

SUMMARY

[0005] This disclosure relates to therapeutic regimens for treatment of a disorder, such as cancer. More specifically, this disclosure relates to therapeutic regimens for treatment of a disorder, such as cancer, by administration of allogeneic CAR-T-cells. Features of the methods, including the timing of the doses and numbers of cells administered, provide various advantages, such as improved efficacy and/or lower toxicity, for example, due to increased exposure of the subject to the administered cells.

[0006] Provided are methods for administering to subjects CAR-T cells, for example, to treat diseases and/or disorders in the subjects. The methods generally involve administering single or multiple doses of such cells, and/or administering a subsequent dose to a subject having been previously treated with a prior (e.g., first) dose of such cells. Also provided are cells, compositions, and articles of manufacture for use in such methods.

[0007] In some examples, the methods involve treating an adult subject who has refractory and/or relapsed CD19⁺ B-cell acute lymphoblastic leukemia, comprising administering to the subject at least one dose of allogeneic chimeric antigen receptor (CAR)-T cells (CAR-T cells) comprising an anti-human CD19 4-1BB/CD3zeta CAR (e.g. UCART19 (CD19CAR/RQR8⁺_TCR $\alpha\beta$ -_T-cells), wherein the at least one dose is selected from the group consisting of about 6×10^5 cell/dose, about 6×10^6 cells/dose, about $6-8 \times 10^7$ cells/dose, and about $1.8-2.4 \times 10^8$ cells/dose.

[0008] In some examples, the methods involve treating a pediatric subject who has refractory and/or relapsed CD19⁺ B-cell acute lymphoblastic leukemia, comprising administering to the subject at least one dose of allogeneic chimeric antigen receptor (CAR)-T cells (CAR-T cells) comprising an anti-human CD19 4-1BB/CD3zeta CAR (e.g. UCART19 (CD19CAR/RQR8⁺_TCR $\alpha\beta$ -_T-cells) or UCART19 (CD19CAR/R2⁺_TCR $\alpha\beta$ -_T-cells) or UCART19 (CD19CAR/TCR $\alpha\beta$ -_T-cells), wherein the at least one dose is about $2-8 \times 10^7$ cells/dose.

[0009] In some examples, the methods involve treating a subject who has refractory and/or relapsed Non-Hodgkin's Lymphoma (e.g., large B-cell lymphoma or follicular lymphoma), the method comprising administering to the subject at least one dose of allogeneic chimeric antigen receptor (CAR)-T cells (CAR-T cells) comprising an anti-human CD19 4-1BB/CD3zeta CAR (CD19CAR/RQR8⁺_TCR $\alpha\beta$ -_T-cells), wherein the at least one dose is about 20×10^6 cells/dose

to about 360×10^6 cells/dose, for example about 20×10^6 cells/dose, about 40×10^6 cells/dose, about 80×10^6 cells/dose, about 120×10^6 cells/dose, 240×10^6 cells/dose, or about 360×10^6 cells/dose.

[0009a] In one aspect, the present disclosure refers to use of at least one dose of allogeneic chimeric antigen receptor (CAR)-T cells (CAR-T cells) comprising an anti-human CD19 4-1BB/CD3zeta CAR in the manufacture of a medicament for treating a subject who has refractory and/or relapsed Non-Hodgkin's Lymphoma, wherein the at least one dose is selected from the group consisting of about 120×10^6 cells/dose, about 240×10^6 cells/dose, and about 360×10^6 cells/dose.

