/or preparation steps. The cells for introduction of the recombinant receptor (e.g., CAR) may be isolated from a sample, such as a biological sample, e.g., one obtained from or derived from a subject. In some embodiments, the sample is an apheresis (e.g., leukapheresis) sample.

[00157] In some embodiments, the subject from which the cells are isolated is one not having the cancer or in need of a cell therapy or not to which a cell therapy will be administered. In some embodiments, the cells are isolated from a subject that is different than the subject in need of a cell therapy or to which a cell therapy will be administered. Thus, in some embodiments, the cells are allogeneic to the subject to whom they are administered.

[00158] In some embodiments, the subject from which the cells are isolated is one having the cancer or in need of a cell therapy or to which a cell therapy will be administered. In some embodiments, the cells are isolated from the subject to which a cell therapy will be administered. Thus, in some embodiments, the cells are autologous to the subject to whom they are administered.

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[00159] The samples include tissue, fluid, and other samples taken directly from the subject, as well as samples resulting from one or more processing steps, such as separation, centrifugation, genetic engineering (e.g., transduction with viral vector), washing, and/or incubation. The biological sample can be a sample obtained directly from a biological source or a sample that is processed. Biological samples include, but are not limited to, body fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and sweat, tissue and organ samples, including processed samples derived therefrom.

[00160] In some aspects, the sample from which the cells are derived or isolated is blood or a blood-derived sample, or is or is derived from an apheresis (e.g., a leukapheresis) product. Non-limiting samples include whole blood, peripheral blood mononuclear cells (PBMCs), leukocytes, bone marrow, thymus, tissue biopsy, tumor, leukemia, lymphoma, lymph node, gut associated lymphoid tissue, mucosa associated lymphoid tissue, spleen, other lymphoid tissues, liver, lung, stomach, intestine, colon, kidney, pancreas, breast, bone, prostate, cervix, testes, ovaries, tonsil, or other organ, and/or cells derived therefrom. Samples include, in the context of cell therapy, e.g., adoptive cell therapy, samples from autologous and allogeneic sources.

[00161] The cells in some embodiments are primary cells, e.g., primary human cells. In some embodiments, the cells are immune cells, e.g. primary NK cells.

[00162] In some embodiments, isolation of the cells includes one or more preparation and/or non affinity-based cell separation steps. In some examples, cells are washed, centrifuged, and/or incubated in the presence of one or more reagents, for example, to remove unwanted components, enrich for desired components, lyse or remove cells sensitive to particular reagents. In some examples, cells are separated based on one or more property, such as density, adherent properties, size, sensitivity and/or resistance to particular components.

[00163] In some examples, cells from the circulating blood of a subject are obtained, e.g., by apheresis (e.g., leukapheresis). The samples, in some aspects, contain lymphocytes, including NK cells, T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and/or platelets, and in some aspects contain cells other than red blood cells and platelets.

[00164] In some embodiments, the isolation methods include the separation of different cell types based on the expression or presence in the cell of one or more specific molecules, such as surface markers, e.g., surface proteins, intracellular markers, or nucleic acid. In some embodiments, any known method for separation based on such markers may be used. In some embodiments, the separation is affinity- or immunoaffinity-based separation. For example, the isolation in some aspects includes separation of cells and cell populations based on the cells' expression or expression level of one or more markers, typically cell

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surface markers, for example, by incubation with an antibody or binding partner that specifically binds to such markers, followed generally by washing steps and separation of cells having bound the antibody or binding partner, from those cells having not bound to the antibody or binding partner.

[00165] Such separation steps can be based on positive selection, in which the cells having bound the reagents are retained for further use, and/or negative selection, in which the cells having not bound to the antibody or binding partner are retained. In some examples, both fractions are retained for further use. In some aspects, negative selection can be particularly useful where no antibody is available that specifically identifies a cell type in a heterogeneous population, such that separation is best carried out based on markers expressed by cells other than the desired population.

[00166] The separation need not result in 100% enrichment or removal of a particular cell population or cells expressing a particular marker. For example, positive selection of or enrichment for cells of a particular type, such as those expressing a marker, refers to increasing the number or percentage of such cells, but need not result in a complete absence of cells not expressing the marker. Likewise, negative selection, removal, or depletion of cells of a particular type, such as those expressing a marker, refers to decreasing the number or percentage of such cells, but need not result in a complete removal of all such cells.

[00167] In some examples, multiple rounds of separation steps are carried out, where the positively or negatively selected fraction from one step is subjected to another separation step, such as a subsequent positive or negative selection. In some examples, a single separation step can deplete cells expressing multiple markers simultaneously, such as by incubating cells with a plurality of antibodies or binding partners, each specific for a marker targeted for negative selection. Likewise, multiple cell types can simultaneously be positively selected by incubating cells with a plurality of antibodies or binding partners expressed on the various cell types.

[00168] For example, in some aspects, NK cells or specific subpopulations thereof, such as cells positive or expressing high levels of one or more surface markers, e.g., CD56+, CCR7+, CD16+, CD57+, CD11+, CX3CR1+, a Killer Ig-like receptor (KIR) +, NKp30+, NKp44+, or NKp46+ NK cells, are isolated by positive or negative selection techniques. For example, CD56+ NK cells can be positively selected using anti-CD56 conjugated magnetic beads.

[00169] In some embodiments, the cells (e.g., NK cells) are expanded in culture prior to, during, and/or following genetic engineering. In some embodiments, the cells are expanded in culture prior to genetic engineering. In some embodiments, the cells are expanded in culture following genetic engineering. In some embodiments, the cells are expanded in culture prior to and following genetic engineering. Methods for expanding cells

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are known in the art and include any of those described in US Patent Nos. 7,435,596 and 8,026,097; and Patent Application Nos. PCT/SG2018/050138; PCT/US2020/044033; PCT/US2021/071330; and PCT/US2022/074164.

[00170] In some embodiments, expanding the cells in culture comprises co-culturing the cells with feeder cells. In some embodiments, the feeder cells express IL15 (e.g., membrane-bound IL15) and 4-1BBL. In some embodiments, the feeder cells express membrane-bound interleukin 15 (mbIL15) and 4-1BBL. In some embodiments, the feeder cells do not express MHC I molecules. In some embodiments, the feeder cells do not express MHC II molecules. In some embodiments, the feeder cells are immune cells. In some embodiments, the feeder cells are K562 cells. Engineered feeder cells are disclosed in, for example, International Patent Application PCT/SG2018/050138.

[00171] In some embodiments, expanding the cells in culture comprising culturing the cells in the presence of IL2, IL12, and/or IL18. In some embodiments, the cells are cultured in the presence of IL2. In some embodiments, the cells are cultured in the presence of IL12. In some embodiments, the cells are cultured in the presence of IL18. In some embodiments, the cells are cultured in the presence of IL12 and IL18. In some embodiments, the cells are cultured in the presence of IL2, IL12, and IL18.

[00172] In some embodiments, the preparation methods include steps for freezing, e.g., cryopreserving, the cells, either before or after isolation, engineering, and/or expansion. In some embodiments, the cells are suspended in a freezing solution. In some embodiments, a composition provided herein is cryopreserved (e.g., prior to infusion into a subject). Any of a variety of known freezing solutions and parameters in some aspects may be used.

D. Genetic Editing of Cells

[00173] Provided herein are methods and uses of genetically engineered immune cells, including genetically engineered immune cells that are genetically edited, and/or compositions thereof. In some aspects, the immune cells are genetically edited to increase or decrease expression of a target protein. In some aspects, the immune cells are genetically edited to increase expression of a target protein. In some aspects, the immune cells are genetically edited to decrease expression of a target protein. In some aspects, the methods comprise genetically editing the immune cells, such as to increase or decrease expression of a target protein. In some aspects, the methods comprise genetically editing the immune cells to increase expression of a target protein. In some aspects, the methods comprise genetically editing the immune cells to decrease expression of a target protein. Expression of a target protein can be reduced by disrupting a gene (a target gene) encoding the target protein or a portion thereof.

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[00174] It is contemplated that the immune cells can be genetically edited at any point prior to, during, and/or after the genetic engineering. In some embodiments, the immune cells are genetically edited prior to the genetic engineering. In some embodiments, the immune cells are genetically edited contemporaneously with the genetic engineering. In some embodiments, the immune cells are genetically edited after the genetic engineering.

[00175] As discussed below, in several embodiments, genetic editing is employed to reduce or eliminate expression of a target protein, for example by disrupting a gene encoding the protein. In several embodiments, genetic editing can reduce transcription of a target gene by about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97%, about 98%, about 99%, or more (including any amount between those listed). In several embodiments, genetic editing reduces transcription of a target gene by at least about 30%. In several embodiments, genetic editing reduces transcription of a target gene by at least about 40%. In several embodiments, genetic editing reduces transcription of a target gene by at least about 50%. In several embodiments, genetic editing reduces transcription of a target gene by at least about 60%. In several embodiments, genetic editing reduces transcription of a target gene by at least about 70%. In several embodiments, genetic editing reduces transcription of a target gene by at least about 80%. In several embodiments, genetic editing reduces transcription of a target gene by at least about 90%. In several embodiments, genetic editing reduces transcription of a target gene by at least about 95%. In several embodiments, genetic editing reduces transcription of a target gene by at least about 99%. In several embodiments, the gene is completely knocked out, such that transcription of the target gene is eliminated (undetectable).

[00176] In several embodiments, genetic editing can reduce expression of a target protein by about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97%, about 98%, about 99%, or more (including any amount between those listed). In several embodiments, genetic editing reduces expression of a target protein by at least about 30%. In several embodiments, genetic editing reduces expression of a target protein by at least about 40%. In several embodiments, genetic editing reduces expression of a target protein by at least about 50%. In several embodiments, genetic editing reduces expression of a target protein by at least about 60%. In several embodiments, genetic editing reduces expression of a target protein by at least about 70%. In several embodiments, genetic editing reduces expression of a target protein by at least about 80%. In several embodiments, genetic editing reduces expression of a target protein by at least about 90%. In several embodiments, genetic editing reduces expression of a target protein by at least about 95%. In several embodiments, genetic editing reduces expression of a target protein by at least about 99%. In several embodiments, the gene is completely knocked out, such that expression of the target protein is eliminated (undetectable).

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[00177] In several embodiments, genetic editing is used to “knock in” or otherwise increase transcription of a target gene. In several embodiments, transcription of a target gene is increased by about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97%, about 98%, about 99%, or more (including any amount between those listed). In several embodiments, transcription of a target gene is increased by at least about 30%. In several embodiments, transcription of a target gene is increased by at least about 40%. In several embodiments, transcription of a target gene is increased by at least about 50%. In several embodiments, transcription of a target gene is increased by at least about 60%. In several embodiments, transcription of a target gene is increased by at least about 70%. In several embodiments, transcription of a target gene is increased by at least about 80%. In several embodiments, transcription of a target gene is increased by at least about 90%. In several embodiments, transcription of a target gene is increased by at least about 100%.

[00178] In several embodiments, genetic editing is used to “knock in” or otherwise enhance expression of a target protein. In several embodiments, expression of a target protein can be enhanced by about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97%, about 98%, about 99%, or more (including any amount between those listed). In several embodiments, expression of a target protein is increased by at least about 30%. In several embodiments, expression of a target protein is increased by at least about 40%. In several embodiments, expression of a target protein is increased by at least about 50%. In several embodiments, expression of a target protein is increased by at least about 60%. In several embodiments, expression of a target protein is increased by at least about 70%. In several embodiments, expression of a target protein is increased by at least about 80%. In several embodiments, expression of a target protein is increased by at least about 90%. In several embodiments, expression of a target protein is increased by at least about 100%.

[00179] As discussed in more detail below, a variety of approaches can be employed in a given embodiment to improve or alter one or more characteristics of immune cells. Genetic editing can be used to reduce, eliminate (e.g., knockout), or increase expression of a target gene. For example, the transcription of the target gene and/or the translation of a protein encoded by the target gene (e.g., a target protein) can be reduced, eliminated (e.g., knocked out), or increased. The target gene can be implicated in the immune functionality of the cell or be a part of a signaling pathway for which an increase or decrease in function is desired. Further detailed below are various gene targets. Disruption of certain genes in immune cells (e.g., NK cells) can increase activity and/or persistence of those immune cells.

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i. Methods of Genetic Editing

[00180] In several embodiments, genetic editing (whether knock out or knock in) of a target gene is accomplished through targeted introduction of DNA breakage, and a subsequent DNA repair mechanism. In several embodiments, double strand breaks of DNA are repaired by non-homologous end joining (NHEJ), wherein enzymes are used to directly join the DNA ends to one another to repair the break. NHEJ is an error-prone process. In general, in the absence of a repair template, the NHEJ process re-ligates the ends of the cleaved DNA strands, which frequently results in nucleotide deletions and insertions at the cleavage site. In several embodiments, however, double strand breaks are repaired by homology directed repair (HDR), which is advantageously more accurate, thereby allowing sequence specific breaks and repair. HDR uses a homologous sequence as a template for regeneration of missing DNA sequences at the break point, such as a vector with the desired genetic elements (e.g., an insertion element to disrupt the coding sequence of a TCR subunit) within a sequence that is homologous to the flanking sequences of a double strand break. This will result in the desired change (e.g., insertion) being inserted at the site of the DSB. The HDR pathway can occur by way of the canonical HDR pathway or the alternative HDR pathway. Unless otherwise indicated, the term “HDR” or “homology-directed repair” as used herein encompasses both canonical HDR and alternative HDR.

[00181] Canonical HDR or “canonical homology-directed repair” or “cHDR,” are used interchangeably, and refers to the process of repairing DNA damage using a homologous nucleic acid (e.g., an endogenous homologous sequence, such as a sister chromatid; or an exogenous nucleic acid, such as a donor template). Canonical HDR typically acts when there has been a significant resection at the DSB, forming at least one single-stranded portion of DNA. In a normal cell, canonical HDR typically involves a series of steps such as recognition of the break, stabilization of the break, resection, stabilization of single-stranded DNA, formation of a DNA crossover intermediate, resolution of the crossover intermediate, and ligation. The canonical HDR process requires RAD51 and BRCA2, and the homologous nucleic acid, e.g., repair template, is typically double-stranded. In canonical HDR, a double-stranded polynucleotide, e.g., a double-stranded repair template, is introduced, which comprises a sequence that is homologous to the targeting sequence, and which will either be directly integrated into the targeting sequence or will be used as a template to insert the sequence, or a portion the sequence, of the repair template into the target gene. After resection at the break, repair can progress by different pathways, e.g., by the double Holliday junction model (also referred to as the double strand break repair, or DSBR, pathway), or by the synthesis-dependent strand annealing (SDSA) pathway.

[00182] In the double Holliday junction model, strand invasion occurs by the two single stranded overhangs of the targeting sequence to the homologous sequences in the

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double-stranded polynucleotide, e.g., double stranded donor template, which results in the formation of an intermediate with two Holliday junctions. The junctions migrate as new DNA is synthesized from the ends of the invading strand to fill the gap resulting from the resection. The end of the newly synthesized DNA is ligated to the resected end, and the junctions are resolved, resulting in the insertion at the targeting sequence, or a portion of the targeting sequence that includes the gene variant. Crossover with the polynucleotide, e.g., repair template, may occur upon resolution of the junctions.

[00183] In the SDSA pathway, only one single stranded overhang invades the polynucleotide, e.g., donor template, and new DNA is synthesized from the end of the invading strand to fill the gap resulting from resection. The newly synthesized DNA then anneals to the remaining single stranded overhang, new DNA is synthesized to fill in the gap, and the strands are ligated to produce the modified DNA duplex.

[00184] Alternative HDR, or “alternative homology-directed repair,” or “alternative HDR,” are used interchangeably, and refers, in some embodiments, to the process of repairing DNA damage using a homologous nucleic acid (e.g., an endogenous homologous sequence, such as a sister chromatid; or an exogenous nucleic acid, such as a repair template). Alternative HDR is distinct from canonical HDR in that the process utilizes different pathways from canonical HDR, and can be inhibited by the canonical HDR mediators, RAD51 and BRCA2. Moreover, alternative HDR is also distinguished by the involvement of a single-stranded or nicked homologous nucleic acid template, e.g., repair template, whereas canonical HDR generally involves a double-stranded homologous template. In the alternative HDR pathway, a single strand template polynucleotide, e.g., repair template, is introduced. A nick, single strand break, or DSB at the cleavage site, for altering a desired target site, e.g., a gene variant in a target gene, is mediated by a nuclease molecule, and resection at the break occurs to reveal single stranded overhangs. Incorporation of the sequence of the template polynucleotide, e.g., repair template, to alter the target site of the DNA typically occurs by the SDSA pathway, as described herein. In some embodiments, HDR is carried out by introducing, into a cell, one or more agent(s) capable of inducing a DSB, and a repair template, e.g., a single-stranded oligonucleotide. The introducing can be carried out by any suitable delivery. The conditions under which HDR is allowed to occur can be any conditions suitable for carrying out HDR in a cell.

[00185] In several embodiments, gene editing is accomplished by one or more of a variety of engineered nucleases. In several embodiments, restriction enzymes are used, particularly when double strand breaks are desired at multiple regions. In several embodiments, a bioengineered nuclease is used. Depending on the embodiment, one or more of a Zinc Finger Nuclease (ZFN), transcription-activator like effector nuclease (TALEN), meganuclease and/or clustered regularly interspaced short palindromic repeats

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(CRISPR/Cas9) system are used to specifically edit the genes encoding one or more of the TCR subunits.

[00186] Meganucleases are characterized by their capacity to recognize and cut large DNA sequences (from 14 to 40 base pairs). In several embodiments, a meganuclease from the LAGLIDADG family is used, and is subjected to mutagenesis and screening to generate a meganuclease variant that recognizes a unique sequence(s), such as a specific site in a target gene, or any other target gene disclosed herein. In several embodiments, two or more meganucleases, or functions fragments thereof, are fused to create a hybrid enzyme that recognizes a desired target sequence within the target gene.

[00187] In contrast to meganucleases, ZFNs and TALEN function based on a non-specific DNA cutting catalytic domain which is linked to specific DNA sequence recognizing peptides such as zinc fingers or transcription activator-like effectors (TALEs). Advantageously, the ZFNs and TALENs thus allow sequence-independent cleavage of DNA, with a high degree of sequence-specificity in target recognition. Zinc finger motifs naturally function in transcription factors to recognize specific DNA sequences for transcription. The C-terminal part of each finger is responsible for the specific recognition of the DNA sequence. While the sequences recognized by ZFNs are relatively short, (e.g., ~3 base pairs), in several embodiments, combinations of 2, 3, 4, 5, 6, 7, 8, 9, 10 or more zinc fingers whose recognition sites have been characterized are used, thereby allowing targeting of specific sequences. The combined ZFNs are then fused with the catalytic domain(s) of an endonuclease, such as FokI (optionally a FokI heterodimer), to induce a targeted DNA break.

[00188] Transcription activator-like effector nucleases (TALENs) are specific DNA-binding proteins that feature an array of 33 or 34-amino acid repeats. Like ZFNs, TALENs are a fusion of a DNA cutting domain of a nuclease to TALE domains, which allow for sequence-independent introduction of double stranded DNA breaks with highly precise target site recognition. TALENs can create double strand breaks at the target site that can be repaired by error-prone non-homologous end-joining (NHEJ), resulting in gene disruptions through the introduction of small insertions or deletions. Advantageously, TALENs are used in several embodiments, at least in part due to their higher specificity in DNA binding, reduced off-target effects, and ease in construction of the DNA-binding domain.