[0010] In some examples, the methods involve (a) administering to a subject with a disease a first dose of allogeneic CAR-T cells; and (b) administering to the subject a subsequent dose of allogeneic CAR-T cells. In other examples, the methods are carried out by administering to the subject the subsequent dose or doses as in (b), to a subject that has been previously administered the first dose as in (a). In some examples, the CAR-T cells are tumor antigen-specific CAR-T cells. In some examples, the CAR-T cells are CD19-specific CAR-T cells (e.g., UCART19 (CD19CAR/RQR8+_TCR $\alpha\beta$ -T-cells)). In some examples, the disease is a tumor. In some examples, it is a cancer, malignancy, neoplasm, or other proliferative disease or disorder, such as leukemia, lymphoma, e.g., chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL (e.g., relapsed/refractory ALL)), non-Hodgkin's lymphoma, acute myeloid leukemia, diffuse large B-cell lymphoma (DLBCL), multiple myeloma, follicular lymphoma, mantle cell lymphoma, indolent B cell lymphoma, B cell malignancies, cancers of the colon, lung (e.g., small and non-small cell lung cancer), liver, breast, prostate, ovarian, skin, melanoma, bone, and brain cancer, ovarian cancer, epithelial cancers, renal cell carcinoma, pancreatic adenocarcinoma, Hodgkin lymphoma, cervical carcinoma, colorectal cancer, glioblastoma, neuroblastoma, Ewing sarcoma, medulloblastoma, osteosarcoma, synovial sarcoma, head and neck squamous cell carcinoma (HNSCC), and/or mesothelioma. In some examples, the disease is a leukemia or lymphoma. In some examples, the disease is acute lymphoblastic leukemia. In some examples, the disease is relapsed or refractory acute lymphoblastic leukemia. In some examples, the disease is non-Hodgkin lymphoma (NHL). In some examples, the disease is relapsed or refractory large B-cell lymphoma. In some examples, the disease is relapsed or refractory follicular lymphoma.

[0011] In some examples the disease is acute lymphoblastic leukemia (ALL). In some examples the disease is pediatric acute lymphoblastic leukemia (ALL). In some examples the disease is an advanced lymphoid malignancy such as B-cell acute lymphoblastic leukemia (B-ALL). In some examples, the disease is refractory B-ALL, e.g. adult refractory B-ALL. In some examples, the disease is relapsed B-ALL, e.g. adult relapsed B-ALL. In some examples, the disease is extramedullary disease associated, for example, with one or more of the cancers described herein. In some examples, the subject exhibits detectable molecular

disease and/or minimum residual disease at the time of the administration of the subsequent dose. In some examples, the subject exhibits one or more symptoms of the disease at the time of the administration of the subsequent dose. In some examples, the disease is a cancer and the subject exhibits relapse at the time of initiation of the administration of the subsequent dose. In some examples, the disease is a leukemia or lymphoma and the subject exhibits greater than 5% blast cells in the bone marrow at the time of the administration of the subsequent dose.

[0012] In some examples, the disease is a cancer and the subject exhibits morphologic disease. In some examples, the disease is a leukemia or lymphoma and the subject exhibits extramedullary disease (i.e., presence of blasts outside bone marrow) at the time of the administration of the subsequent dose. In some examples, the disease is a leukemia or lymphoma and the subject exhibits greater than 5% blast cells in the bone marrow at the time of the administration of the subsequent dose. In other examples, the disease is a cancer and the subject does not exhibit morphologic disease at the time of initiation of the administration of the subsequent dose. In some examples, the disease is a leukemia or lymphoma and the subject does not exhibit greater than 5% blast cells in the bone marrow at the time of the administration of the subsequent dose.

[0013] In some examples, the methods involve (a) administering to a subject having a disease a first dose of allogeneic CAR-T cells. In some examples, the first dose contains about 1×10^4 cells, about 5×10^4 cells, about 1×10^5 cells, about 5×10^5 cells, about 1×10^6 cells, about 5×10^6 cells, about 6×10^6 cells, about 1×10^7 cells, about 6×10^7 cells, about 1×10^8 , about 1.8×10^8 cells, or about 4.8×10^8 cells. In some examples, the first dose contains about 20×10^6 cells to about 360×10^6 cells, for example about 20×10^6 cells, about 40×10^6 cells, about 80×10^6 cells, about 120×10^6 cells, 240×10^6 cells, or about 360×10^6 cells. In some examples, the methods further involve (b) administering to the subject a subsequent dose of CAR-T cells at a time point that is at least or more than about 5 weeks after and less than about 24 weeks after initiation of the administration in (a).

[0014] In some examples, a subject with relapsed/refractory ALL is administered a first and subsequent dose of allogeneic CAR-T cells each containing about 6×10^6 cells, and the subsequent dose of CAR-T cells in (b) is administered about 99 days after initiation of the administration in (a).

[0015] In some examples, the methods further involve the administration of additional subsequent or subsequent doses, such that a first and multiple subsequent doses are administered, e.g., in accordance with the dosing amounts and timing schedules as specified for the first and subsequent doses. In some examples, the first of one or more subsequent doses is administered at a time that is at least or greater than 5 weeks after the initiation of the administration of the subsequent dose. In some examples, the administration of the first, second, and subsequent doses includes administering at least three of the doses within about 5 weeks. In some examples, the subsequent dose is administered at about 16 weeks following the initiation of administration of the first dose, and an additional subsequent or subsequent dose is administered at week 17 following the initiation of administration of the first dose. In some examples, additional subsequent doses are administered at week 17 and/or week 34 following the initiation of administration of the first dose.

[0016] In some examples, the time of administering the subsequent dose(s) is further one at which the subject does not exhibit an immune response, e.g., does not exhibit a detectable adaptive host immune response specific for the CAR-T after said first (or prior) dose.

[0017] In some examples, the time between the administration of the first dose (initial dose), e.g., the initiation of the administration of the first or prior dose, and the initiation of the administration of the subsequent dose (e.g., the initiation of the administration of the subsequent dose) is greater than about 4 weeks, e.g., greater than about 5, 6, 7, 8, or 9 weeks, e.g., greater than about 20 weeks, e.g., between about 9 and about 35 weeks, between about 14 and about 28 weeks, between 15 and 27 weeks, or between 16 weeks and about 18 weeks; and/or at or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27 weeks. In some examples, administration of the subsequent dose (e.g., initiation thereof) is more than about 5 weeks after and less than about 24 weeks after administration of the first or prior dose (e.g., initiation thereof). In some examples, the administration of the subsequent dose is initiated 17 weeks following the initiation of the first dose. In some examples, the time between administration of the first and the subsequent dose (e.g., initiation thereof) or prior and next subsequent dose is greater than about 5 weeks and less than about 24 weeks, such as between 10 and 24 weeks, such as about 17 weeks. In some examples, the time between administration of the first and the subsequent dose (e.g., initiation thereof) is about 17 weeks. In some examples, administration of the subsequent dose (e.g., initiation thereof) is more than about 7 days after and less than about 365 days after administration

of the first or prior dose (e.g., initiation thereof). In some examples, administration of the subsequent dose (e.g., initiation thereof) is more than about 30 days after and less than about 110 days after administration of the first or prior dose (e.g., initiation thereof).

[0018] In some examples, the subject exhibits an absence of persistence of CAR-T cells at the time of the administration of the subsequent dose. In some examples, the subject exhibits a suboptimal response at the time of the administration of the subsequent dose. In some examples, a suboptimal response may comprise any one or more of the following: (i) complete response (CR), complete response with incomplete recovery of blood count (CRi), with detectable minimal residual disease (leukemic patients) and absence of cytogenetic response; (ii) marrow complete response; (iii) partial response; or (iv) stable response.

[0019] In some examples, administration of the first dose leads to amelioration of one or more symptoms of the disease in the subject following administration of the first dose. In some examples, at the time of the administration of the subsequent dose, the subject has relapsed and/or one or more symptoms of the disease have increased following an initial amelioration (e.g., remission) experienced after the first dose.