[00189] CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) are genetic elements that bacteria use as protection against viruses. The repeats are short sequences that originate from viral genomes and have been incorporated into the bacterial genome. Cas (CRISPR associated proteins) process these sequences and cut matching viral DNA sequences. By introducing plasmids containing Cas genes and specifically constructed CRISPRs into eukaryotic cells, the eukaryotic genome can be cut at

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any desired position. Additional information on CRISPR can be found in US Patent Publication No. 2014/0068797, which is incorporated by reference herein.

[00190] In several embodiments, CRISPR is used to disrupt a target gene. Depending on the embodiment and which target gene is to be edited, a Class 1 or Class 2 Cas is used. In several embodiments, a Class 1 Cas is used, and the Cas type is selected from the following types: I, IA, IB, IC, ID, IE, IF, IU, III, IIIA, IIIB, IIIC, IIID, IV IVA, IVB, and combinations thereof. In several embodiments, the Cas is selected from the group consisting of Cas3, Cas8a, Cas5, Cas8b, Cas8c, Cas10d, Cse1, Cse2, Csy1, Csy2, Csy3, GSU0054, Cas10, Csm2, Cmr5, Cas10, Csx11, Csx10, Csf1, and combinations thereof. In several embodiments, the Cas is Cas3. In several embodiments, a Class 2 Cas is used, and the Cas type is selected from the following types: II, IIA, IIB, IIC, V, VI, and combinations thereof. In several embodiments, the Cas is selected from the group consisting of Cas9, Csn2, Cas4, Cas12a (previously known as Cpf1), C2c1, C2c3, Cas13a (previously known as C2c2), Cas13b, Cas13c, CasX, CasY and combinations thereof. In some embodiments, the Cas is Cas9. In some embodiments, class 2 CasX is used, wherein CasX can form a complex with a guide nucleic acid and wherein the complex can bind to a target DNA, and wherein the target DNA comprises a non-target strand and a target strand. In some embodiments, class 2 CasY is used, wherein CasY is capable of binding and modifying a target nucleic acid and/or a polypeptide associated with target nucleic acid.

ii. Target Genes

[00191] In some embodiments, the immune cells are genetically edited at a target gene. In several embodiments, editing of a target gene advantageously imparts to the edited cells enhanced expansion, cytotoxicity and/or persistence. For example, in some embodiments, immune cells are genetically edited to increase IL15 levels and/or signaling. Without wishing to be bound by theory, it is contemplated that genetically editing the cells to increase IL15 levels and/or signaling may obviate the need to provide a lymphodepleting therapy containing fludarabine to subjects prior to administration of genetically engineered cells. Specifically, it is contemplated that the increased IL15 bioavailability afforded by fludarabine is not necessary in immune cells genetically edited to increase IL15 levels and/or signaling. Thus, in some embodiments, the immune cells (e.g., NK cells) are genetically edited at a target gene to increase IL15 levels, such as by reducing or eliminating expression of the cytokine-inducible SH2-containing protein (Cis) (e.g., by disrupting the CISH gene encoding Cis). In some aspects, the immune cells (e.g., NK cells) are genetically edited at a target gene to increase IL15 signaling, such as by reducing or eliminating expression of the cytokine-inducible SH2-containing protein (Cis) (e.g., by disrupting the CISH gene encoding Cis).

[00192] By way of non-limiting example, IL15 is a positive regulator of NK cells, which as disclosed herein, can enhance one or more of NK cell homing, NK cell migration, NK

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cell expansion/proliferation, NK cell cytotoxicity, and/or NK cell persistence. In CD8⁺ T cells, CISH actively silences TCR signaling to maintain tumor tolerance, and CISH has been shown to be a downstream negative regulator of IL-15 receptor signaling (Palmer et al., J. Exp. Med. (2015) 212(12):2095-2113). In NK and T cells, CISH plays a role in checkpoint maturation and proliferation (Delconte et al., Nature Immunol (2016) 17:816-24). Thus, according to several embodiments, genetically editing CISH increases the persistence, proliferation, and/or cytotoxicity, or otherwise enhances the efficacy, of immune cells (e.g., NK cells) as disclosed herein.

[00193] In several embodiments, CISH genetic editing activates or inhibits a wide variety of pathways. The CIS protein is a negative regulator of IL15 signaling by way of, for example, inhibiting JAK-STAT signaling pathways. These pathways would typically lead to transcription of IL15-responsive genes (including CISH). In several embodiments, disruption of CISH disinhibits JAK-STAT (e.g., JAK1-STAT5) signaling and there is enhanced transcription of IL15-responsive genes. In several embodiments, disruption of CISH yields enhanced signaling through mammalian target of rapamycin (mTOR), with corresponding increases in expression of genes related to cell metabolism and respiration. In several embodiments, disruption of CISH yields IL15 induced increased expression of IL-2R α (CD25), but not IL-15R α or IL-2/15R β , enhanced NK cell membrane binding of IL15 and/or IL2, increased phosphorylation of STAT-3 and/or STAT-5, and elevated expression of the antiapoptotic proteins, such as Bcl-2. In several embodiments, CISH disruption results in IL15-induced upregulation of selected genes related to mitochondrial functions (e.g., electron transport chain and cellular respiration) and cell cycle. Thus, in several embodiments, CISH disruption by genetic editing enhances the NK cell cytotoxicity and/or persistence, at least in part via metabolic reprogramming. In several embodiments, negative regulators of cellular metabolism, such as TXNIP, are downregulated in response to CISH disruption. In several embodiments, promoters for cell survival and proliferation including BIRC5 (Survivin), TOP2A, CKS2, and RACGAP1 are upregulated after CISH disruption, whereas antiproliferative or proapoptotic proteins such as TGFB1, ATM, and PTCH1 are downregulated. In several embodiments, CISH disruption alters the state (e.g., activates or inactivates) signaling via or through one or more of CXCL-10, IL2, TNF, IFNg, IL13, IL4, Jnk, PRF1, STAT5, PRKCQ, IL2 receptor Beta, SOCS2, MYD88, STAT3, STAT1, TBX21, LCK, JAK3, IL& receptor, ABL1, IL9, STAT5A, STAT5B, Tcf7, PRDM1, and/or EOMES.

[00194] In several embodiments, CISH editing endows an NK cell with enhanced ability to home to a target site. In several embodiments, CISH editing endows an NK cell with enhanced ability to migrate, e.g., within a tissue in response to, for example chemoattractants or away from repellants. In several embodiments, CISH editing endows an

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NK cell with enhanced ability to be activated, and thus exert, for example, anti-tumor effects. In several embodiments, CISH editing endows an NK cell with enhanced proliferative ability, which in several embodiments, allows for generation of robust NK cell numbers from a donor blood sample. In addition, in such embodiments, NK cells edited for CISH and engineered to express a CAR are more readily, robustly, and consistently expanded in culture. In several embodiments, CISH genetic editing endows an NK cell with enhanced cytotoxicity. In several embodiments, the editing of CISH synergistically enhances the cytotoxic effects of immune cells that express a CAR.

[00195] In several embodiments, CIS expression is knocked down or knocked out through genetic editing of the CISH gene, for example, by use of CRISPR-Cas editing. Thus, in some embodiments, the immune cells (e.g., NK cells) are genetically edited at the CISH gene. Small interfering RNA, antisense RNA, TALENs or zinc fingers are used in other embodiments. Information on CISH editing can be found, for example, in International Patent Application Nos. PCT/US2023/060850 and PCT/US2020/035752, which are each incorporated in their entirety by reference herein.

[00196] In several embodiments, genetic editing reduces transcription of CISH by about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97%, about 98%, about 99%, or more (including any amount between those listed). In several embodiments, genetic editing reduces transcription of CISH by at least about 30%. In several embodiments, genetic editing reduces transcription of CISH by at least about 40%. In several embodiments, genetic editing reduces transcription of CISH by at least about 50%. In several embodiments, genetic editing reduces transcription of CISH by at least about 60%. In several embodiments, genetic editing reduces transcription of CISH by at least about 70%. In several embodiments, genetic editing reduces transcription of CISH by at least about 80%. In several embodiments, genetic editing reduces transcription of CISH by at least about 90%. In several embodiments, genetic editing reduces transcription of CISH by at least about 95%. In several embodiments, genetic editing reduces transcription of CISH by at least about 99%.

[00197] In several embodiments, genetic editing can reduce expression of Cis by about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97%, about 98%, about 99%, or more (including any amount between those listed). In several embodiments, genetic editing reduces expression of Cis by about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97%, about 98%, about 99%, or more (including any amount between those listed). In several embodiments, genetic editing reduces expression of Cis by at least about 30%. In several embodiments, genetic editing reduces expression of Cis by at least about 40%. In several embodiments, genetic editing reduces

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expression of Cis by at least about 50%. In several embodiments, genetic editing reduces expression of Cis by at least about 60%. In several embodiments, genetic editing reduces expression of Cis by at least about 70%. In several embodiments, genetic editing reduces expression of Cis by at least about 80%. In several embodiments, genetic editing reduces expression of Cis by at least about 90%. In several embodiments, genetic editing reduces expression of Cis by at least about 95%. In several embodiments, genetic editing reduces expression of Cis by at least about 99%.

[00198] In some embodiments, immune cells (e.g., NK cells) are genetically edited to reduce or eliminate expression of an alternative or additional target gene, for example, transforming growth factor-beta receptor 2 protein (TGFB β 2) and/or Casitas B-lineage lymphoma-b protein (Cbl-b). In some embodiments, the immune cells are genetically edited to reduce expression of TGFB β 2. In some embodiments, the immune cells are genetically edited to reduce expression of Cbl-b. The expression of any other target protein, or any combination of target proteins, can be decreased or eliminated, such as by disrupting the gene(s) encoding the target protein(s).

E. Composition and Formulations

[00199] Also provided are compositions including immune cells (e.g., NK cells) genetically engineered to express a CD19-directed CAR, including pharmaceutical compositions and formulations. Also provided are compositions comprising genetically engineered NK cells that express any of the CD19-directed CARs described herein, including pharmaceutical compositions and formulations.

[00200] Provided are pharmaceutical formulations comprising genetically engineered NK cells expressing a CD19-directed CAR, a plurality of genetically engineered NK cells expressing a CD19-directed CAR, and/or additional agents for combination treatment or therapy. The pharmaceutical compositions and formulations generally include one or more optional pharmaceutically acceptable carrier or excipient. Thus, in some embodiments, the pharmaceutical composition comprises a pharmaceutically acceptable carrier. In some embodiments, the composition includes at least one additional therapeutic agent.

[00201] In any of the provided embodiments, a composition comprising immune cells (e.g., NK cells) comprises a cryopreservative. In some embodiments, a composition comprising immune cells (e.g., NK cells) was previously cryopreserved.

[00202] The term “pharmaceutical formulation” refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[00203] A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A

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pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[00204] In some aspects, the choice of carrier is determined in part by the particular cell, binding molecule, and/or antibody, and/or by the method of administration. Accordingly, there are a variety of suitable formulations. For example, the pharmaceutical composition can contain preservatives. Suitable preservatives may include, for example, methylparaben, propylparaben, sodium benzoate, and benzalkonium chloride. In some aspects, a mixture of two or more preservatives is used. The preservative or mixtures thereof are typically present in an amount of about 0.0001% to about 2% by weight of the total composition. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

[00205] In some aspects, a buffer is included in the composition. Suitable buffering agents include, for example, citric acid, sodium citrate, phosphoric acid, potassium phosphate, and various other acids and salts. In some aspects, a mixture of two or more buffers is used. The buffering agent or mixtures thereof are typically present in an amount of from about 0.001% to about 4% by weight of the total composition. Methods for preparing administrable pharmaceutical compositions are known.

[00206] Formulations of the antibodies described herein can include lyophilized formulations and aqueous solutions. The formulation or composition may also contain more than one active ingredient useful for the particular indication, disease, or condition being treated with the binding molecules or cells, preferably those with activities complementary to the binding molecule or cell, where the respective activities do not adversely affect one another. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended. Thus, in some embodiments, the pharmaceutical composition further includes other pharmaceutically active agents or drugs, such as

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chemotherapeutic agents, e.g., methotrexate or rituximab. In some embodiments, the cells or antibodies are administered in the form of a salt, e.g., a pharmaceutically acceptable salt. Suitable pharmaceutically acceptable acid addition salts include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric, and sulphuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic acids, for example, p-toluenesulphonic acid.

[00207] The pharmaceutical composition in some embodiments contains the engineered cells in amounts effective to treat or prevent the disease or condition, such as a therapeutically effective or prophylactically effective amount. Therapeutic or prophylactic efficacy in some embodiments is monitored by periodic assessment of treated subjects. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and can be determined. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[00208] Formulations include those for oral, intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. In some embodiments, the agent or cell population is administered to the subject by intravenous, intraperitoneal, or subcutaneous injection using peripheral systemic delivery.

[00209] In some embodiments, the compositions are provided as sterile liquid formulations (e.g., isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions), which in some aspects may be buffered to a selected pH. Liquid formulations are generally easier to prepare than gels, other viscous compositions, and solid compositions. In addition, liquid compositions are somewhat more convenient to administer, particularly by injection. The liquid composition can comprise a carrier, which can be a solvent or dispersion medium containing, for example, water, saline, phosphate buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol), and suitable mixtures thereof.

[00210] Sterile injectable solutions can be prepared by incorporating the agent or cell into a solvent, such as an admixture with a suitable carrier, diluent, or excipient (e.g., sterile water, saline, glucose, dextrose, and the like). Formulations for in vivo administration are typically sterile. Sterility can be readily achieved, for example, by filtration through sterile filtration membranes. In some embodiments, the dose of engineered cells administered was previously cryopreserved. In some embodiments, the dose of engineered cells administered is in a cryopreserved composition. In some aspects, the composition is administered after thawing the cryopreserved composition.

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F. Combination Agents

[00211] In some embodiments, the genetically engineered cells are administered as part of a combination treatment, such as simultaneously with or sequentially with, in any order, another therapeutic intervention, such as an antibody or engineered cell or receptor or agent, such as a cytotoxic or therapeutic agent. The genetically engineered cells in some embodiments are co-administered with one or more additional therapeutic agents or in connection with another therapeutic intervention, either simultaneously or sequentially in any order. In some contexts, the cells are co-administered with another therapy sufficiently close in time such that the cell populations enhance the effect of one or more additional therapeutic agents, or vice versa. In some embodiments, the cells are administered prior to, concurrently with, and/or subsequent to the one or more additional therapeutic agents. In some embodiments, the cells are administered prior to the one or more additional therapeutic agents. In some embodiments, the cells are administered concurrently with the one or more additional therapeutic agents. In some embodiments, the cells are administered after the one or more additional therapeutic agents. In some embodiments, a lymphodepleting therapy is administered prior to, concurrently with, and/or subsequent to the one or more additional therapeutic agents. In some embodiments, a lymphodepleting therapy is administered prior to the one or more additional therapeutic agents. In some embodiments, a lymphodepleting therapy is administered concurrently with the one or more additional therapeutic agents. In some embodiments, the lymphodepleting therapy is administered after the one or more additional therapeutic agents.

[00212] In some embodiments, the additional therapeutic agent includes chemotherapy, radiation therapy, surgery, transplantation, adoptive cell therapy, antibodies, cytotoxic agents, chemotherapeutic agents, cytokines, growth inhibitory agents, anti-hormonal agents, kinase inhibitors, anti-angiogenic agents, cardioprotectants, immunostimulatory agents, immunosuppressive agents, immune checkpoint inhibitors, antibiotics, angiogenesis inhibitors, metabolic modulators or other therapeutic agents or any combination thereof. In some embodiments, the additional therapeutic agent is a protein, a peptide, a nucleic acid, a small molecule agent, a cell, a toxin, a lipid, a carbohydrate or combinations thereof, or any other type of therapeutic agent, e.g. radiation. In some embodiments, the additional therapeutic agent includes surgery, chemotherapy, radiation therapy, transplantation, administration of cells expressing a recombinant receptor, e.g., CAR, kinase inhibitor, immune checkpoint inhibitor, mTOR pathway inhibitor, immunosuppressive agents, immunomodulators, antibodies, immunoablative agents, antibodies and/or antigen binding fragments thereof, antibody conjugates, other antibody therapies, cytotoxins, steroids, cytokines, peptide vaccines, hormone therapy, antimetabolites, metabolic modulators, alkylating agents, anthracyclines, vinca alkaloids, proteasome inhibitors, protein kinase

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inhibitors, and/or other types of immunotherapy. In some embodiments, the additional therapeutic agent or treatment is bone marrow transplantation, T cell ablative therapy using chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, and/or antibody therapy.

[00213] In several embodiments, the therapeutic agent is an NK cell engager (e.g., a molecule that binds both an antigen expressed by cells of the cancer and an antigen expressed by NK cells). In several embodiments, the NK cell engager binds to an activating receptor on an NK cell and an antigen expressed by cells of the cancer. In some embodiments, the activating receptor on the NK cell is selected from the group consisting of CD16, NKp30, NKp46, NKG2D, and any combination thereof.

[00214] In some embodiments, the additional agent includes an immune checkpoint inhibitor. Immune checkpoint inhibitors include any agent that blocks or inhibits in a statistically significant manner, the inhibitory pathways of the immune system. Such inhibitors may include small molecule inhibitors or may include antibodies, or antigen binding fragments thereof, that bind to and block or inhibit immune checkpoint receptors, ligands and/or receptor-ligand interaction. In some embodiments, modulation, enhancement and/or stimulation of particular receptors can overcome immune checkpoint pathway components. Illustrative immune checkpoint molecules that may be targeted for blocking, inhibition, modulation, enhancement and/or stimulation include, but are not limited to, PD-1 (CD279), PD-L1 (CD274, B7-H1), CTLA-4, LAG-3 (CD223), TIM-3, 4-1BB (CD137), 4-1BBL (CD137L), GITR (TNFRSF18, AITR), CD40, OX40 (CD134, TNFRSF4), B7-H3, B7-H4, B7H3, B7H4, VISTA, KIR, 2B4, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), TIGIT, and LAIR1.

[00215] In some embodiments, the additional agent is a CD20 inhibitor, e.g., an anti-CD20 antibody (e.g., an anti-CD20 mono- or bi-specific antibody) or a fragment thereof, antibody-drug conjugate (ADC), or engineered toxin body (ETB). In some embodiments, the CD20 inhibitor is an anti-CD20 antibody. In some embodiments, the CD20 inhibitor is an anti-CD20 antibody or an antigen-binding fragment thereof. Exemplary anti-CD20 antibodies include rituximab (RITUXAN®) and obinutuzumab (GAZYVA®). In some embodiments, the additional agent is or includes rituximab. In some embodiments, the additional agent is or includes obinutuzumab. In several embodiments, the antibody targets CD20. In several embodiments, the anti-CD20 antibody is rituximab. In some embodiments, biosimilar rituximab-abbs, rituximab-arxx, and/or rituximab-pvvr are used. In several embodiments, ocrelizumab, ofatumumab, obinutuzumab, ibritumomab, ibritumomab or combinations thereof are used. In several embodiments, the dose of the anti-CD20 antibody ranges from between about 150 mg/m² and about 500 mg/m², including about 150 mg/m², about 200 mg/m², about 250 mg/m², about 300 mg/m², about 350 mg/m², about 375 mg/m², about 400 mg/m², about

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425 mg/m², about 450 mg/m², or about 500 mg/m² (or any dose between those listed). In several embodiments, the dose of the anti-CD20 antibody is about 375 mg/m². In some embodiments, the anti-CD20 antibody is rituximab and the dose is about 375 mg/m². In several embodiments, the dose of the anti-CD20 antibody is about 500 mg/m². In some embodiments, the anti-CD20 antibody is rituximab and the dose is about 500 mg/m². In several embodiments, the anti-CD20 antibody will be administered 1, 2, 3, or more times. In several embodiments, the anti-CD20 antibody will be administered 1, 2, 3, 4 or more days prior to administration of cells at the initial time point in a dosing cycle. In some embodiments, a single dose of 375 mg/m² rituximab is administered during the dosing cycle. In some embodiments, a single dose of 375 mg/m² rituximab is administered during the first dosing cycle, and a single dose of 500 mg/m² rituximab is administered during each subsequent dosing cycle. In some embodiments, the single dose of rituximab is administered prior to administration of the engineered immune cells, (e.g., about 3 days prior to administration). In some embodiments, the single dose of rituximab is administered about 3 days prior to administration of the engineered immune cells. In several embodiments, the anti-CD20 antibody will be administered 3 days prior to administration of cells. In some embodiments, the CD20 inhibitor is an ADC. In some embodiments, the CD20 inhibitor is an ETB. In some embodiments, the CD20 inhibitor is a small molecule.

[00216] In some embodiments, the additional agent is a CD22 inhibitor, e.g., an anti-CD22 antibody (e.g., an anti-CD22 mono- or bi-specific antibody) or a fragment thereof, antibody-drug conjugate (ADC), or engineered toxin body (ETB). In some embodiments, the CD22 inhibitor is an anti-CD22 antibody or an antigen-binding fragment thereof. In some embodiments, the CD22 inhibitor is an ADC. In some embodiments, the CD22 inhibitor is an ETB. In some embodiments, the CD22 inhibitor is a small molecule.

[00217] In some embodiments, the additional agent is an EGFR inhibitor, e.g., an anti-EGFR antibody (e.g., an anti-EGFR mono- or bi-specific antibody) or a fragment thereof, antibody-drug conjugate (ADC), or engineered toxin body (ETB). In some embodiments, the EGFR inhibitor is an anti-EGFR antibody. In some embodiments, the EGFR inhibitor is an anti-EGFR antibody or an antigen-binding fragment thereof. Exemplary anti-EGFR antibodies include cetuximab, panitumumab (VECTIBIX®), nimotuzumab, and necitumumab (PORTRAZZA™). In several embodiments, the anti-EGFR antibody comprises cetuximab. In several embodiments, the anti-EGFR antibody is cetuximab. In several embodiments, the anti-EGFR antibody comprises panitumumab. In several embodiments, the anti-EGFR antibody is panitumumab. In several embodiments, the anti-EGFR antibody comprises nimotuzumab. In several embodiments, the anti-EGFR antibody is nimotuzumab. In several embodiments, the anti-EGFR antibody comprises necitumumab. In several embodiments, the anti-EGFR antibody is necitumumab. In several embodiments, the anti-

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EGFR antibody will be administered 1, 2, 3, or more times. In several embodiments, the anti-EGFR antibody will be administered 1, 2, 3, 4 or more days prior to administration of cells at the initial time point in a dosing cycle. In some embodiments, a single dose of an anti-EGFR antibody is administered during the dosing cycle. In some embodiments, the anti-EGFR antibody is administered prior to, concurrent with, and/or subsequent to administration of the engineered immune cells. In some embodiments, the anti-EGFR antibody is administered prior to administration of the engineered immune cells, (e.g., about 3 days prior to administration). In some embodiments, the anti-EGFR antibody is administered concurrent with administration of the engineered immune cells. In some embodiments, the anti-EGFR antibody is administered subsequent to administration of the engineered immune cells. In some embodiments, the additional agent is or includes cetuximab. In some embodiments, the EGFR inhibitor is an ADC. In some embodiments, the EGFR inhibitor is an ETB. In some embodiments, the EGFR inhibitor is a small molecule.

[00218] The dose of the additional agent can be any therapeutically effective amount, e.g., any dose amount described herein, and the appropriate dosage of the additional agent may depend on the type of disease to be treated, the type, dose and/or frequency of the recombinant receptor, cell and/or composition administered, the severity and course of the disease, whether the recombinant receptor, cell and/or composition is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the recombinant receptor, cell and/or composition, and the discretion of the attending physician. The recombinant receptor, cell and/or composition and/or the additional agent and/or therapy can be administered to the patient at one time, repeated or administered over a series of treatments.

II. Articles of Manufacture and Kits

[00219] Also provided are articles of manufacture or kits containing the provided genetically engineered NK cells and/or compositions comprising the same. The articles of manufacture may include a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, test tubes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. In some embodiments, the container has a sterile access port. Non-limiting examples of containers include intravenous solution bags and vials, including those with stoppers pierceable by a needle for injection. The article of manufacture or kit may further include a package insert indicating that the composition can be used to treat a particular condition such as a condition described herein (e.g., cancer). Alternatively, or additionally, the article of manufacture or kit may further include another or the same container comprising a pharmaceutically-acceptable buffer. It may further include other materials such as other buffers, diluents, filters, needles, and/or syringes.

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[00220] The label or package insert may indicate that the composition is used for treating a disease (e.g., cancer) in an individual. The label or a package insert, which is on or associated with the container, may indicate directions for reconstitution and/or use of the formulation. The label or package insert may further indicate that the formulation is useful or intended for subcutaneous, intravenous, or other modes of administration for treating or preventing a disease (e.g., cancer) in an individual.

[00221] The container in some embodiments holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the disease (e.g., cancer). The article of manufacture or kit may include (a) a first container with a composition contained therein (i.e., first medicament), wherein the composition includes the engineered NK cells; and (b) a second container with a composition contained therein (i.e., second medicament), wherein the composition includes a further agent, such as a cytotoxic or otherwise therapeutic agent, and which article or kit further comprises instructions on the label or package insert for treating the subject with the second medicament, in an effective amount.

III. Method of Treatment, Uses, Administration and Dosing

[00222] Some embodiments relate to a method of treating, ameliorating, inhibiting, or preventing cancer with a cell or immune cell comprising a chimeric antigen receptor, as disclosed herein. In some embodiments, the method includes treating or preventing cancer. In some embodiments, the method includes administering a therapeutically effective amount of immune cells expressing a tumor-directed chimeric antigen receptor as described herein. Examples of types of cancer that may be treated as such are described herein.

[00223] Disclosed herein are methods of treating cancer in a subject. In some embodiments, the methods comprise administering to the subject any one of the CD19 binding domains disclosed herein, any one of the CD19-directed CARs disclosed herein, or any one of the CAR-expressing cells disclosed herein, or any combination thereof.

[00224] Also disclosed herein are uses of any one of the CD19 binding domains disclosed herein, any one of the CD19-directed CARs disclosed herein, any one of the cells disclosed herein, or any combination thereof for the treatment of cancer.

[00225] Also disclosed herein are uses of any one of the CD19 binding domains disclosed herein, any one of the CD19-directed CARs disclosed herein, any one of the cells disclosed herein, or any combination thereof in the manufacture of a medicament for the treatment of cancer.

[00226] In certain embodiments, treatment of a subject with a genetically engineered cell(s) described herein achieves one, two, three, four, or more of the following effects, including, for example: (i) reduction or amelioration the severity of disease or symptom

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associated therewith; (ii) reduction in the duration of a symptom associated with a disease; (iii) protection against the progression of a disease or symptom associated therewith; (iv) regression of a disease or symptom associated therewith; (v) protection against the development or onset of a symptom associated with a disease; (vi) protection against the recurrence of a symptom associated with a disease; (vii) reduction in the hospitalization of a subject; (viii) reduction in the hospitalization length; (ix) an increase in the survival of a subject with a disease; (x) a reduction in the number of symptoms associated with a disease; and (xi) an enhancement, improvement, supplementation, complementation, or augmentation of the prophylactic or therapeutic effect(s) of another therapy. Each of these comparisons are versus, for example, a different therapy for a disease, which includes a cell-based immunotherapy for a disease using cells that do not express the constructs disclosed herein. Advantageously, the engineered NK and/or T cells disclosed herein further enhance one or more of the above.

[00227] In particular, methods of treating subjects with dosing regimens as provided herein and described in the Working Examples (e.g., with NK cells engineered to express a CD19 CAR) may result in unexpected efficacy and safety, including an efficacy and safety profile allowing for outpatient administration. It is contemplated that such unexpected effects are due, at least in part, to providing an increased number of genetically engineered NK cells during a time period in which a subject's immune response has not yet fully recovered following lymphodepleting therapy, thereby allowing increased engraftment of adoptively transferred immune cells (e.g., NK cells). Such a time period may include, for example, within about 7 days of administration of the genetically engineered NK cells and/or within about 14 days of administration of a first dose of lymphodepleting therapy. For example, by providing higher doses of genetically engineered NK cells, an increased number of doses, or both, within this time period, the efficacy of genetically engineered NK cells may be improved. Further, the opportunity to provide higher and/or an increased number of doses of genetically engineered NK cells is afforded by the lack of graft vs. host disease and toxicity associated with NK cells. This is in contrast with, for example, cytokine release syndrome (CRS) and neurotoxicity frequently observed with adoptive transfer of engineered T cells, such as CAR T cells.

[00228] Further, it is contemplated herein that the relatively limited persistence of CAR NK cells in vivo, combined with the methods of treatment described herein, may allow for modification of the standard lymphodepletion regimens used with CAR T cell therapies, such that the risk of potential toxicities can be mitigated. Multiple studies have shown that adequate suppression of the host immune system correlates with responses in cell therapy clinical trials (Miller et al., *Blood* (2005) 105(8):3051-57; Turtle et al., *J. Clin. Oncol.* (2017) 35(26):3010-20). Thus, lymphodepleting therapy has been an integral part of CAR T cell clinical trials. Similarly, use of high-dose lymphodepletion regimens prior to adoptive transfer

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of NK cell therapies has yielded in vivo NK cell expansion and persistence, whereas low-dose lymphodepletion regimens did not (Kilgour et al., *Front. Immunol.* (2023) 14:1166038). In particular, a lymphodepletion regimen of fludarabine and cyclophosphamide has historically been associated with the ability to detect adoptively transferred immune cells. The benefit of eliminating lymphocytes via a fludarabine-containing lymphodepleting therapy may be realized not just through reduced rejection of adoptively transferred immune cells, but also through improved availability of cytokines such as interleukin 15 (IL15) (Gauthier et al., *Blood* (2020) 136(Supp. 1):37–38).

[00229] However, CD19 CAR-expressing NK cells as provided herein are expected to have much of their activity shortly after administration to a subject, such that the primary benefit of lymphodepletion for NK cells may be from cyclophosphamide, which has activity against lymphocytes earlier than fludarabine. For example, the nadir of white blood cell counts has been reported to be approximately 13 days after fludarabine treatment (FLUDARA® USPI 2010) compared to approximately 9 days after cyclophosphamide treatment (Buckner et al., *Cancer* (1972) 29(2):357-65). In connection with this, where multiple doses of CD19 CAR-expressing NK cells are provided in a single dosing cycle, the provision of all doses within the peak activity window of cyclophosphamide (e.g., within about 7-10 days of the administration of cyclophosphamide) may be particularly advantageous. The inventors therefore contemplate the combination of a cyclophosphamide-only lymphodepleting therapy in combination with a dosing cycle in which all doses of CD19 CAR-expressing NK cells are provided within about 7-10 days of administration of the cyclophosphamide-only lymphodepleting therapy. For example, a single dose of cyclophosphamide can be provided about 3 days prior to administration of the first dose of CD19 CAR NK cells (Day -3), and the doses of CD19 CAR NK cells can be provided on Days 0, 3, and 7. Similarly, a single dose of cyclophosphamide can be provided about 3 days prior to administration of the first dose of CD19 CAR NK cells (Day -3), and the doses of CD19 CAR NK cells can be provided on Days 0, 2, and 4 or on Days 0, 2, and 5. To this end, the inventors contemplate that a dosing cycle comprising administration of a single dose of cyclophosphamide on Day -3 and administration of the CD19 CAR NK cells on Days 0, 2, and 4 could be particularly convenient in an outpatient setting, where cyclophosphamide could be provided e.g., on Friday, and doses of CD19 CAR NK cells could be provided e.g., on the following Monday, Wednesday, and Friday. Without wishing to be bound by theory, the provision of each of the doses of CD19 CAR-expressing NK cells within about 7-10 days of the administration of cyclophosphamide may allow for improved peak concentration and/or persistence of the NK cells as compared to a dosing regimen in which one or more doses of the dosing cycle are provided later in time.

[00230] Further, increased cytokine (e.g., IL15) bioavailability may be unnecessary with CD19 CAR-expressing NK cells expressing membrane-bound interleukin

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15 (mbIL15), including those as provided herein. Similar considerations could apply to NK cells genetically engineered to increase IL15 signaling (e.g., via knockout of the CISH gene). As fludarabine may increase not only short-term toxicity (Hay et al., Blood (2017) 130(21):2295-2306) but also the potential for secondary malignancies, removing fludarabine from lymphodepleting therapy may improve the risk-benefit profile. Thus, it is contemplated that use of the CD19 CAR-expressing NK cells as provided herein to treat a cancer may not require a fludarabine-containing lymphodepletion regimen, as is commonly used for hematologic malignancies. Rather, a lymphodepletion regimen of only cyclophosphamide may be sufficient to achieve efficacy and reduce potential toxicities associated with LD.

[00231] Doses of immune cells such as NK cells can be determined for a given subject based on their body mass, disease type and state, and desired aggressiveness of treatment, but range, depending on the embodiments, from about 10⁵ cells per kg to about 10¹² cells per kg (e.g., 10⁵-10⁷, 10⁷-10¹⁰, 10¹⁰-10¹² and overlapping ranges therein). In several embodiments, a range of immune cells such as NK cells is administered, for example between about 1 x 10⁸ cells/kg to about 1 x 10⁷ cells/kg. In several embodiments, a range of immune cells such as NK cells is administered, for example between about 1 x 10⁹ CAR-expressing immune cells to about 3 x 10⁹ CAR-expressing immune cells.

[00232] In several embodiments, multiple doses are used, for example, two, three, four, or more doses within a dosing cycle. In some embodiments, a dosing cycle comprises administration of two doses of NK cells. In some embodiments, a dosing cycle consists of two doses of NK cells. In several embodiments, a dosing cycle comprises administration of three doses of NK cells. In several embodiments, a dosing cycle consists of three doses of NK cells. In several embodiments, a dosing cycle comprises administration of four doses of NK cells. In several embodiments, a dosing cycle consists of four doses of NK cells. In several embodiments, a dosing cycle comprises administration of five doses of NK cells. In several embodiments, a dosing cycle consists of five doses of NK cells. Such multi-dose cycles can be repeated one or more times, as needed to treat a cancer or disease progression.

[00233] In some embodiments, all doses of the dosing cycle are administered to the subject within about 10 days, within about 9 days, within about 8 days, within about 7 days, within about 6 days, within about 5 days, or within about 4 days. In some embodiments, all doses of the dosing cycle are administered to the subject within about 10 days. In some embodiments, all doses of the dosing cycle are administered to the subject within about 9 days. In some embodiments, all doses of the dosing cycle are administered to the subject within about 8 days. In some embodiments, all doses of the dosing cycle are administered to the subject within about 7 days. In some embodiments, all doses of the dosing cycle are administered to the subject within about 6 days. In some embodiments, all doses of the dosing

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cycle are administered to the subject within about 5 days. In some embodiments, all doses of the dosing cycle are administered to the subject within about 4 days. In some embodiments, all doses of a dosing cycle are administered to the subject within about 13 days, within about 12 days, within about 11 days, within about 10 days, within about 9 days, within about 8 days, or within about 7 days of administration of a lymphodepleting therapy to a subject. In some embodiments, all doses of a dosing cycle are administered to the subject within about 13 days of a lymphodepleting therapy to a subject. In some embodiments, all doses of a dosing cycle are administered to the subject within about 12 days of a lymphodepleting therapy to a subject. In some embodiments, all doses of a dosing cycle are administered to the subject within about 11 days of a lymphodepleting therapy to a subject. In some embodiments, all doses of a dosing cycle are administered to the subject within about 10 days of a lymphodepleting therapy to a subject. In some embodiments, all doses of a dosing cycle are administered to the subject within about 9 days of a lymphodepleting therapy to a subject. In some embodiments, all doses of a dosing cycle are administered to the subject within about 8 days of a lymphodepleting therapy to a subject. In some embodiments, all doses of a dosing cycle are administered to the subject within about 7 days of a lymphodepleting therapy to a subject. In some embodiments, the dosing cycle consists of three doses. In some embodiments, the lymphodepleting therapy does not comprise fludarabine. In some embodiments, the lymphodepleting therapy consists of cyclophosphamide.

[00234] In several embodiments, between about 1×10^9 to about 5×10^9 CAR-expressing NK cells are provided in each dose. In several embodiments, a dose comprises about 1×10^9 CAR-expressing NK cells. In several embodiments, a dose comprises about 1.5×10^9 CAR-expressing NK cells. In several embodiments, between about 2×10^9 to about 5×10^9 CAR-expressing NK cells are provided in each dose. In several embodiments, a dose comprises about 2×10^9 CAR-expressing NK cells. In several embodiments, a dose comprises about 2.5×10^9 CAR-expressing NK cells. In several embodiments, a dose comprises about 3×10^9 CAR-expressing NK cells. In several embodiments, a dose comprises about 3.5×10^9 CAR-expressing NK cells. In several embodiments, a dose comprises about 4×10^9 CAR-expressing NK cells. In several embodiments, a dose comprises about 4.5×10^9 CAR-expressing NK cells. In several embodiments, a dose comprises about 5×10^9 CAR-expressing NK cells.

[00235] In several embodiments, between about 1×10^9 CAR-expressing NK cells and about 5×10^9 CAR-expressing NK cells are administered three times over a 28-day cycle. In several embodiments, between about 2×10^9 CAR-expressing NK cells and about 5×10^9 CAR-expressing NK cells are administered three times over a 28-day cycle. In several embodiments, 1×10^9 CAR-expressing NK cells are administered three times over a 28-day cycle. In several embodiments, 1.5×10^9 CAR-expressing NK cells are administered three

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times over a 28-day cycle. In several embodiments, 2×10^9 CAR-expressing NK cells are administered three times over a 28-day cycle. In several embodiments, 2.5×10^9 CAR-expressing NK cells are administered three times over a 28-day cycle. In several embodiments, 3×10^9 CAR-expressing NK cells are administered three times over a 28-day cycle. In several embodiments, 3.5×10^9 CAR-expressing NK cells are administered three times over a 28-day cycle. In several embodiments, 4×10^9 CAR-expressing NK cells are administered three times over a 28-day cycle. In several embodiments, 4.5×10^9 CAR-expressing NK cells are administered three times over a 28-day cycle. In several embodiments, 5×10^9 CAR-expressing NK cells are administered three times over a 28-day cycle.

[00236] In several embodiments, at least about 3×10^9 CAR-expressing NK cells are administered in a dosing cycle. In several embodiments, at least about 4.5×10^9 CAR-expressing NK cells are administered in a dosing cycle. In several embodiments, at least about 6×10^9 CAR-expressing NK cells are administered in a dosing cycle. In several embodiments, at least about 7.5×10^9 CAR-expressing NK cells are administered in a dosing cycle. In several embodiments, at least about 9×10^9 CAR-expressing NK cells are administered in a dosing cycle.

[00237] In some embodiments, each dose of NK cells is administered between about 2-4 days apart. In some embodiments, a second dose of NK cells is administered about 2-4 days after administration of the first dose. In some embodiments, a third dose of NK cells is administered about 2-4 days after administration of the second dose.