[0020] In some examples, the subsequent dose of cells contains CAR-T cells in an amount sufficient for amelioration of the disease in the subject. In some examples, the administration of the subsequent dose leads to a further amelioration of the disease in the subject. In some examples, administration of the subsequent dose leads to amelioration of the disease in the subject as compared with immediately prior to initiation of the administration of the subsequent dose. In some examples, administration of the subsequent dose leads to MRD negativity. In some examples, the method ameliorates the disease to a greater degree and/or for a greater period of time as compared to a method with an alternative dosing regimen wherein the subject is administered the cells in the first dose and the cells in the subsequent dose in a single dose. The amelioration may comprise a reduction in total number of cells, e.g., tumor cells, of the disease in the subject, in an organ of the subject, in a tissue of the subject, or in a bodily fluid of the subject. The amelioration may comprise a reduction in molecular detection by flow cytometry or quantitative PCR, mass or volume of a tumor, and/or a reduction in number and/or extent of metastases. In some examples, the amelioration comprises improvement in survival of the subject, e.g., increased time of survival or incident-free, progression-free, or relapse-free survival.

[0021] In some examples, the disease persists following the administration of the first dose and/or the administration of the first dose is not sufficient to eradicate the disease in the subject. In some examples, the administration of said subsequent dose leads to amelioration of the disease in the subject as compared with immediately prior to initiation of the administration of the subsequent dose.

[0022] In some examples, the administration of the first dose does not induce severe cytokine release syndrome (CRS) in the subject. The severity of cytokine release syndrome (CRS), may be assessed according to the modified grading described by Lee DW, et al., Blood 2014;124(2):188-195, which is incorporated by reference herein in its entirety. In some examples, administration of the first dose does not induce CRS in the subject. In some examples, based on clinical data, administration of the first dose does not induce severe CRS in a majority of subjects. In some examples, the administration of the first dose does not induce CRS encompassing a combination of (1) persistent fever (fever of at least 38 degrees Celsius for at least three days) and (2) a serum level of C reactive protein (CRP) of at least at or about 20 mg/dL, and/or does not induce CRS encompassing hypotension requiring the use of two or more vasopressors or respiratory failure requiring mechanical ventilation.

[0023] In some examples, administration of the first dose does not induce grade 3 or higher neurotoxicity in the subject. In some examples, based on clinical data, administration of the first dose does not induce grade 3 or higher neurotoxicity in a majority of subjects. In some examples, symptoms associated with a clinical risk of neurotoxicity and/or grade 3 or higher neurotoxicity include confusion, delirium, expressive aphasia, obtundation, myoclonus, lethargy, altered mental status, convulsions, seizure-like activity, seizures (optionally as confirmed by electroencephalogram [EEG]), elevated levels of beta amyloid ($A\beta$), elevated levels of glutamate, and elevated levels of oxygen radicals.

[0024] In some examples, the subject has been treated with a therapeutic agent targeting the tumor prior to the administration of the first dose. In some examples, the subject is refractory or non-responsive to said therapeutic agent at the time of the administration of the first dose and/or the subsequent dose. In some examples, subsequent to administration of the first dose and before said administration of the subsequent dose, or prior to administration of the first dose, the methods further include assessing a serum level of a factor indicative of CRS, a symptom of the disease, and/or an indicator of a host anti-recombinant receptor (e.g., anti-CAR) immune response in the

subject, such as a humoral or cell-mediated immune response. In some such examples, the symptom of the disease detected is or comprises a total number of cells of the disease in the subject, in an organ of the subject, in a tissue of the subject, or in a bodily fluid of the subject, molecular detection by flow cytometry or quantitative PCR, mass or volume of a solid tumor, or number or extent of metastases.