[00238] In some embodiments, a second dose of NK cells is administered about 2-4 days after administration of the first dose, and a third dose of NK cells is administered about 2-4 days after administration of the second dose. In some embodiments, a second dose of NK cells is administered about 3 days after administration of the first dose. In some embodiments, a third dose of NK cells is administered about 4 days after administration of the second dose. In some embodiments, a second dose of NK cells is administered about 3 days after administration of the first dose, and a third dose of NK cells is administered about 4 days after administration of the second dose.

[00239] In several embodiments, a dosing cycle comprises administration of three doses of NK cells (e.g., over a 28-day period), wherein the second dose is administered about 2-4 days after administration of the first dose, the third dose is administered about 2-4 days after administration of the second dose, and each dose comprises about 1×10^9 CAR-expressing NK cells. In several embodiments, a dosing cycle comprises administration of three doses of NK cells (e.g., over a 28-day period), wherein the second dose is administered about 3 days after administration of the first dose, the third dose is administered about 4 days after

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administration of the second dose, and each dose comprises about 1×10^9 CAR-expressing NK cells.

[00240] In several embodiments, a dosing cycle comprises administration of three doses of NK cells (e.g., over a 28-day period), wherein the second dose is administered about 2-4 days after administration of the first dose, the third dose is administered about 2-4 days after administration of the second dose, and each dose comprises about 1.5×10^9 CAR-expressing NK cells. In several embodiments, a dosing cycle comprises administration of three doses of NK cells (e.g., over a 28-day period), wherein the second dose is administered about 3 days after administration of the first dose, the third dose is administered about 4 days after administration of the second dose, and each dose comprises about 1.5×10^9 CAR-expressing NK cells.

[00241] In several embodiments, a dosing cycle comprises administration of three doses of NK cells (e.g., over a 28-day period), wherein the second dose is administered about 2-4 days after administration of the first dose, the third dose is administered about 2-4 days after administration of the second dose, and each dose comprises about 2×10^9 CAR-expressing NK cells. In several embodiments, a dosing cycle comprises administration of three doses of NK cells (e.g., over a 28-day period), wherein the second dose is administered about 3 days after administration of the first dose, the third dose is administered about 4 days after administration of the second dose, and each dose comprises about 2×10^9 CAR-expressing NK cells.

[00242] In several embodiments, the first dose is administered on the first day of the dosing cycle (e.g., Day 0). In some embodiments, the second dose is administered about 2 days after administration of the first dose (e.g., Day 2). In several embodiments, the second dose is administered about 3 days after administration of the first dose (e.g., Day 3). In several embodiments, the second dose is administered about 4 days after administration of the first dose (e.g., Day 4). In some embodiments, the second dose is administered on Day 2 of the dosing cycle. In some embodiments, the second dose is administered on Day 3 of the dosing cycle. In some embodiments, the second dose is administered on Day 4 of the dosing cycle. In several embodiments, the third dose is administered about 2 days after administration of the second dose. In several embodiments, the third dose is administered about 3 days after administration of the second dose. In several embodiments, the third dose is administered about 4 days after administration of the second dose (e.g., Day 7). In some embodiments, the third dose is administered on Day 4 of the dosing cycle. In some embodiments, the third dose is administered on Day 5 of the dosing cycle. In some embodiments, the third dose is administered on Day 6 of the dosing cycle. In some embodiments, the third dose is administered on Day 7 of the dosing cycle.

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[00243] In some embodiments, each dose is separated by between about 24 hours and about 72 hours. In some embodiments, each dose is separated by at least about 24 hours. In some embodiments, each dose is separated by about 24 hours. In some embodiments, each dose is separated by at least about 48 hours. In some embodiments, each dose is separated by about 48 hours. In some embodiments, each dose is separated by at least about 72 hours. In some embodiments, each dose is separated by about 72 hours.

[00244] In several embodiments, the first dose is administered on the first day of the dosing cycle (e.g., Day 0), the second dose is administered 2-4 after administration of the first dose, and the third dose is administered 2-4 days after administration of the second dose.

[00245] In several embodiments, the first dose is administered on the first day of the dosing cycle (e.g., Day 0), the second dose is administered about 2 days after administration of the first dose (e.g., Day 2), and the third dose is administered about 2 days after administration of the second dose (e.g., Day 4). In some embodiments, about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 2, and about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 4. In some embodiments, about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 2, and about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 4. In some embodiments, about 2.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 2.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 2, and about 2.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 4. In some embodiments, about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 2, and about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 4.

[00246] In several embodiments, the first dose is administered on the first day of the dosing cycle (e.g., Day 0), the second dose is administered about 2 days after administration of the first dose (e.g., Day 2), and the third dose is administered about 3 days after administration of the second dose (e.g., Day 5). In some embodiments, about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 2, and about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 5. In some embodiments, about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 2, and about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 5. In some embodiments, about 2.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 2.5×10^9

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NK cells (e.g., CAR-expressing NK cells) are administered on Day 2, and about 2.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 5. In some embodiments, about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 2, and about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 5.

[00247] In several embodiments, the first dose is administered on the first day of the dosing cycle (e.g., Day 0), the second dose is administered about 3 days after administration of the first dose (e.g., Day 3), and the third dose is administered about 2 days after administration of the second dose (e.g., Day 5). In some embodiments, about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 3, and about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 5. In some embodiments, about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 3, and about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 5. In some embodiments, about 2.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 2.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 3, and about 2.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 5. In some embodiments, about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 3, and about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 5.

[00248] In several embodiments, the first dose is administered on the first day of the dosing cycle (e.g., Day 0), the second dose is administered about 3 days after administration of the first dose (e.g., Day 3), and the third dose is administered about 3 days after administration of the second dose (e.g., Day 6). In some embodiments, about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 3, and about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 6. In some embodiments, about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 3, and about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 6. In some embodiments, about 2.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 2.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 3, and about 2.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 6. In some embodiments, about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 3, and about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 6.

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[00249] In several embodiments, a dosing cycle comprises administration of three doses of NK cells (e.g., over a 28-day period), wherein the second dose is administered about 2-4 days after administration of the first dose, the third dose is administered about 2-4 days after administration of the second dose, and each dose comprises about 2.5×10^9 CAR-expressing NK cells. In several embodiments, a dosing cycle comprises administration of three doses of NK cells (e.g., over a 28-day period), wherein the second dose is administered about 3 days after administration of the first dose, the third dose is administered about 4 days after administration of the second dose, and each dose comprises about 2.5×10^9 CAR-expressing NK cells.

[00250] In several embodiments, a dosing cycle comprises administration of three doses of NK cells (e.g., over a 28-day period), wherein the second dose is administered about 2-4 days after administration of the first dose, the third dose is administered about 2-4 days after administration of the second dose, and each dose comprises about 3×10^9 CAR-expressing NK cells. In several embodiments, a dosing cycle comprises administration of three doses of NK cells (e.g., over a 28-day period), wherein the second dose is administered about 3 days after administration of the first dose, the third dose is administered about 4 days after administration of the second dose, and each dose comprises about 3×10^9 CAR-expressing NK cells.

[00251] In some embodiments, about 1×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 1×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 3, and about 1×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 7. In some embodiments, about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 3, and about 1.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 7. In some embodiments, about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 3, and about 2×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 7. In some embodiments, about 2.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 2.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 3, and about 2.5×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 7. In some embodiments, about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 0, about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 3, and about 3×10^9 NK cells (e.g., CAR-expressing NK cells) are administered on Day 7.

[00252] In some embodiments, the first dose is administered on about Day 0 of the dosing cycle. In some embodiments, the second dose is administered on about Day 2 of the dosing cycle. In some embodiments, the second dose is administered on about Day 3 of

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the dosing cycle. In some embodiments, the third dose is administered on about Day 4 of the dosing cycle. In some embodiments, the third dose is administered on about Day 5 of the dosing cycle. In some embodiments, the third dose is administered on about Day 6 of the dosing cycle. In some embodiments, the third dose is administered on about Day 7 of the dosing cycle. In some embodiments, each dose is separated by between about 24 hours and about 72 hours. In some embodiments, each dose is separated by at least about 24 hours. In some embodiments, each dose is separated by about 24 hours. In some embodiments, each dose is separated by at least about 48 hours. In some embodiments, each dose is separated by about 48 hours. In some embodiments, each dose is separated by at least about 72 hours. In some embodiments, each dose is separated by about 72 hours.

[00253] In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 2 of the dosing cycle, and the third dose is administered on about Day 4 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 2 of the dosing cycle, and the third dose is administered on about Day 5 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 3 of the dosing cycle, and the third dose is administered on about Day 5 of the dosing cycle. In some embodiments, the first dose is administered on about Day 0 of the dosing cycle, the second dose is administered on about Day 3 of the dosing cycle, and the third dose is administered on about Day 6 of the dosing cycle.

[00254] In some embodiments, a dose of NK cells of the dosing cycle is administered on an outpatient basis. In some embodiments, two doses of NK cells of the dosing cycle are administered on an outpatient basis. In some embodiments, each dose of NK cells of the dosing cycle is administered on an outpatient basis.

[00255] In several embodiments, the administration of engineered NK cells is preceded by one or more preparatory treatments. In several embodiments, the administration of engineered NK cells is preceded by lymphodepletion. Thus, in some embodiments, a subject is administered a lymphodepleting therapy prior to administration of a dosing cycle. In several embodiments, each dosing cycle is preceded by lymphodepletion. Thus, also provided herein is a method of preparing a subject having a cancer for treatment with a composition comprising natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds to CD19, the method comprising administering a lymphodepleting therapy to the subject prior to administration of the composition to the subject. In some embodiments, the lymphodepleting therapy comprises administration of cyclophosphamide and does not comprise administration of fludarabine. Thus, in some aspects, provided herein is a method of preparing a subject having a cancer for treatment with a composition comprising

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natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds to CD19, the method comprising administering a lymphodepleting therapy consisting of cyclophosphamide to the subject prior to administration of the composition to the subject.

[00256] In several embodiments, a combination of chemotherapeutic agents is used for lymphodepletion. In several embodiments, a single chemotherapeutic agent is used for lymphodepletion. In several embodiments, wherein a combination of chemotherapeutic agents is used, agents with different mechanisms of actions are optionally used. In several embodiments, different classes of agents are optionally used. In several embodiments, an antimetabolic agent is used. In several embodiments, the antimetabolic agent inhibits and/or prevents cell replication.

[00257] In several embodiments, cyclophosphamide, an alkylating agent that reduces tumor growth, is used in lymphodepletion. In several embodiments, the lymphodepletion comprises cyclophosphamide. In some embodiments, the lymphodepletion comprises cyclophosphamide and does not comprise fludarabine. Thus, in some aspects, provided herein is a method of preparing a subject having a cancer for treatment with a composition comprising natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds to CD19, the method comprising administering a lymphodepleting therapy consisting of cyclophosphamide to the subject prior to administration of the composition to the subject.

[00258] In several embodiments, a dose of between about 200 and 600 mg/m² cyclophosphamide is administered, including doses of about 200 mg/m², about 225 mg/m², about 250 mg/m², about 275 mg/m², about 300 mg/m², about 325 mg/m², about 350 mg/m², about 400 mg/m², about 450 mg/m², about 475 mg/m², about 500 mg/m², about 525 mg/m², about 550 mg/m², about 600 mg/m², or about 700 mg/m², or any dose between those listed. In several embodiments, a dose of about 300 mg/m² cyclophosphamide is administered. In several embodiments, a dose of about 400 mg/m² cyclophosphamide is administered. In several embodiments, a dose of about 500 mg/m² cyclophosphamide is administered. In several embodiments, the dose of cyclophosphamide is given daily for days (e.g., prior to CAR-NK administration). In several embodiments, the dose of cyclophosphamide is given daily for at least about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, or about 7 days (e.g., prior to CAR-NK administration). In several embodiments, the cyclophosphamide is given daily for 3 days. In several embodiments, if necessary, the dose can be split and given, for example, twice daily. In several embodiments, the cyclophosphamide is given daily for 3 days, starting 5 days prior to the first administration of a CD-19 CAR-expressing immune cell. In several embodiments, the cyclophosphamide is given at a dose of about 300 mg/m² daily for 3 days, starting 5 days prior to the first

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administration of a CD-19 CAR-expressing immune cell. In several embodiments, the cyclophosphamide is given at a dose of about 500 mg/m² daily for 3 days, starting 5 days prior to the first administration of a CD19 CAR-expressing immune cell.

[00259] In several embodiments, the cyclophosphamide is administered in combination with another agent.

[00260] In several embodiments, the additional agent is also an antimetabolite. In several embodiments, the additional agent inhibits one or more of DNA polymerase alpha, ribonucleotide reductase and/or DNA primase, thus inhibiting DNA synthesis. In several embodiments, the additional agent is fludarabine. In several embodiments, a dose of between about 5.0 mg/m² – about 200 mg/m² fludarabine is administered, including doses of about 5.0 mg/m², about 10.0 mg/m², about 15.0 mg/m², about 20.0 mg/m², about 25.0 mg/m², about 30.0 mg/m², about 35.0 mg/m², about 40.0 mg/m², about 45.0 mg/m², about 50.0 mg/m², about 60.0 mg/m², about 70.0 mg/m², about 80.0 mg/m², about 90.0 mg/m², about 100.0 mg/m², about 125.0 mg/m², about 150.0 mg/m², about 175.0 mg/m², about 200.0 mg/m², or any dose between those listed. In several embodiments, a dose of about 10 mg/m² fludarabine is administered. In several embodiments, a dose of about 20 mg/m² fludarabine is administered. In several embodiments, a dose of about 30 mg/m² fludarabine is administered. In several embodiments, a dose of about 40 mg/m² fludarabine is administered. In several embodiments, a dose of about 50 mg/m² fludarabine is administered. In several embodiments, a dose of about 100 mg/m² fludarabine is administered. In several embodiments, the dose of fludarabine is given daily for at least about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, or about 7 days. In several embodiments, the dose of fludarabine is given daily for about 3 days. In several embodiments, about 30 mg/m² fludarabine is given daily for about 3 days. In several embodiments, about 30 mg/m² fludarabine is given daily for about 5 days. In several embodiments, if necessary, the dose can be split and given, for example, twice daily.

[00261] In several embodiments, about 300 mg/m² cyclophosphamide and about 30 mg/m² fludarabine is each given daily for about 3 days. In several embodiments, prior to each dosing cycle, about 300 mg/m² cyclophosphamide and about 30 mg/m² fludarabine is each given daily for about 3 days. In several embodiments, about 500 mg/m² cyclophosphamide and about 30 mg/m² fludarabine is each given daily for about 3 days. In several embodiments, prior to each dosing cycle, about 500 mg/m² cyclophosphamide and about 30 mg/m² fludarabine is each given daily for about 3 days.

[00262] In certain embodiments, a dose of a genetically engineered cell(s) described herein or composition thereof is administered to a subject every day, every other day, every couple of days, every third day, once a week, twice a week, three times a week, or once every two weeks. In other embodiments, two, three or four doses of a genetically

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engineered cell(s) described herein or composition thereof is administered to a subject every day, every couple of days, every third day, once a week or once every two weeks. In some embodiments, a dose(s) of a genetically engineered cell(s) described herein or composition thereof is administered for 2 days, 3 days, 5 days, 7 days, 14 days, 21 days, or 28 days. In certain embodiments, a dose of a genetically engineered cell(s) described herein or composition thereof is administered for 1 month, 1.5 months, 2 months, 2.5 months, 3 months, 4 months, 5 months, 6 months or more. In several embodiments, a dosing period is set and a certain number of doses is administered within that time period. For example, in several embodiments, a dosing cycle is 28 days in length with doses of engineered immune cells given on day 0, day 3, and day 7.

[00263] In several embodiments, a subject is administered a lymphodepleting therapy at least one time prior to administration of genetically engineered cells as disclosed herein. In several embodiments, the lymphodepleting therapy is administered before one or more additional doses of engineered cells are administered. In several embodiments, a dosing regimen is used that comprises lymphodepletion followed by a dosing cycle. In several embodiments, the dosing cycle itself is approximately 14, 21, 28, 35, 42 or more days. In several embodiments, each dose of the dosing cycle is administered less than a week apart from each other. In several embodiments, a subject receives a first dose on day 0 of the cycle, a second dose on day 3 of the cycle and a third dose on day 7 of the cycle. In several such embodiments, a 28-day cycle is used with primary outcome measures evaluated at day 28. In several embodiments, lymphodepletion is performed prior to the inception of each dosing cycle, if subsequent dosing cycles are required (e.g., the subject requires further treatment). For example, in several embodiments, a subject undergoes lymphodepletion, receives a plurality of doses of engineered cells according to a cycle, is evaluated at the end of the cycle time and, if deemed necessary undergoes a second lymphodepletion followed by a second dosing cycle. In several embodiments where multiple dosing cycles are used, a lymphodepleting therapy is only administered prior to the first dosing cycle. In several embodiments, fludarabine/cyclophosphamide is used to achieve lymphodepletion. In several embodiments, the lymphodepleting therapy comprises administration of cyclophosphamide (500 mg/m²) and fludarabine (30mg/m²), each administered daily for 5 days. Depending on the embodiment, different concentrations may be used. In such embodiments where multiple dosing cycles are used, a first and a second dosing cycle need not be the same (e.g., a first cycle may have 2 doses, while a second uses three doses). Depending on the subject 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more dosing cycles are performed.

[00264] Advantageously, in several embodiments, the therapies and dosing regimens provided for herein provide effective anti-cancer treatment without certain CAR-T cell toxicities, such as cytokine release syndrome, immune effector cell-associated

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neurotoxicity syndrome (ICANS) or neurotoxicity, or graft-versus host disease. In several embodiments, complete remission is achieved. In several embodiments, complete response (CR) is achieved. In several embodiments, partial response (PR) is achieved. In several embodiments, stable disease (SD) or limited progression of disease is accomplished.

[00265] Clinical outcomes can be assessed by any of the methods known in the art, including based on the Lugano classification with lymphoma response to immunomodulatory therapy criteria (LYRIC) refinement for subjects with non-Hodgkin lymphoma (NHL); the 2018 International Workshop on Chronic Lymphocytic Leukemia (iwCLL) guidelines for subjects with chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL); Version 1.2020 National Comprehensive Cancer Network (NCCN) for subjects with B-cell acute lymphoblastic leukemia (B-ALL); or 6th International Workshop on Waldenström macroglobulinemia (WM) for subjects with WM. See Cheson et al., *Blood* (2016) 128(21):2489-96; Cheson et al., *J Clin Oncol* (2014) 32(27):3059-68; Hallek et al., *Blood* (2018) 131(25):2745-60; NCCN Guidelines for Acute Lymphoblastic Leukemia 1.2020; and Owen et al., *Br J Haematol* (2013) 160(2):171-6.

[00266] In some embodiments, also provided herein are nucleic acid and amino acid sequences that have sequence identity and/or homology of at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% (and ranges therein) as compared with the respective nucleic acid or amino acid sequences of SEQ ID NOS: 1-41 (or combinations of two or more of SEQ ID NOS: 1-41) and that also exhibit one or more of the functions as compared with the respective SEQ ID NOS: 1-41 (or combinations of two or more of SEQ ID NOS: 1-41) including but not limited to, (i) enhanced proliferation, (ii) enhanced activation, (iii) enhanced cytotoxic activity against cells presenting ligands to which NK cells harboring receptors encoded by the nucleic acid and amino acid sequences bind, (iv) enhanced homing to tumor or infected sites, (v) reduced off target cytotoxic effects, (vi) enhanced secretion of immunostimulatory cytokines and chemokines (including, but not limited to IFN γ , TNF α , IL-22, CCL3, CCL4, and CCL5), (vii) enhanced ability to stimulate further innate and adaptive immune responses, and (viii) combinations thereof.

[00267] Additionally, in several embodiments, there are provided amino acid sequences that correspond to any of the nucleic acids disclosed herein, while accounting for degeneracy of the nucleic acid code. Furthermore, those sequences (whether nucleic acid or amino acid) that vary from those expressly disclosed herein, but have functional similarity or equivalency are also contemplated within the scope of the present disclosure. The foregoing includes mutants, truncations, substitutions, or other types of modifications.

[00268] In several embodiments, polynucleotides encoding the disclosed cytotoxic receptor complexes are mRNA. In some embodiments, the polynucleotide is DNA.

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In some embodiments, the polynucleotide is operably linked to at least one regulatory element for the expression of the cytotoxic receptor complex.

[00269] Additionally provided, according to several embodiments, is a vector comprising the polynucleotide encoding any of the polynucleotides provided for herein, wherein the polynucleotides are optionally operatively linked to at least one regulatory element for expression of a cytotoxic receptor complex. In several embodiments, the vector is a retrovirus.

[00270] Further provided herein are engineered immune cells (such as NK and/or T cells) comprising the polynucleotide, vector, or cytotoxic receptor complexes as disclosed herein. Further provided herein are engineered NK cells comprising the polynucleotide, vector, or cytotoxic receptor complexes as disclosed herein. Further provided herein are compositions comprising a mixture of engineered immune cells (such as NK cells and/or engineered T cells), each population comprising the polynucleotide, vector, or cytotoxic receptor complexes as disclosed herein. Further provided herein are compositions comprising engineered NK cells comprising the polynucleotide, vector, or cytotoxic receptor complexes as disclosed herein. In some embodiments, the composition comprises a pharmaceutically acceptable carrier. In some embodiments, the composition comprises a cryopreservative.

IV. Subjects

[00271] Some embodiments of the compositions and methods described herein relate to administering immune cells comprising a chimeric antigen receptor to a subject with cancer.

[00272] In several embodiments, the subject has large B-cell lymphoma (LBCL). In several embodiments, the subject has aggressive LBCL. In several embodiments, the subject has Non-Hodgkin lymphoma (NHL). In several embodiments, the subject has diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), marginal zone lymphoma (MZL), mantle cell lymphoma (MCL), or B-cell acute lymphoblastic leukemia (B-ALL). In several embodiments, the subject has diffuse large B-cell lymphoma (DLBCL). In several embodiments, the subject has follicular lymphoma (FL). In several embodiments, the subject has high grade FL (e.g., FL grade 3b). In several embodiments, the subject has indolent lymphoma (IL). In several embodiments, the subject has grade 1, 2, or 3a FL. In several embodiments, the subject has marginal zone lymphoma (MZL). In several embodiments, the subject has mantle cell lymphoma (MCL). In several embodiments, the subject has B-cell acute lymphoblastic leukemia (B-ALL). In several embodiments, the subject has Waldenström macroglobulinemia (WM). In several embodiments, the subject has Chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL). In several embodiments, the subject has CLL. In several embodiments, the subject has SLL. In several embodiments, the subject

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has primary mediastinal large B cell lymphoma (PMBCL). In some embodiments, the cancer is a relapsed/refractory (r/r) cancer.

[00273] In some embodiments, the subject has marrow-localized disease (e.g., $\leq 5\%$ peripheral blasts without other evidence of extramedullary disease including lymphoblastic lymphoma). In some embodiments, the subject has $\leq 5\%$ peripheral blasts. In some embodiments, the subject has $< 5\%$ peripheral blasts. In some embodiments, the subject has r/r B-ALL. In some embodiments, the subject has r/r B-ALL with $\leq 5\%$ peripheral blasts. In some embodiments, the subject has r/r B-ALL with $< 5\%$ peripheral blasts. In some embodiments, the subject does not have evidence of extramedullary disease. In some embodiments, the subject does not have evidence of extramedullary disease. In some embodiments, the subject does not have evidence of extramedullary disease including lymphoblastic lymphoma. In some embodiments, the subject does not have other evidence of extramedullary disease including lymphoblastic lymphoma.

[00274] In some embodiments, the subject has a histologically or cytologically confirmed diagnosis of r/r B cell NHL, CLL, or B-ALL. In some embodiments, the diagnosis is defined by WHO 2016 classification criteria.

[00275] In some embodiments, the subject has measurable disease as defined by any of the methods for diagnosing and staging known in the art, including WHO 2016 classification for r/r B cell NHL or B-ALL (Quintanilla-Martinez, Hematological Oncology (2017) 35:37-4); Lugano classification for NHL (Cheson et al., J. Clin. Oncol (2014) 32(27):3059-68); iwCLL for CLL and SLL (Hallek et al., Blood (2018) 131(25):2745-60); and Second International Workshop on Waldenström Macroglobulinemia for WM (Owen et al., Semin Oncol (2003) 30(2):110-15).

[00276] In some embodiments, the subject has been treated with a previous line of therapy. In some embodiments, the subject is relapsed/refractory (R/R) to a previous line of therapy. In some embodiments, the previous line of therapy comprises one previous line of therapy. In some embodiments, the subject has MCL, the previous line of therapy is one previous line of therapy, and the one previous line of therapy is not CAR T cells. In some embodiments, the subject has WM, and the previous line of therapy is one previous line of therapy. In some embodiments, the previous line of therapy comprises two previous lines of therapy. In some embodiments, the previous line of therapy comprises three previous lines of therapy. In some embodiments, the previous line of therapy comprises four previous lines of therapy. In some embodiments, the subject did not respond to or relapsed within 12 months of completion of the prior line of therapy. In some embodiments, the subject did not respond to the prior line of therapy. In some embodiments, the subject relapsed within 12 months of completion of the prior line of therapy.

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[00277] In some embodiments, the subject has been treated with at least two lines of prior therapy. In some embodiments, if the subject (i) has MCL, (ii) has not been previously treated with CAR T cells; or (iii) has WM, the subject has been previously treated with at least one prior line of therapy.

[00278] In some embodiments, the previous line of therapy comprises an inhibitor of Bruton's tyrosine kinase (BTKi). In some embodiments, the subject has been previously treated with a BTKi. In some embodiments, the subject has a cancer for which a BTKi is approved, and the previous line of therapy comprises a BTKi. In some embodiments, the subject is R/R to a BTKi. In some embodiments, the BTKi comprises ibrutinib. In some embodiments, the BTKi is ibrutinib. In some embodiments, the subject has been previously treated with ibrutinib. In some embodiments, the subject is R/R to ibrutinib.

[00279] In some embodiments, the previous line of therapy comprises a tyrosine kinase inhibitor. In some embodiments, the subject has Philadelphia chromosome (Ph+) B-ALL and the previous line of therapy comprises a tyrosine kinase inhibitor.

[00280] In some embodiments, the previous line of therapy comprises a Bcl-2 inhibitor. In some embodiments, the subject has been previously treated with a Bcl-2 inhibitor. In some embodiments, the subject has CLL or SLL, and the previous line of therapy comprises a Bcl-2 inhibitor. In some embodiments, the subject is R/R to a Bcl-2 inhibitor. In some embodiments, the Bcl-2 inhibitor comprises venetoclax. In some embodiments, the Bcl-2 inhibitor is venetoclax. In some embodiments, the subject has been previously treated with venetoclax. In some embodiments, the subject is R/R to venetoclax.

[00281] In some embodiments, the previous line of therapy comprises a BTKi and a Bcl-2 inhibitor. In some embodiments, the subject has been previously treated with a BTKi and a Bcl-2 inhibitor. In some embodiments, the subject is R/R to a BTKi and a Bcl-2 inhibitor. In some embodiments, the BTKi is ibrutinib. In some embodiments, the Bcl-2 inhibitor is venetoclax.

[00282] In some embodiments, the previous line of therapy comprises a CD20-targeted therapy and a cytotoxic chemotherapy (e.g., anthracycline). In some embodiments, the CD20-targeted therapy is an anti-CD20 antibody. In some embodiments, the anti-CD20 antibody is an anti-CD20 monoclonal antibody. In some embodiments, the anti-CD20 antibody comprises rituximab. In some embodiments, the anti-CD20 antibody is rituximab. In some embodiments, the cytotoxic chemotherapy comprises anthracycline. In some embodiments, the cytotoxic chemotherapy is anthracycline. In some embodiments, the subject has been previously treated with an anti-CD20 monoclonal antibody and a cytotoxic therapy (e.g., anthracycline). In some embodiments, the subject is R/R to an anti-CD20 monoclonal antibody and a cytotoxic therapy (e.g., anthracycline). In some embodiments, if the previous line of

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therapy comprises a CD20-targeted therapy, cells of the cancer are CD20+ (e.g., as assessed locally).

[00283] In some embodiments, the previous line of therapy comprises a CD19-directed therapy. In some embodiments, the subject has been previously treated with a CD19-directed therapy. In some embodiments, the previous line of therapy comprises chimeric antigen receptor (CAR) T cells. In some embodiments, the subject has been previously treated with CAR T cells (CAR T exposed). In some embodiments, the subject has been previously treated with anti-CD19 CAR T cells. In some embodiments, the CAR T cells are autologous CAR T cells. In some embodiments, the subject has been previously treated with autologous CAR T cells. In some embodiments, the subject has been previously treated with autologous anti-CD19 CAR T cells. In some embodiments, if the previous line of therapy comprises a CD19-directed therapy, cells of the cancer are CD19+ (e.g., as assessed locally).

[00284] In some embodiments, the previous line of therapy does not comprise a CD19-directed therapy. In some embodiments, the subject has not been previously treated with a CD19-directed therapy. In some embodiments, the previous line of therapy does not comprise chimeric antigen receptor (CAR) T cells. In some embodiments, the subject has not been previously treated with CAR T cells (CAR T naïve). In some embodiments, the subject has not been previously treated with autologous CAR T cells. In some embodiments, the subject has not been previously treated with anti-CD19 CAR T cells. In some embodiments, the subject has not been previously treated with autologous anti-CD19 CART T cells.

[00285] In some embodiments, the subject is a human. In some embodiments, the subject is an adult. In some embodiments, the subject is at least 18 years of age.

[00286] In some embodiments, the subject has an Eastern Cooperative Oncology Group Performance Status (ECOG) of 0, 1, or 2. In some embodiments, the subject has an Eastern Cooperative Oncology Group Performance Status (ECOG) of 0 or 1. In some embodiments, the subject has an ECOG of 0. In some embodiments, the subject has an ECOG of 1. In some embodiments, the subject has an ECOG of 2.

[00287] In some embodiments, the subject has adequate organ function. In some embodiments, adequate organ function comprises a platelet count $\geq 30,000/\mu\text{L}$. In some embodiments, the subject has a white blood cell count of less than or equal to $109/\text{L}$. In some embodiments, adequate organ function comprises serum creatinine value $\leq 1.5 \times$ upper limit of normal (ULN). In some embodiments, adequate organ function comprises total bilirubin value $\leq 1.5 \times$ ULN or $\leq 3.0 \times$ ULN for subjects with hereditary benign hyperbilirubinemia. In some embodiments, adequate organ function comprises aspartate aminotransferase (AST)/serum glutamic-oxaloacetic transaminase (SGOT) value $\leq 3 \times$ ULN and alanine aminotransferase (ALT)/serum glutamic pyruvic transaminase (SGPT) value $\leq 3 \times$ ULN. In some embodiments, adequate organ function comprises baseline international normalized

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ratio (INR) ≤ 2 or activated partial thromboplastin time (aPTT) of ≤ 2 times ULN. In some embodiments, adequate organ function comprises, the subject does not require oxygen therapy.

[00288] In some embodiments, the subject does not have Burkitt lymphoma. In some embodiments, the subject does not have primary central nervous system (CNS) lymphoma. In some embodiments, the subject does not have Richter's transformation. In some embodiments, the subject does not have Richter's transformation to Hodgkin lymphoma. In some embodiments, the subject does not have any evidence of active CNS malignancy.

V. Cancer Types

[00289] Some embodiments of the compositions and methods described herein relate to administering immune cells comprising a tumor-directed chimeric antigen receptor and/or tumor-directed chimeric receptor to a subject with cancer.

[00290] Cancers derived from B-cell lineages are a worldwide healthcare burden. More than 500,000 new cases of non-Hodgkin lymphoma (NHL) (median age 69 years) and 50,000 new cases of acute lymphoblastic leukemia (ALL) (median age 16 years) are expected in the world each year (seer.cancer.gov, Smith Br J Cancer. 2015;112(9):1575-84, Solomon, paper presented at: 11th International Conference on Hematology & Hematological Oncology; November 08-09, 2017). Despite progress in treatment, many patients diagnosed with these heterogeneous groups of cancers still succumb to their diseases. Approximately 30% to 50% of newly diagnosed patients with aggressive large cell lymphomas are not cured by first line treatment (Gisselbrecht J Clin Oncol. 2010 Sep 20;28(27):4184-90; Kenkre Curr Oncol Rep. 2008;10:393-403; Sehn Blood 2006;109(5):1857-1861; Sinha Expert Opin Investig Drugs 2011 May 20(5):669-80). Similarly, more than half of adults with ALL will ultimately relapse (Malard 2020).

[00291] In some embodiments, the cancer is a hematologic malignancy. In some embodiments, the cancer is a leukemia or a lymphoma. In some embodiments, the lymphoma is a double hit/expressor lymphoma. In some embodiments, the lymphoma is a triple hit/expressor lymphoma. In some embodiments, the cancer comprises Richter's transformation.

[00292] Various embodiments provided for herein include treatment or prevention of various malignancies, such as non-Hodgkin lymphoma, B-cell lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), mantle cell lymphoma, marginal zone lymphomas, Burkitt lymphoma, lymphoplasmacytic lymphoma, hairy cell leukemia, primary central nervous system lymphoma, primary intraocular lymphoma. In some embodiments, the cancer is non-Hodgkin lymphoma. In some embodiments, the cancer is B-cell lymphoma. In some embodiments, the cancer is diffuse large B-cell lymphoma. In some embodiments, the cancer

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is follicular lymphoma. In some embodiments, the cancer is chronic lymphocytic leukemia. In some embodiments, the cancer is chronic myelogenous leukemia. In some embodiments, the cancer is mantle cell lymphoma. In some embodiments, the cancer is marginal zone lymphoma. Additional types of cancer include, but are not limited to, Hodgkin lymphoma, acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), adrenocortical carcinoma, Kaposi sarcoma, lymphoma, gastrointestinal cancer, appendix cancer, central nervous system cancer, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, brain tumors (including but not limited to astrocytomas, spinal cord tumors, brain stem glioma, craniopharyngioma, ependymoblastoma, ependymoma, medulloblastoma, medulloepithelioma), breast cancer, bronchial tumors, cervical cancer, colon cancer, chronic myeloproliferative disorders, ductal carcinoma, endometrial cancer, esophageal cancer, gastric cancer, renal cell cancer, leukemia, oral cancer, nasopharyngeal cancer, liver cancer, lung cancer (including but not limited to, non-small cell lung cancer, (NSCLC) and small cell lung cancer), pancreatic cancer, bowel cancer, lymphoma, melanoma, ocular cancer, ovarian cancer, pancreatic cancer, prostate cancer, pituitary cancer, uterine cancer, and vaginal cancer.

[00293] In some embodiments, the cancer is a B cell-derived NHL, such as an aggressive large B cell lymphoma (LBCL). In some embodiments, the LBCL is diffuse large B cell lymphoma (DLBCL) not otherwise specified; high grade B cell lymphoma; DLBCL derived from follicular lymphoma (FL) (FL grade 3b); DLBCL derived from Richter's transformation to DLBCL from chronic lymphocytic leukemia (CLL); primary mediastinal LBCL; and DLBCL derived from Waldenström macroglobulinemia (WM). In some embodiments, the cancer is a NHL. In some embodiments, the cancer is a LBCL. In some embodiments, the cancer is an aggressive LBCL. In some embodiments, the cancer is DLBCL. In some embodiments, the cancer is FL grade 3b.

[00294] In some embodiments, the cancer is an indolent lymphoma (IL). In some embodiments, the IL is a low grade FL (FL grades 1, 2, and 3a), MCL, or MZL. In some embodiments, the IL is a low grade FL (FL grades 1, 2, and 3a). In some embodiments, the IL is MCL. In some embodiments, the IL is MZL. In some embodiments, the cancer is a low grade FL (FL grades 1, 2, and 3a). In some embodiments, the cancer is FL grade 1. In some embodiments, the cancer is FL grade 2. In some embodiments, the cancer is FL grade 3a. In some embodiments, the cancer is MCL. In some embodiments, the cancer is MZL.

[00295] In some embodiments, the cancer is CLL or SLL. In some embodiments, the cancer is CLL. In some embodiments, the cancer is SLL. In some embodiments, the cancer is B-ALL.

[00296] In some embodiments, the cancer is relapsed/refractory (R/R). In some embodiments, the cancer is R/R NHL. In some embodiments, the cancer is R/R LBCL. In

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some embodiments, the cancer is R/R CLL. In some embodiments, the cancer is R/R SLL. In some embodiments, the cancer is R/R B-ALL.

[00297] In some embodiments, cells of the cancer express CD19. In some embodiments, cells of the cancer express CD19 at the time of administration of a dose (e.g., the first dose) of genetically engineered NK cells. Expression of CD19 can be determined by any methods known in the art, including by flow cytometry.

[00298] In some embodiments, cells of the cancer express CD20. In some embodiments, cells of the cancer express CD20 at the time of administration of a dose (e.g., the first dose) of genetically engineered NK cells. Expression of CD20 can be determined by any methods known in the art, including by flow cytometry.

[00299] In some embodiments, the cancer has been previously treated with CAR T cells (CAR T exposed). In some embodiments, the cancer is relapsed/refractory to CAR T cells. In some embodiments, the CAR T cells are anti-CD19 CAR T cells. In some embodiments, the CAR T cells are autologous CAR T cells. In some embodiments, the CAR T cells are autologous anti-CD19 CAR T cells. Thus, in some embodiments, the cancer has been previously treated with autologous anti-CD19 CAR T cells. In some embodiments, the cancer is R/R to autologous anti-CD19 CAR T cells. In some embodiments, the cancer has not been previously treated with CAR T cells (CAR T naïve). In some embodiments, the cancer has not been previously treated with anti-CD19 CAR T cells, optionally autologous anti-CD19 CAR T cells. Thus, in some embodiments, the cancer is not R/R to anti-CD19 CAR T cells.