[0025] In some examples, the methods include assessing a symptom of the disease prior to administration of the subsequent dose, and based on the result of the assessment, determining the subsequent dose of cells to be administered to the subject. In some examples, if the assessment determines that the subject has morphologic disease, the subject is administered a subsequent dose containing less than, greater than, or about the same number of allogeneic CAR-T cells as the number of allogeneic CAR-T cells in the first dose. In some examples, if the assessment determines that the subject has minimal residual disease, the subject is administered a subsequent dose containing an increased number of allogeneic CAR-T cells as compared to the first dose. In some examples, the subsequent dose comprises about the same number of allogeneic CAR-T cells as the number in the first dose. In some examples, the number of allogeneic CAR-T cells per kilogram administered in the subsequent dose is less than or is the same or about the same as the number of allogeneic CAR-T cells per kilogram administered in the first dose. In other examples, the number of allogeneic CAR-T cells administered in the subsequent dose is greater than the number of allogeneic CAR-T cells administered in the first dose. In some examples, the subsequent dose comprises an increased number of such cells as compared to the first dose, such as at least 2-fold, 5-fold, or 10-fold greater than the number in the first dose. In some examples, the number of CAR-T cells per kilogram administered in the subsequent dose is at least at or about 2 times or at or about 3 times greater than the number of receptor-expressing (e.g., CAR-expressing) cells per kilogram administered in the first dose.

[0026] In some examples, the population of allogeneic CAR-T cells in the first dose expands in the subject following administration of the first dose and/or following the administration of the subsequent dose. In some examples, the expansion is evidenced by an increase in serum C-reactive protein (CRP) level following the administration of the first dose and/or subsequent dose as compared to just prior to the administration. In some examples, the expansion is evidenced by PK as assessed by, e.g., without limitation, flow cytometry. In some examples, the expansion is evidenced by an increase in a level of CAR-encoding nucleic acid in the serum, as

measured by qPCR, following the administration of the first dose and/or subsequent dose as compared to just prior to the administration. In some examples, the increase is at least 1, 2, or 3-fold.

[0027] In some examples, the cells of the first dose and the cells of the second or subsequent dose are derived from the same donor. In some examples, the cells of the first dose and the cells of the second or subsequent dose are derived from different donors.

[0028] In some examples, the first and/or subsequent dose is not a split dose. For example, in some examples, the cells of the first dose are administered in a single pharmaceutical composition comprising the cells of the first dose and/or the cells of the subsequent dose are administered in a single pharmaceutical composition comprising the cells of the subsequent dose. In other examples, the first and/or subsequent dose is a split dose, for example, where the cells of the first dose are administered in a plurality of compositions, collectively comprising the cells of the first dose, over a period of no more than three days; and/or the subsequent dose is a split dose, where the cells of the subsequent dose are administered in a plurality of compositions, collectively comprising the cells of the subsequent dose, over a period of no more than three days.

[0029] In some examples, the methods include administering a subsequent dose of allogeneic CAR-T cells to a subject previously administered a first dose of allogeneic CAR-T cells. In some examples, the subsequent dose of cells is administered at a time point that is at least or more than about 5 weeks after and less than about 24 weeks after initiation of the first dose. In some examples, the number of allogeneic CAR-T cells administered in the subsequent dose is the same as the first dose. In some examples, the CAR-T cells are CD19-specific CAR-T cells (e.g., UCART19).

[0030] In some examples, the number of cells administered in the first dose is between about 0.5×10^6 cells and 5×10^8 cells, between about 0.75×10^6 cells and 8×10^7 cells or between about 5×10^6 cells and 7×10^6 cells, each inclusive. In some examples, the number of cells administered in the first dose of CD19-specific CAR-T cells is between about 0.5×10^6 cells and 1×10^9 cells, between about 1×10^5 cells and 3×10^8 cells or between about 6×10^5 cells and 2.4×10^8 cells, each inclusive. In some examples, the number of cells administered in the first dose of UCART19 cells is about 6×10^5 cells, about 6×10^6 cells, about 6×10^7 cells, about 8×10^7 cells, about 1.8×10^8 cells, or about 2.4×10^8 cells. In some examples, the number of cells administered in the first dose of UCART19 cells is about 6×10^6 cells.