[00300] In some embodiments, the cancer is aggressive LBCL that has not been previously treated with CAR T cells (CAR T naïve), optionally autologous CAR T cells. In some embodiments, the cancer is aggressive LBCL that has not been previously treated with anti-CD19 CAR T cells (CAR T naïve), optionally autologous anti-CD19 CAR T cells. In some embodiments, the cancer is a R/R LBCL that has not been previously treated with CAR T cells (CAR T naïve), optionally autologous CAR T cells. In some embodiments, the cancer is a R/R LBCL that has not been previously treated with anti-CD19 CAR T cells (CAR T naïve), optionally autologous anti-CD19 CAR T cells. In some embodiments, the cancer is a R/R NHL that has not been previously treated with CAR T cells (CAR T naïve), optionally autologous CAR T cells. In some embodiments, the cancer is a R/R NHL that has not been previously treated with anti-CD19 CAR T cells (CAR T naïve), optionally autologous anti-CD19 CAR T cells. In some embodiments, the cancer is MCL that has not been previously treated with CAR T cells (CAR T naïve), optionally autologous CAR T cells. In some embodiments, the cancer is MCL that has not been previously treated with anti-CD19 CAR T cells (CAR T naïve), optionally autologous anti-CD19 CAR T cells. In some embodiments, the cancer is an IL that has not been previously treated with CAR T cells (CAR T naïve), optionally autologous CAR T cells. In some embodiments, the cancer is an IL that has not been previously treated with

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anti-CD19 CAR T cells (CAR T naïve), optionally autologous anti-CD19 CAR T cells. In some embodiments, the cancer is B-ALL that has not been previously treated with CAR T cells (CAR T naïve), optionally autologous CAR T cells. In some embodiments, the cancer is B-ALL that has not been previously treated with anti-CD19 CAR T cells (CAR T naïve), optionally autologous anti-CD19 CAR T cells.

[00301] In some embodiments, the cancer is aggressive LBCL that has been previously treated with CAR T cells (CAR T exposed), optionally autologous CAR T cells. In some embodiments, the cancer is aggressive LBCL that has been previously treated with anti-CD19 CAR T cells (CAR T exposed), optionally autologous anti-CD19 CAR T cells. In some embodiments, the cancer is a R/R LBCL that has been previously treated with CAR T cells (CAR T exposed), optionally autologous CAR T cells. In some embodiments, the cancer is a R/R LBCL that has been previously treated with anti-CD19 CAR T cells (CAR T exposed), optionally autologous anti-CD19 CAR T cells. In some embodiments, the cancer is a R/R NHL that has been previously treated with CAR T cells (CAR T exposed), optionally autologous CAR T cells. In some embodiments, the cancer is a R/R NHL that has been previously treated with anti-CD19 CAR T cells (CAR T exposed), optionally autologous anti-CD19 CAR T cells. In some embodiments, the cancer is MCL that has been previously treated with CAR T cells (CAR T exposed), optionally autologous CAR T cells. In some embodiments, the cancer is MCL that has been previously treated with anti-CD19 CAR T cells (CAR T exposed), optionally autologous anti-CD19 CAR T cells. In some embodiments, the cancer is an IL that has been previously treated with CAR T cells (CAR T exposed), optionally autologous CAR T cells. In some embodiments, the cancer is an IL that has been previously treated with anti-CD19 CAR T cells (CAR T exposed), optionally autologous anti-CD19 CAR T cells. In some embodiments, the cancer is B-ALL that has been previously treated with CAR T cells (CAR T exposed), optionally autologous CAR T cells. In some embodiments, the cancer is B-ALL that has been previously treated with anti-CD19 CAR T cells (CAR T exposed), optionally autologous anti-CD19 CAR T cells.

[00302] In some embodiments, the cancer is a LBCL (e.g., DLBCL) that has been previously treated with an anti-CD20 monoclonal antibody and a cytotoxic chemotherapy (e.g., anthracycline). In some embodiments, the cancer is a LBCL (e.g., DLBCL) that has been previously treated with an anti-CD20 monoclonal antibody and anthracycline. In some embodiments, the cancer is an IL that has been previously treated with an anti-CD20 monoclonal antibody and a cytotoxic chemotherapy (e.g., anthracycline). In some embodiments, the cancer is an IL that has been previously treated with an anti-CD20 monoclonal antibody and anthracycline.

[00303] In some embodiments, the cancer is a MCL, CLL, SLL, or WM that has been previously treated with an inhibitor of Bruton's tyrosine kinase (BTKi) (e.g., ibrutinib). In

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some embodiments, the cancer is a MCL that has been previously treated with a BTKi (e.g., ibrutinib). In some embodiments, the cancer is a MCL that has been previously treated with a BTKi (e.g., ibrutinib) and anti-CD19 CAR T cells. In some embodiments, the cancer is a CLL that has been previously treated with a BTKi (e.g., ibrutinib). In some embodiments, the cancer is a SLL that has been previously treated with a BTKi (e.g., ibrutinib). In some embodiments, the cancer is a WM that has been previously treated with a BTKi (e.g., ibrutinib).

[00304] In some embodiments, the cancer is a CLL or SLL that has been previously treated with a Bcl-2 inhibitor (e.g., venetoclax). In some embodiments, the cancer is a CLL that has been previously treated with a Bcl-2 inhibitor (e.g., venetoclax). In some embodiments, the cancer is a SLL that has been previously treated with a Bcl-2 inhibitor (e.g., venetoclax). In some embodiments, the cancer is a CLL or SLL that has been previously treated with a Bcl-2 inhibitor (e.g., venetoclax) and a BTKi (e.g., ibrutinib). In some embodiments, the cancer is a CLL that has been previously treated with a Bcl-2 inhibitor (e.g., venetoclax) and a BTKi (e.g., ibrutinib). In some embodiments, the cancer is a SLL that has been previously treated with a Bcl-2 inhibitor (e.g., venetoclax) and a BTKi (e.g., ibrutinib).

DEFINITIONS

[00305] Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

[00306] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

[00307] The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

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[00308] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC).

[00309] An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[00310] "Isolated nucleic acid encoding an anti-CD19 antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[00311] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[00312] The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Polypeptides, including the antibodies and antibody chains and other peptides, e.g., linkers and CD19-binding peptides, may include amino acid residues including natural and/or non-natural amino acid residues. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. In some aspects, the polypeptides may contain modifications with respect to a native or natural sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[00313] As used herein, "percent (%) amino acid sequence identity" and "percent identity" and "sequence identity" when used with respect to an amino acid sequence (reference polypeptide sequence) is defined as the percentage of amino acid residues in a candidate sequence (e.g., the subject antibody or fragment) that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and

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introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[00314] An amino acid substitution may include replacement of one amino acid in a polypeptide with another amino acid. Amino acid substitutions may be introduced into a binding molecule, e.g., antibody, of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, or decreased immunogenicity.

[00315] Amino acids generally can be grouped according to the following common side-chain properties.

[00316] (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

[00317] (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

[00318] (3) acidic: Asp, Glu;

[00319] (4) basic: His, Lys, Arg;

[00320] (5) residues that influence chain orientation: Gly, Pro;

[00321] (6) aromatic: Trp, Tyr, Phe.

[00322] Non-conservative amino acid substitutions will involve exchanging a membrane of one of these classes for another class.

[00323] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

[00324] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[00325] As used herein, a "subject" is a mammal, such as a human or other animal, and typically is human. In some embodiments, the subject, e.g., patient, to whom the agent or agents, cells, cell populations, or compositions are administered, is a mammal, typically a primate, such as a human. In some embodiments, the primate is a monkey or an ape. The subject can be male or female and can be any suitable age, including infant, juvenile,

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adolescent, adult, and geriatric subjects. In some embodiments, the subject is a non-primate mammal, such as a rodent.

[00326] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to complete or partial amelioration or reduction of a disease or condition or disorder, or a symptom, adverse effect or outcome, or phenotype associated therewith. Desirable effects of treatment include, but are not limited to, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. The terms do not imply complete curing of a disease or complete elimination of any symptom or effect(s) on all symptoms or outcomes.

[00327] "Preventing" (and grammatical variations thereof such as "prevent" or "prevention") as used herein, includes providing prophylaxis with respect to the occurrence or recurrence of a disease in a subject that may be predisposed to the disease but has not yet been diagnosed with the disease. In some embodiments, the provided cells and compositions are used to delay development of a disease or to slow the progression of a disease.

[00328] A "therapeutically effective amount" of an agent, e.g., a pharmaceutical formulation or cells, refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result, such as for treatment of a disease, condition, or disorder, and/or pharmacokinetic or pharmacodynamic effect of the treatment. The therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the subject, and the populations of cells administered. In some embodiments, the provided methods involve administering the cells and/or compositions at effective amounts, e.g., therapeutically effective amounts.

[00329] As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. For example, "a" or "an" means "at least one" or "one or more." It is understood that aspects, embodiments, and variations described herein include "comprising," "consisting," and/or "consisting essentially of" aspects, embodiments and variations.

[00330] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

[00331] Throughout this disclosure, various aspects of the claimed subject matter are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible

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limitation on the scope of the claimed subject matter. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the claimed subject matter. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the claimed subject matter, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the claimed subject matter. This applies regardless of the breadth of the range.

[00332] As used herein, a "composition" refers to any mixture of two or more products, substances, or compounds, including cells. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

[00333] As used herein, a statement that a cell or population of cells is "positive" for a particular marker refers to the detectable presence on or in the cell of a particular marker, typically a surface marker. When referring to a surface marker, the term refers to the presence of surface expression as detected by flow cytometry, for example, by staining with an antibody that specifically binds to the marker and detecting said antibody, wherein the staining is detectable by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control under otherwise identical conditions and/or at a level substantially similar to that for cell known to be positive for the marker, and/or at a level substantially higher than that for a cell known to be negative for the marker.

[00334] As used herein, a statement that a cell or population of cells is "negative" for a particular marker refers to the absence of substantial detectable presence on or in the cell of a particular marker, typically a surface marker. When referring to a surface marker, the term refers to the absence of surface expression as detected by flow cytometry, for example, by staining with an antibody that specifically binds to the marker and detecting said antibody, wherein the staining is not detected by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control under otherwise identical conditions, and/or at a level substantially lower than that for cell known to be positive for the marker, and/or at a level substantially similar as compared to that for a cell known to be negative for the marker.

NON-LIMITING EMBODIMENTS

[00335] Among the embodiments provided herein are:

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1. A method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

the second dose is administered to the subject between 2-4 days after administration of the first dose to the subject;

the third dose is administered to the subject between 2-4 days after administration of the second dose to the subject; and

each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

2. A method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

all three doses are administered to the subject within between about 4 days and about 10 days; and

each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

3. A method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

all three doses are administered to the subject within between about 4 days and about 10 days;

the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle; and

the lymphodepleting therapy comprises cyclophosphamide and does not comprise fludarabine.

4. A method of preparing a subject having a cancer for treatment with natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, the method comprising administering a lymphodepleting therapy to the subject prior to administration of a first dosing cycle of the genetically engineered NK cells to the subject, wherein:

the first dosing cycle comprises a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells,

each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells; and

all three doses are administered to the subject within between about 4 days and about 10 days.

5. The method of any one of Embodiments 2-4, wherein the second dose is administered to the subject between 2-4 days after administration of the first dose to the subject and the third dose is administered to the subject between 2-4 days after administration of the second dose to the subject.

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6. The method of any one of Embodiments 3-5, wherein each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

7. The method of any one of Embodiments 1-6, wherein each of the first, second, and third doses comprises about 1.5×10^9 CAR-expressing NK cells.

8. A method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

the second dose is administered to the subject between 2-4 days after administration of the first dose to the subject;

the third dose is administered to the subject between 2-4 days after administration of the second dose to the subject; and

each of the first, second and third doses comprises at least about 2×10^9 CAR-expressing NK cells.

9. The method of any one of Embodiments 1-6 and Embodiment 8, wherein each of the first, second and third doses comprises about 2×10^9 CAR-expressing NK cells.

10. The method of any one of Embodiments 1-6 and Embodiment 8, wherein each of the first, second and third doses comprises about 2.5×10^9 CAR-expressing NK cells.

11. The method of any one of Embodiments 1, 2, and 5-10, wherein the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle.

12. The method of any one of Embodiments 1-11 wherein the first dosing cycle is followed by an additional dosing cycle.

13. The method of any one of Embodiments 1-12, wherein, if the subject exhibits a clinical response, optionally a complete response (CR), following the first dosing cycle, the method comprises an additional dosing cycle as consolidation treatment.

14. The method of any one of Embodiments 1-13, wherein, if the subject exhibits a clinical response following a dosing cycle and subsequently exhibits disease progression, the method comprises an additional dosing cycle as retreatment.

15. The method of any one of Embodiments 1-14, wherein the method comprises between one dosing cycle and five dosing cycles.

16. The method of any one of Embodiments 1-15, wherein the subject is administered a lymphodepleting therapy prior to each dosing cycle.

17. The method of any one of Embodiments 1-16, wherein each dosing cycle is between about 14 days and about 35 days.

18. The method of any one of Embodiments 1-17, wherein each dosing cycle is about 28 days.

19. The method of any one of Embodiments 3-7 and 9-18, wherein the lymphodepleting therapy comprises cyclophosphamide, optionally wherein a dose of cyclophosphamide is between about 300 mg/m^2 and about 1000 mg/m^2 .

20. The method of any one of Embodiments 3-7 and 9-19, wherein the lymphodepleting therapy comprises a single dose of cyclophosphamide.

21. The method of Embodiment 20, wherein the single dose of cyclophosphamide is administered to the subject about 3 days prior to administration of the dosing cycle.

22. The method of Embodiment 20 or Embodiment 21, wherein the single dose of cyclophosphamide is about 1000 mg/m^2 .

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23. The method of any one of Embodiments 3-7 and 9-19, wherein the lymphodepleting therapy comprises three doses of cyclophosphamide.

24. The method of Embodiment 23, wherein each dose of cyclophosphamide is about 500 mg/m².

25. The method of Embodiment 23 or Embodiment 24, wherein a dose of cyclophosphamide is administered to the subject on each of about 5 days prior, 4 days prior, and 3 days prior to administration of the first dose of the dosing cycle.

26. The method of any one of Embodiments 4-7 and 9-25, wherein the lymphodepleting therapy does not comprise fludarabine.

27. The method of any one of Embodiments 4-7 and 9-25, wherein the lymphodepleting therapy comprises fludarabine, optionally wherein a dose of fludarabine is between about 20 mg/m² and about 40 mg/m².

28. The method of Embodiment 27, wherein the lymphodepleting therapy comprises three doses of fludarabine.

29. The method of Embodiment 27 or Embodiment 28, wherein each dose of fludarabine is about 30 mg/m².

30. The method of any one of Embodiments 27-29, wherein a dose of fludarabine is administered to the subject on each of about 5 days prior, 4 days prior, and 3 days prior to administration of the first dose of the dosing cycle.

31. The method of any one of Embodiments 1-30, further comprising administration of a therapeutic agent that targets CD20.

32. The method of any one of Embodiments 1-31, wherein the subject is administered a therapeutic agent that targets CD20.

33. The method of Embodiment 31 or Embodiment 32, wherein the therapeutic agent is an anti-CD20 monoclonal antibody.

34. The method of Embodiment 33, wherein the anti-CD20 antibody is rituximab.

35. The method of any one of Embodiments 31-34, wherein the therapeutic agent that targets CD20 is administered in an amount between about 150 mg/m² and about 500 mg/m².

36. The method of any one of Embodiments 31-35, wherein the therapeutic agent that targets CD20 is administered in an amount of about 375 mg/m².

37. The method of any one of Embodiments 31-36, wherein the therapeutic agent is administered to the subject at least one time and the at least one time is at least 2 days prior to administration of the first dose of the dosing cycle.

38. The method of any one of Embodiments 31-35, wherein the therapeutic agent is administered to the subject one time 3 days prior to administration of the first dose of the dosing cycle.

39. The method of any one of Embodiments 3-7 and 9-38, wherein the first dose of genetically engineered NK cells is administered to the subject about 2 to 5 days after administration of the lymphodepleting therapy has concluded, optionally wherein the first dose of the genetically engineered NK cells is administered to the subject about 3 days after administration of the lymphodepleting therapy has concluded.

40. The method of any one of Embodiments 3-7 and 9-39, wherein all three doses of the genetically engineered NK cells are administered to the subject within about 10 days after administration of the lymphodepleting therapy has concluded.

41. A method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of

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the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

the second dose is administered to the subject about 3 days after administration of the first dose to the subject,

the third dose is administered to the subject about 4 days after administration of the second dose to the subject;

each of the first, second and third doses comprises between about 1.0×10^9 CAR-expressing NK cells and about 2×10^9 CAR-expressing NK cells;

the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle; and

the lymphodepleting therapy consists of a dose of about 500 mg/m² of cyclophosphamide on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle.

42. A method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

the second dose is administered to the subject about 3 days after administration of the first dose to the subject,

the third dose is administered to the subject about 4 days after administration of the second dose to the subject;

each of the first, second and third doses comprises about 1.5×10^9 CAR-expressing NK cells;

the subject is administered a lymphodepleting therapy comprising (i) a dose of about 500 mg/m² of cyclophosphamide on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle and (ii) a dose of about 30 mg/m² of fludarabine on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle; and

the subject is administered a dose of about 375 mg/m² of rituximab about 3 days prior to administration of the first dose of the dosing cycle.

43. A method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

the second dose is administered to the subject about 3 days after administration of the first dose to the subject,

the third dose is administered to the subject about 4 days after administration of the second dose to the subject;

each of the first, second and third doses comprises about 2×10^9 CAR-expressing NK cells or about 2.5×10^9 CAR-expressing NK cells;

the subject is administered a lymphodepleting therapy comprising (i) a dose of about 500 mg/m² of cyclophosphamide on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle and (ii) a dose of about 30 mg/m² of fludarabine on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle; and

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the subject is administered a dose of about 375 mg/m² of rituximab about 3 days prior to administration of the first dose of the dosing cycle.

44. A method of preparing a subject having a cancer for treatment with a composition comprising natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds to CD19, the method comprising administering a lymphodepleting therapy to the subject prior to administration of the composition to the subject, wherein the lymphodepleting therapy consists of cyclophosphamide.

45. The method of any one of Embodiments 1-44, wherein the cancer is a CD19-expressing cancer.

46. The method of any one of Embodiments 1-45, wherein the cancer is a blood cancer.

47. The method of any one of Embodiments 1-46, wherein the cancer is a leukemia or a lymphoma.

48. The method of any one of Embodiments 1-47, wherein the cancer is a B cell cancer.

49. The method of any one of Embodiments 1-48, wherein the cancer is a Non-Hodgkin lymphoma (NHL).

50. The method of any one of Embodiments 1-49, wherein the cancer is a large B-cell lymphoma (LBCL), optionally an aggressive LBCL.

51. The method of any one of Embodiments 1-50, wherein the cancer is diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), marginal zone lymphoma (MZL), mantle cell lymphoma (MCL), Waldenström macroglobulinemia (WM), or B-cell acute lymphoblastic leukemia (B-ALL).

52. The method of any one of Embodiments 1-48, wherein the cancer is a chronic lymphocytic leukemia (CLL) or a small lymphocytic lymphoma (SLL).

53. The method of any one of Embodiments 1-52, wherein the cancer is a relapsed/refractory (R/R) cancer.

54. The method of any one of Embodiments 1-53, wherein the subject has less than or equal to 5% peripheral blasts, optionally wherein the subject has no evidence of extramedullary disease.

55. The method of any one of Embodiments 1-54, wherein the subject has received at least 1 but not more than 7 lines of previous therapy, optionally wherein the subject has received at least 1 but not more than 4 lines of previous therapy.

56. The method of any one of Embodiments 1-55, wherein the subject has received at least one line of previous therapy.

57. The method of any one of Embodiments 1-56, wherein the subject has received at least two lines of previous therapy.

58. The method of any one of Embodiments 55-57, wherein a line of previous therapy comprises an anti-CD20 monoclonal antibody and a cytotoxic chemotherapy, optionally wherein the cytotoxic therapy is anthracycline.

59. The method of any one of Embodiments 55-58, wherein a line of previous therapy comprises chimeric antigen receptor-expressing T (CAR T) cells, optionally wherein a line of previous therapy comprises autologous anti-CD19 CAR T cells.

60. The method of any one of Embodiments 55-59, wherein a line of previous therapy does not comprise CAR T cells, optionally wherein a line of previous therapy does not comprise autologous anti-CD19 CAR T cells.

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61. The method of any one of Embodiments 55-60, wherein a line of previous therapy comprises an inhibitor of Bruton's tyrosine kinase (BTKi), optionally wherein the BTKi is ibrutinib.

62. The method of any one of Embodiments 55-61, wherein a line of previous therapy comprises an inhibitor of Bcl-2, optionally wherein the Bcl-2 inhibitor is venetoclax.

63. The method of any one of Embodiments 1-58, wherein the CAR comprises:
(a) an antigen-binding moiety that targets CD19;
(b) a transmembrane domain; and
(c) an intracellular signaling domain comprising an OX40 domain and a CD3zeta domain.

64. The method of Embodiment 63, wherein the antigen-binding moiety comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein:

the VH comprises a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 24, 25, and 26, respectively; and the VL comprises a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 27, 28, and 29, respectively;

the VH comprises a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 30, 31, and 32, respectively; and the VL comprises a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 33, 34, and 29, respectively;

the VH comprises the amino acid sequence set forth in SEQ ID NO: 35 and/or the VL comprises the amino acid sequence set forth in SEQ ID NO:36; and/or

the antigen-binding domain is an scFv comprising the amino acid sequence of SEQ ID NO:37.

65. The method of any one of Embodiments 1-64, wherein the genetically engineered NK cells are also engineered to express membrane-bound interleukin 15 (mbIL15).

66. The method of Embodiment 65, wherein the mbIL15 has at least 95% sequence identity to SEQ ID NO: 23 or 40.

67. The method of any one of Embodiments 1-66, wherein the dosing cycle does not result in cytokine release syndrome, immune effector cell-associated neurotoxicity syndrome (ICANS)/neurotoxicity, and/or graft versus host disease.

68. The method of any one of Embodiments 1-67, wherein the genetically engineered NK cells are allogeneic with respect to the subject.

69. The method of any one of Embodiments 1-68, wherein a dosing cycle is administered to the subject on an outpatient basis.

70. The method of any one of Embodiments 1-68, wherein at least one dose of each dosing cycle is administered to the subject on an outpatient basis.

71. The method of any one of Embodiments 1-70, wherein each dose of each dosing cycle is administered to the subject on an outpatient basis.

72. The method of any one of Embodiments 1-71, wherein, among subjects treated according to the method, the overall response rate (ORR) is at least about 50%, at least about 60%, at least about 70%, or at least about 80%.

73. The method of any one of Embodiments 1-72, wherein at least about 50%, at least about 60%, at least about 70%, or at least about 80% of subjects treated according to the method exhibit a complete response (CR).

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74. Use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein:

the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19; and

the NK cells are for administration as a first dosing cycle comprising a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

the second dose is for administration to the subject between 2-4 days after administration of the first dose to the subject,

the third dose is for administration to the subject between 2-4 days after administration of the second dose to the subject; and

each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

75. Use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein:

the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19; and

the NK cells are for administration as a first dosing cycle comprising a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein all three doses are administered to the subject within between about 4 days and about 10 days; and

each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

76. Use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein:

the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19;

the NK cells are for administration as a first dosing cycle comprising a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein all three doses are administered to the subject within between about 4 days and about 10 days; and

the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle, wherein the lymphodepleting therapy comprises cyclophosphamide and does not comprise fludarabine.

78. Use of a lymphodepleting therapy in the manufacture of a medication for preparing a subject having a cancer for treatment with natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, wherein the lymphodepleting therapy is for administration to the subject prior to administration of a first dosing cycle of the genetically engineered NK cells to the subject, wherein:

the first dosing cycle comprises a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells,

each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells; and

all three doses are for administration to the subject within between about 4 days and about 10 days.

79. The use of any one of Embodiments 76-78, wherein the second dose is for administration to the subject between 2-4 days after administration of the first dose to the

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subject and the third dose is for administration to the subject between 2-4 days after administration of the second dose to the subject.

80. Use of a lymphodepleting therapy for preparing a subject having a cancer for treatment with a composition comprising natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds to CD19, wherein the lymphodepleting therapy consists of cyclophosphamide.

81. The use of any one of Embodiments 76, 79, and 80, wherein each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

82. The use of any one of Embodiments 74-81, wherein each of the first, second and third doses comprises about 1.5×10^9 CAR-expressing NK cells.

83. Use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein:

the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19; and

the NK cells are for administration as a first dosing cycle comprising a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

the second dose is for administration to the subject between 2-4 days after administration of the first dose to the subject,

the third dose is for administration to the subject between 2-4 days after administration of the second dose to the subject; and

each of the first, second and third doses comprises at least about 2×10^9 CAR-expressing NK cells.

84. The use of any one of Embodiments 74-81 and Embodiment 83, wherein each of the first, second and third doses comprises about 2×10^9 CAR-expressing NK cells.

85. The use of any one of Embodiments 74-81 and Embodiment 83, wherein each of the first, second and third doses comprises about 2.5×10^9 CAR-expressing NK cells.

86. Use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein:

the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19; and

the NK cells are for administration as a first dosing cycle comprising a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

the second dose is for administration to the subject between 2-4 days after administration of the first dose to the subject,

the third dose is for administration to the subject between 2-4 days after administration of the second dose to the subject, and

each of the first, second and third doses comprises between about 1.0×10^9 CAR-expressing NK cells and about 2×10^9 CAR-expressing NK cells;

the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle; and

the lymphodepleting therapy consists of a dose of about 500 mg/m² of cyclophosphamide on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle.

87. Use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein:

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the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19;

the NK cells are for administration as a first dosing cycle comprising a first dose of the generally engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

the second dose is for administration to the subject about 3 days after administration of the first dose to the subject,

the third dose is for administration to the subject about 4 days after administration of the second dose to the subject;

each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells;

the subject was administered a lymphodepleting therapy comprising (i) a dose of about 500 mg/m^2 of cyclophosphamide on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle and (ii) a dose of about 30 mg/m^2 of fludarabine on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle; and

the subject was administered a dose of about 375 mg/m^2 of rituximab about 3 days prior to administration of the first dose of the dosing cycle.

88. The use of Embodiment 86 or Embodiment 87, wherein each of the first, second and third doses comprises about 1.5×10^9 CAR-expressing NK cells.

89. Use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein:

the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19;

the NK cells are for administration as a first dosing cycle comprising a first dose of the generally engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

the second dose is for administration to the subject about 3 days after administration of the first dose to the subject,

the third dose is for administration to the subject about 4 days after administration of the second dose to the subject;

each of the first, second and third doses comprises about 2×10^9 CAR-expressing NK cells or about 2.5×10^9 CAR-expressing NK cells;

the subject was administered a lymphodepleting therapy comprising (i) a dose of about 500 mg/m^2 of cyclophosphamide on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle and (ii) a dose of about 30 mg/m^2 of fludarabine on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle; and

the subject was administered a dose of about 375 mg/m^2 of rituximab about 3 days prior to administration of the first dose of the dosing cycle.

90. The use of any one of Embodiments 86, 87, and 89, wherein each of the first, second and third doses comprises about 2×10^9 CAR-expressing NK cells.

91. The use of Embodiment 87 or Embodiment 89, wherein each of the first, second and third doses comprises about 2.5×10^9 CAR-expressing NK cells.

92. The use of any one of Embodiments 74-91, wherein the CAR-expressing NK cells also express a membrane-bound interleukin-15 (mbIL15).

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EXAMPLES

[00336] The following are non-limiting descriptions of experimental methods and materials that will be used in examples disclosed below.

Example 1 – Administration of anti-CD19 CAR-expressing NK cells to subjects with cancer

[00337] Therapeutic anti-CD19 CAR-expressing NK cell compositions (CD19 CAR NK cells) were administered to subjects with cancer (e.g., B-cell malignancies) in accordance with a non-limiting dosing regimen.

[00338] Primary NK cells were isolated by immunoaffinity-based selection from leukapheresis samples from healthy donors and cultured in the presence of a stimulatory cell line. Isolated NK cells were subsequently transduced with a viral vector (e.g., retroviral vector) encoding a non-limiting example of a CD19-directed CAR (see Figure 1), expanded in culture, and cryopreserved. The CD19-directed CAR contains an extracellular anti-CD19 scFv (e.g., SEQ ID NO:37), a CD8alpha hinge (e.g., SEQ ID NO: 6) and transmembrane domain (e.g., SEQ ID NO:8), and an intracellular signaling domain containing an OX40 co-stimulatory signaling region (e.g., SEQ ID NO:14) and a CD3zeta signaling domain (e.g., SEQ ID NO:18). The viral vector further contains a sequence encoding a membrane-bound interleukin-15 (mbIL15; e.g., SEQ ID NO:40), which is separated from the CAR-encoding sequence by a sequence encoding a T2A ribosomal skip sequence (e.g., SEQ ID NO:20, encoded by SEQ ID NO:19). The cryopreserved NK cell compositions (CD19 CAR NK cells) were thawed prior to intravenous administration to subjects. All subjects have relapsed/refractory CD19+ B-cell malignancies including large B-cell lymphoma (LBCL, including diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma grade 3b (FL3b)), mantle cell lymphoma (MCL), follicular lymphoma (FL) grades 1, 2, and 3a, and marginal zone lymphoma (MZL); have received two or more prior lines of therapy; have an ECOG status of 0-2; and are either naïve or exposed to CD19 CAR T cell therapy.

[00339] Subjects were administered a lymphodepleting (LD) therapy prior to administration of the dosing cycle. The LD therapy consists of either 500 mg/m² Cy on each of Days -3, -4, and -5 (Figure 2A) or 1000 mg/m² Cy on Day -3 (Figure 2B), the Cy alone or in combination with 30 mg/m² fludarabine (Flu) on each of Days -5, -4, and -3. The CD19 CAR NK cells were administered beginning on Day 0 of a 28-day dosing cycle. In particular, subjects are administered a dose of 1.5 x 10⁹, 2 x 10⁹, or 2.5 x 10⁹ CD19 CAR NK cells on each of Days 0, 3, and 7. Optionally, subjects are administered a single dose of 375 mg/m² rituximab on Day -3. A schematic of the non-limiting dosing regimens is shown in Figures 2A-2B. Outcome measures are optionally assessed, such as on Day 27. Subjects who exhibited clinical benefit from treatment with a dosing cycle were eligible for an additional dosing cycle to deepen or consolidate response. Subjects who exhibited initial clinical benefit from treatment with a dosing cycle and subsequently exhibited disease progression (e.g., relapse)

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were eligible for retreatment with an additional dosing cycle. Subjects may be administered a maximum of five dosing cycles, with each dosing cycle optionally preceded by LD therapy and rituximab. In addition, subjects with ongoing cytopenias may be eligible to receive a dosing cycle preceded by Cy-only LD therapy.

[00340] Primary endpoints may include any of the following: (1) incidence, nature, and severity of treatment related adverse events will be evaluated with an adverse event defined as any unfavorable and unintended sign including clinically significant abnormal laboratory findings, symptom or disease measured (e.g., 30 days) after the last dose of the NK cells; and (2) proportion of subjects experiencing dose-limiting toxicities of the NK cells.

[00341] Secondary outcome measures may include any of the following: (1) pharmacokinetic parameters in the context of the immune system, including but not limited to maximum concentration (C_{max}), time to reach maximum concentration (T_{max}), area under the concentration-time curve (AUC), half-life (t_{1/2}), and duration of persistence of the CD19 CAR-NK cells in the peripheral blood and other target tissues such as bone marrow; (2) humoral and cellular immunogenicity against the CD19 CAR NK cells; (3) changes in serum cytokine levels such as interferon-gamma (IFN-γ) and other host responses to CD19 CAR NK cells in peripheral; (4) best overall response rates in dose finding and safety lead-in cohorts; and/or (5) other antitumor measurements, which may include duration of response (DOR), time-to-first response, time-to-best response, bridge-to-transplant rate, event-free survival (EFS), progression free survival (PFS), and overall survival (OS) using standard disease specific response assessment criteria.

[00342] In particular, two subjects that were administered 500 mg/m² Cy and 30 mg/m² Flu on each of Days -5, -4, and -3 followed by a first dosing cycle comprising a dose of 1.5 x 10⁹ CD19 CAR NK cells on each of Days 0, 3, and 7 exhibited ongoing cytopenias. The two subjects were subsequently administered a second dosing cycle of CD19 CAR NK cells preceded by lymphodepletion with Cy alone (500 mg/m² Cy on each of Days -5, -4, and -3). The concentration of CD19 CAR NK cells was assessed in the peripheral blood of the subjects at various time points during and between the first and second cycles. As shown in Figure 3 (assessment time points indicated by dots), both subjects achieved similar CD19 CAR NK concentrations in the first and second cycles, despite the second cycle being preceded by Cy-only lymphodepletion. These data are consistent with a finding that a Cy-only lymphodepletion achieves sufficient exposure of the CD19 CAR NK cells as provided herein.

[00343] It is contemplated that various combinations or sub combinations of the specific features and aspects of the embodiments disclosed above may be made and still fall within one or more of the inventions. Further, the disclosure herein of any particular feature, aspect, method, property, characteristic, quality, attribute, element, or the like in connection with an embodiment can be used in all other embodiments set forth herein. Accordingly, it

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should be understood that various features and aspects of the disclosed embodiments can be combined with or substituted for one another in order to form varying modes of the disclosed inventions. Thus, it is intended that the scope of the present inventions herein disclosed should not be limited by the particular disclosed embodiments described above. Moreover, while the invention is susceptible to various modifications, and alternative forms, specific examples thereof have been shown in the drawings and are herein described in detail. It should be understood, however, that the invention is not to be limited to the particular forms or methods disclosed, but to the contrary, the invention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the various embodiments described and the appended claims. Any methods disclosed herein need not be performed in the order recited. The methods disclosed herein include certain actions taken by a practitioner; however, they can also include any third-party instruction of those actions, either expressly or by implication. In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

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Sequences

SEQ ID NO	Sequence	Description
1	GGGSGGGSGGGGS	linker
2	GGGSGGGGS	linker
3	GSTSGSGKPGSGEGSTKG	linker
4	MALPVTALLPLALLHAARP	CD8a signal sequence
5	accacgacgccagcgcgcgaccaccaacaccggcgccaccatcgctcgagcccctgtccctgcgccagaggcgtgccggccagcggcgggggcgagtcacacgaggggctggacttcgcctgtgat	Human CD8a hinge (nt)
6	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD	Human CD8a hinge (aa)
7	atctacatctggcgccctggccgggactgtggggtccttctcctgtcactggttatcacc	Human CD8a transmembrane (nt)
8	IYIWAPLAGTCGVLLSLVIT	Human CD8a transmembrane (aa)
9	actaccacaccgccccgaggccacctacgccggcaccgactatgccagtcaccccctctctctgcgcccgaggcttgccggcctgcggctgggggggtccacaccggggcctggattttgcgtgcgatatatacatctgggcacctcttgccggcaccctgcggagtgtctctctcactcgttattacgctgtactgc	Human CD8a hinge/transmembrane (nt)
10	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLSLVIT	Human CD8a hinge/transmembrane (aa)
11	FWVLVVVGGVLACYSLLVTVAFIIFWVRS	Human CD28 transmembrane (aa)
12	KRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS	Human CD28 intracellular (aa)
13	cggagggaccagaggctgcccccgatgccacaagccccctgggggaggcagttccgga ccccatccaagaggagcaggccgacgccactccaccctggccaagatc	Human OX40 (nt)
14	RRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI	Human OX40 (aa)
15	aaacggggcagaaagaaactcctgtatatattcaacaaccatttatgagaccagtacaaactactcaagaggaagalggctgtagctgccgattccagaagaagaaggaggatgtgaactg	Human 4-1BB (nt)

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SEQ ID NO	Sequence	Description
16	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	Human 4-1BB (aa)
17	agagtgaagttcagcaggagcgcagacgcccccgctaccagcagggccagaaccagctct ataacgagctcaatctaggacgaagagaggagtagcatgltttggacaagagacgtggccgg gacctlgagatgggggaaagccgagaaggaagaaccctcaggaaggcctgtacaatgaa ctgcagaaagataagatggcggaggcctacagtgagattgggatgaaaggcgagcgccgga ggggcaaggggcacgatggcctttaccaggggtctcagtacagccaccaaggacacctacgac gcccttcacatgcaggccctgccccctcgc	Human CD3zeta (nt)
18	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGGLY QGLSTATKDTYDALHMQALPPR	Human CD3zeta (aa)
19	ggctctggcgaggggaaggggtccctgcttacttgcggcgacgtcgaagagaatcccgggtccg	Human T2A (nt)
20	GSGEGRGSLLTCGDVEENPGP	Human T2A (aa)
21	aactgggtcaacgtgattagcgattgaagaaaaatcaggaccttatacagtctatgcatattgac gtlacactgtatactgagagtgtacacccgtcctgtaaggtaacggccatgaaatgctttctc tgagctccaggtcatcagcttgagctcgggacgcaagcatccacgatacgggtgaaaacct calcatccttgcaacaactctctcatctaataagaaacgttacagagagtgggtgtaaggagtg cgaagagltggaagaaaaaacalcaagaatttctcaatccttcgttcacalagtgcaaatgtt cattaacacgtcc	Human IL-15 (nt)
22	NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQ VISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEELEEKNIK EFLQSFVHIVQMFINTS	Human IL-15 (aa)
23	NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQ VISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEELEEKNIK EFLQSFVHIVQMFINTSTTTTPAPRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYC	mbIL15 (aa)
24	GVSLPDYG	CD19 CDR-H1
25	IWGSETT	CD19 CDR-H2
26	AKHYYYGGSYAMDY	CD19 CDR-H3
27	QDISKY	CD19 CDR-L1
28	HT	CD19 CDR-L2
29	QQGNTLPYT	CD19 CDR-L3
30	DYGVS	CD19 CDR-H1
31	VIWGSETTYNSALKS	CD19 CDR-H2
32	HYYYGGSYAMDY	CD19 CDR-H3
33	RASQDISKYLN	CD19 CDR-L1
34	HTSRLHS	CD19 CDR-L2

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SEQ ID NO	Sequence	Description
35	QVQLQESGPGLVKPSQTLSTCTVSGVSLPDYGVSWIRQPPGKGLE WIGVIWGSETTYYN SALKSRLTISKDNSKNQVSLKLSSVTAADTAVY YCAKHYYYGGSYAMDYWGQGTSTVTVSS	CD19 VH
36	DIQMTQSPSSLSASVGDRTITCRASQDISKYLNWYQQKPGGTVKLL IYHTSRLHSGVPSRFSGSGSGTDFTLTISLQPEDATYFCQQGNTLP YTFGGGTKLEIT	CD19 VL
37	DIQMTQSPSSLSASVGDRTITCRASQDISKYLNWYQQKPGGTVKLL IYHTSRLHSGVPSRFSGSGSGTDFTLTISLQPEDATYFCQQGNTLP YTFGGGTKLEITGGGGSGGGSGGGGSQVQLQESGPGLVKPSQTL SLTCTVSGVSLPDYGVSWIRQPPGKGLEWIGVIWGSETTYYN SALK SRLTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYYYGGSYAMDYW GQGTSTVTVSS	CD19 scFv
38	DIQMTQSPSSLSASVGDRTITCRASQDISKYLNWYQQKPGGTVKLL IYHTSRLHSGVPSRFSGSGSGTDFTLTISLQPEDATYFCQQGNTLP YTFGGGTKLEITGGGGSGGGSGGGGSQVQLQESGPGLVKPSQTL SLTCTVSGVSLPDYGVSWIRQPPGKGLEWIGVIWGSETTYYN SALK SRLTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYYYGGSYAMDYW GQGTSTVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYWAPLAGTCGVLLLSLVITLYCRRDQRLPPDAHKPPG GGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDRRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRRGKGDGLYQGLSTATKDTYDALHMQALPPR	CD19 CAR
39	PPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTS DGPTQ QLTWSRESPLKPFLKLSLGLPGLGIHMRPLAIWLFIFNV SQQMGGFY LCQPGPPSEKAWQPWTVNVEGSGELFRWNVSDLGGLGCGLKNR SSEGPSSPSGKLMSPKLYVWAKDRPEIWEGEPPCLPPRDSL NQSLS QDLTMAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELK DDRPARDMWV METGLLLPRATAQDAGKYYCHRGNTMSFHLEITA RPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRKR KRMTDPTRRRFFKVTPPPGSGPQNQYGNVLSLPTPTSGLGRAQRWA AGLGGTAPSYGNPSSDVQADGALGSRSPPGVGPEEEEEGEGYEEPD SEEDSEFYENDSNLQDQLSQDGSYENPEDEPLGPEDEDSFSNA ESYENEDEELTQPVARTMDFLSPHGSADWPSREATSLGSQSYEDM RGILYAAPQLRSIRGQPGPNHEEDADSYENMDNPDGPDPAWGGGG RMGTWSTR	human CD19 (UniProt No. P15391)

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SEQ ID NO	Sequence	Description
40	NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQ VISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEELEEKNIK EFLQSFVHIVQMFINTSTTTPAPRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVIT	mbIL15 (aa)
41	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYC	Human CD8a hinge /transmembrane (aa)

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WHAT IS CLAIMED IS:

1. A method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

the second dose is administered to the subject between 2-4 days after administration of the first dose to the subject;

the third dose is administered to the subject between 2-4 days after administration of the second dose to the subject; and

each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

2. The method of Claim 1, wherein the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle.

3. The method of Claim 2, wherein the lymphodepleting therapy comprises cyclophosphamide and does not comprise fludarabine, optionally, wherein the lymphodepleting therapy consists of cyclophosphamide.

4. A method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

the second dose is administered to the subject between 2-4 days after administration of the first dose to the subject;

the third dose is administered to the subject between 2-4 days after administration of the second dose to the subject;

the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle; and

the lymphodepleting therapy comprises cyclophosphamide and does not comprise fludarabine.

5. The method of Claim 4, wherein each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

6. A method of preparing a subject having a cancer for treatment with natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, the method comprising administering a lymphodepleting therapy to the subject prior to administration of a first dosing cycle of the genetically engineered NK cells to the subject, wherein:

the first dosing cycle comprises a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells;

each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells; and

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all three doses are administered to the subject within between about 4 days and about 10 days.

7. The method of Claim 6, wherein the lymphodepleting therapy comprises cyclophosphamide and does not comprise fludarabine, optionally, wherein the lymphodepleting therapy consists of cyclophosphamide.

8. The method of Claim 1, wherein each of the first, second, and third doses comprises about 1.5×10^9 CAR-expressing NK cells, about 2×10^9 CAR-expressing NK cells, or about 2.5×10^9 CAR-expressing NK cells.

9. The method of Claim 1, wherein the first dosing cycle is followed by an additional dosing cycle.

10. The method of Claim 1, wherein, if the subject exhibits a clinical response, optionally a complete response (CR), following the first dosing cycle, the method comprises an additional dosing cycle as consolidation treatment.

11. The method of Claim 1, wherein, if the subject exhibits a clinical response following a dosing cycle and subsequently exhibits disease progression, the method comprises an additional dosing cycle as retreatment.

12. The method of Claim 1, wherein the method comprises between one dosing cycle and five dosing cycles.

13. The method of Claim 12, wherein the subject is administered a lymphodepleting therapy prior to each dosing cycle.

14. The method of Claim 12, wherein each dosing cycle is between about 14 days and about 35 days.

15. The method of Claim 2 or 3, wherein the lymphodepleting therapy comprises cyclophosphamide, optionally wherein a dose of cyclophosphamide is between about 300 mg/m² and about 1000 mg/m².

16. The method of Claim 2 or 3, wherein the lymphodepleting therapy comprises a single dose of cyclophosphamide.

17. The method of Claim 16, wherein the single dose of cyclophosphamide is administered to the subject about 3 days prior to administration of the dosing cycle.

18. The method of Claim 16, wherein the single dose of cyclophosphamide is about 1000 mg/m².

19. The method of Claim 2 or 3, wherein the lymphodepleting therapy comprises three doses of cyclophosphamide.

20. The method of Claim 19, wherein each dose of cyclophosphamide is about 500 mg/m².

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21. The method of Claim 19 or Claim 20, wherein a dose of cyclophosphamide is administered to the subject on each of about 5 days prior, 4 days prior, and 3 days prior to administration of the first dose of the dosing cycle.

22. The method of any one of Claims 2, 6, and 8-21, wherein the lymphodepleting therapy comprises fludarabine, optionally wherein a dose of fludarabine is between about 20 mg/m² and about 40 mg/m².

23. The method of Claim 22, wherein the lymphodepleting therapy comprises three doses of fludarabine.

24. The method of Claim 22 or Claim 23, wherein each dose of fludarabine is about 30 mg/m².

25. The method of any one of Claims 23-24, wherein a dose of fludarabine is administered to the subject on each of about 5 days prior, 4 days prior, and 3 days prior to administration of the first dose of the dosing cycle.

26. The method of any one of Claims 2-25, wherein the first dose of genetically engineered NK cells is administered to the subject about 2 to 5 days after administration of the lymphodepleting therapy has concluded, optionally wherein the first dose of the genetically engineered NK cells is administered to the subject about 3 days after administration of the lymphodepleting therapy has concluded.

27. The method of any one of Claims 2-26, wherein all three doses of the genetically engineered NK cells are administered to the subject within about 10 days after administration of the lymphodepleting therapy has concluded.

28. The method of any one of Claims 1-27, wherein:

the method further comprises administering a therapeutic agent that targets CD20 to the subject; and/or

the subject is administered a therapeutic agent that targets CD20.

29. The method of Claim 28, wherein the therapeutic agent is an anti-CD20 monoclonal antibody, optionally wherein the anti-CD20 antibody is rituximab.

30. A method of treating a subject having a cancer comprising administering to the subject a first dosing cycle comprising a first dose of natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

the second dose is administered to the subject about 3 days after administration of the first dose to the subject,

the third dose is administered to the subject about 4 days after administration of the second dose to the subject;

each of the first, second and third doses comprises between about 1.0×10^9 CAR-expressing NK cells and about 2×10^9 CAR-expressing NK cells;

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the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle; and

the lymphodepleting therapy consists of a dose of about 500 mg/m² of cyclophosphamide on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle.

31. The method of any one of Claims 1-30, wherein the cancer is a CD19-expressing cancer.

32. The method of any one of Claims 1-31, wherein the cancer is a blood cancer.

33. The method of any one of Claims 1-32, wherein the cancer is a leukemia or a lymphoma.

34. The method of any one of Claims 1-33 wherein the cancer is a B cell cancer.

35. The method of any one of Claims 1-34, wherein the cancer is a Non-Hodgkin lymphoma (NHL).

36. The method of any one of Claims 1-35, wherein the cancer is a large B-cell lymphoma (LBCL), optionally an aggressive LBCL.

37. The method of any one of Claims 1-36, wherein the cancer is diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), marginal zone lymphoma (MZL), mantle cell lymphoma (MCL), Waldenström macroglobulinemia (WM), or B-cell acute lymphoblastic leukemia (B-ALL).

38. The method of any one of Claims 1-34, wherein the cancer is a chronic lymphocytic leukemia (CLL) or a small lymphocytic lymphoma (SLL).

39. The method of any one of Claims 1-38, wherein the cancer is a relapsed/refractory (R/R) cancer.

40. The method of any one of Claims 1-39, wherein the subject has received at least 1 but not more than 7 lines of previous therapy, optionally wherein the subject has received at least 1 but not more than 4 lines of previous therapy.

41. The method of any one of Claims 1-40, wherein the subject has received at least one line of previous therapy or at least two lines of previous therapy.

42. The method of Claim 40 or Claim 41, wherein a line of previous therapy comprises an anti-CD20 monoclonal antibody and a cytotoxic chemotherapy, optionally wherein the cytotoxic therapy is anthracycline.

43. The method of any one of Claims 40-42, wherein a line of previous therapy comprises chimeric antigen receptor-expressing T (CAR T) cells, optionally wherein a line of previous therapy comprises autologous anti-CD19 CAR T cells.

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44. The method of any one of Claims 40-43, wherein a line of previous therapy does not comprise CAR T cells, optionally wherein a line of previous therapy does not comprise autologous anti-CD19 CAR T cells.

45. The method of any one of Claims 40-44, wherein a line of previous therapy comprises an inhibitor of Bruton's tyrosine kinase (BTKi), optionally wherein the BTKi is ibrutinib.

46. The method of any one of Claims 40-45, wherein a line of previous therapy comprises an inhibitor of Bcl-2, optionally wherein the Bcl-2 inhibitor is venetoclax.

47. The method of any one of Claims 1-46, wherein the CAR comprises:
(a) an antigen-binding moiety that targets CD19;
(b) a transmembrane domain; and
(c) an intracellular signaling domain comprising an OX40 domain and a CD3zeta domain.

48. The method of Claim 47, wherein the antigen-binding moiety comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein:
the VH comprises a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 24, 25, and 26, respectively; and the VL comprises a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 27, 28, and 29, respectively;

the VH comprises a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 30, 31, and 32, respectively; and the VL comprises a CDR-1, a CDR-2, and a CDR-3 comprising the amino acid sequences set forth in SEQ ID NOS: 33, 34, and 29, respectively;

the VH comprises the amino acid sequence set forth in SEQ ID NO: 35 and/or the VL comprises the amino acid sequence set forth in SEQ ID NO:36; and/or

the antigen-binding domain is an scFv comprising the amino acid sequence of SEQ ID NO:37.

49. The method of any one of Claims 1-48, wherein the genetically engineered NK cells are also engineered to express membrane-bound interleukin 15 (mbIL15).

50. The method of any one of Claims 1-49, wherein the dosing cycle does not result in cytokine release syndrome, immune effector cell-associated neurotoxicity syndrome (ICANS)/neurotoxicity, and/or graft versus host disease.

51. The method of any one of Claims 1-50, wherein the genetically engineered NK cells are allogeneic with respect to the subject.

52. The method of any one of Claims 1-51, wherein: at least one dose of each dosing cycle is administered to the subject on an outpatient basis, optionally wherein each dose of each dosing cycle is administered to the subject on an outpatient basis; and/or at least one dosing cycle is administered to the subject on an outpatient basis.

53. The method of any one of Claims 1-52, wherein, among subjects treated according to the method: the overall response rate (ORR) is at least about 50%, at least

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about 60%, at least about 70%, or at least about 80%; and/or at least about 50%, at least about 60%, at least about 70%, or at least about 80% of subjects treated according to the method exhibit a complete response (CR).

54. Use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein:

the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19; and

the NK cells are for administration as a first dosing cycle comprising a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein:

the second dose is for administration to the subject between 2-4 days after administration of the first dose to the subject,

the third dose is for administration to the subject between 2-4 days after administration of the second dose to the subject; and

each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

55. Use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein:

the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19;

the NK cells are for administration as a first dosing cycle comprising a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein all three doses are administered to the subject within between about 4 days and about 10 days; and

each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

56. The use of Claim 54 or Claim 55, wherein the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle.

57. Use of a lymphodepleting therapy in the manufacture of a medication for preparing a subject having a cancer for treatment with natural killer (NK) cells genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19, wherein the lymphodepleting therapy is for administration to the subject prior to administration of a first dosing cycle of the genetically engineered NK cells to the subject, wherein:

the first dosing cycle comprises a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells;

each of the first, second and third doses comprises at least about 1×10^9 CAR-expressing NK cells; and

all three doses are for administration to the subject within between about 4 days and about 10 days.

58. The use of Claim 56 or Claim 57, wherein the lymphodepleting therapy comprises cyclophosphamide and does not comprise fludarabine.

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59. Use of natural killer (NK) cells in the manufacture of a medicament for treating a subject having a cancer, wherein:

the NK cells are genetically engineered to express a chimeric antigen receptor (CAR) that binds CD19;

the NK cells are for administration as a first dosing cycle comprising a first dose of the genetically engineered NK cells, a second dose of the genetically engineered NK cells, and a third dose of genetically engineered NK cells, wherein all three doses are administered to the subject within between about 4 days and about 10 days; and

the subject is administered a lymphodepleting therapy prior to administration of the first dosing cycle, wherein the lymphodepleting therapy comprises cyclophosphamide and does not comprise fludarabine.

60. The use of Claim 59, wherein each of the first, second, and third doses comprises at least about 1×10^9 CAR-expressing NK cells.

61. The use of any one of Claims 55-60, wherein the second dose is for administration to the subject between 2-4 days after administration of the first dose to the subject and the third dose is for administration to the subject between 2-4 days after administration of the second dose to the subject.

62. The use of any one of Claims 54-61, wherein each of the first, second and third doses comprises about 1.5×10^9 CAR-expressing NK cells, about 2×10^9 CAR-expressing NK cells, or about 2.5×10^9 CAR-expressing NK cells.

63. The use of any one of Claims 56-62, wherein the lymphodepleting therapy consists of a dose of about 500 mg/m^2 of cyclophosphamide on each of 5 days, 4 days, and 3 days prior to administration of the first dose of the dosing cycle.

64. The use of any one of Claims 54-63, wherein the subject was administered a therapeutic agent that targets CD20 prior to administration of the first dose of the dosing cycle, optionally wherein

the subject was administered a dose of about 375 mg/m^2 of rituximab about 3 days prior to administration of the first dose of the dosing cycle.

65. The use of any one of Claims 54-64, wherein the CAR-expressing NK cells also express a membrane-bound interleukin-15 (mbIL15).

Figure 1

Anti-CD19 scFv	CD8a hinge	CD8a TM	OX40	CD3zeta
Anti-CD19 scFv	CD8a hinge	CD8a TM	OX40	CD3zeta
			2A	mbIL-15

Figure 2A

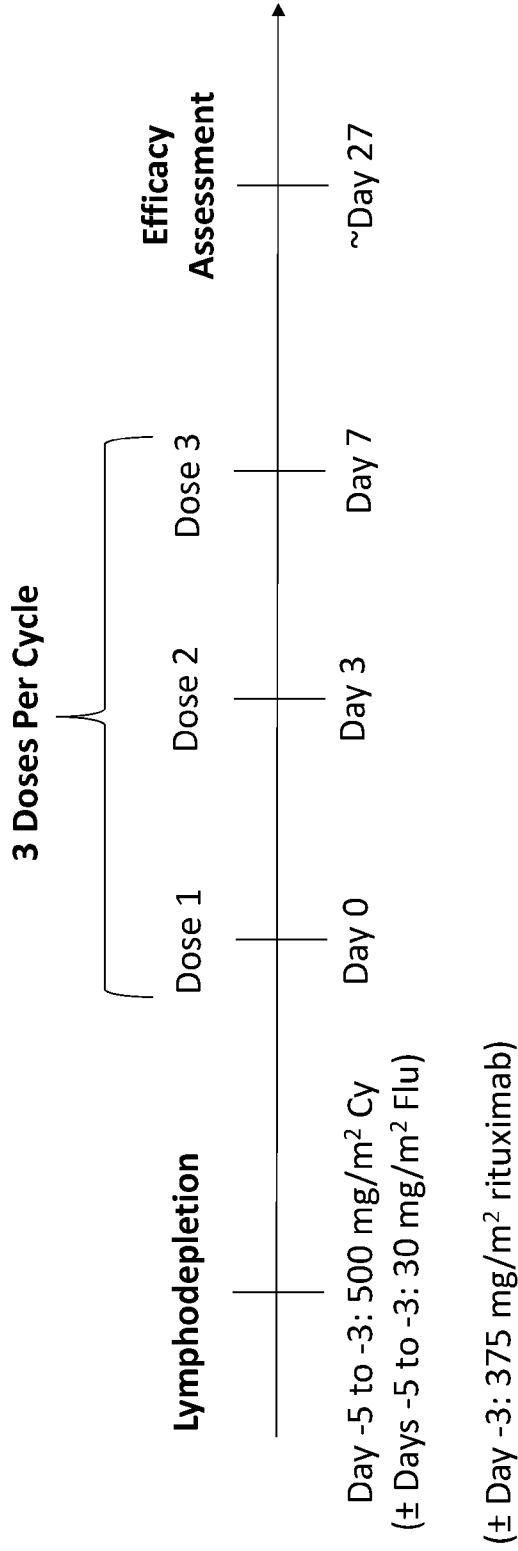


Figure 2B

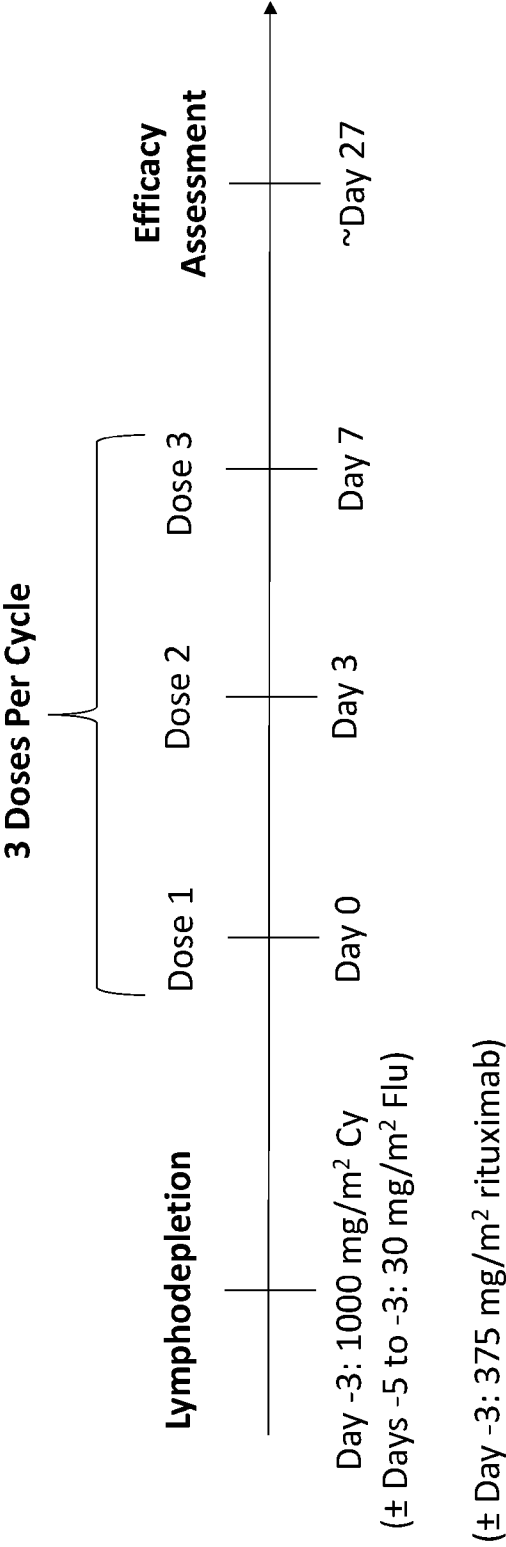


Figure 3