[0031] In some examples, the number of cells administered in the subsequent dose of CD19-specific CAR-T cells is between about 0.5×10^6 cells and 1×10^9 cells, between about 1×10^5 cells and 3×10^8 cells or between about 6×10^5 cells and 2.4×10^8 cells, each inclusive. In some examples, the number of cells administered in the subsequent dose of UCART19 cells is about 6×10^5 cells, about 6×10^6 cells, about 6×10^7 cells, about 8×10^7 cells, about 1.8×10^8 cells, or about 2.4×10^8 cells. In some examples, the number of cells administered in the subsequent dose of UCART19 cells is about 6×10^6 cells. In some examples, the number of cells administered in the first and subsequent dose of UCART19 cells is about 6×10^6 cells.

[0032] In some examples, the methods further include administering a lymphodepletion regimen prior to the first dose and/or prior to the administration of the subsequent dose. In some examples, the lymphodepletion regimen comprises cyclophosphamide, fludarabine, and/or a combination thereof. In some examples, the lymphodepletion regimen comprises one or more of cyclophosphamide, fludarabine, a CD52 antibody (e.g., an antibody comprising the sequence of SEQ ID NO: 8 and/or SEQ ID NO: 10), and/or a combination thereof. In some examples, the administration of the lymphodepletion regimen includes administration of cyclophosphamide and fludarabine prior to the administration of the first dose, and a CD-52 antibody (e.g., an antibody comprising the sequence of SEQ ID NO: 8 and/or SEQ ID NO: 10) after administration of the first dose. In some examples, the administration of the lymphodepletion regimen includes administration of a lymphodepletion regimen prior to the administration of the first dose and optionally not prior to the administration of the subsequent dose. In some examples, the lymphodepletion regimen is administered between 2 and 10 days prior to the administration of the first dose. In some examples, the lymphodepletion regimen is administered between 2 and 10 days prior to the administration of the second dose. In some examples, the lymphodepletion regimen is administered between 2 and 10 days prior to the administration first dose and between 2 and 14 days prior to the administration of the second dose. In some examples, the lymphodepletion regimen is administered over the course of 1, 2, 3, 4, or 5 days.

[0033] In some examples, a lymphodepletion regimen comprises administering about 90-120 mg/m² fludarabine and about 1500 mg/m² cyclophosphamide. In some examples, a lymphodepletion regimen comprises administering about 150 mg/m² fludarabine and about 120 mg/m² cyclophosphamide.

[0034] In some examples, a lymphodepletion regimen comprises administering fludarabine at a dosage of about 30 to 150 mg/m²; cyclophosphamide at a dosage of about 300 to 4000 mg/m²; or a CD52 antibody (e.g., a CD52 antibody comprising the sequence of SEQ ID NO: 8 and/or SEQ ID NO: 10) at a dosage of about 0.3 to 1 mg/kg. In some examples, fludarabine is administered at a dosage of about 30 to 150 mg/m²; cyclophosphamide is administered at a dosage of about 300 to 4000 mg/m²; and a CD52 antibody (e.g., a CD52 antibody comprising the sequence of SEQ ID NO: 8 and/or SEQ ID NO: 10) is administered at a dosage of about 10 to about 13 mg. In some examples, fludarabine is administered at a dosage of about 30 mg/m²/day; cyclophosphamide is administered at a dosage of about 300 mg/m²/day; or a CD52 antibody (e.g., a CD52 antibody comprising the sequence of SEQ ID NO: 8 and/or SEQ ID NO: 10) is administered at a dosage of about 10 to about 13 mg/day. In some examples, fludarabine is administered at a dosage of about 30 mg/m²/day; cyclophosphamide is administered at a dosage of about 300 mg/m²/day; and a CD52 antibody (e.g., a CD52 antibody comprising the sequence of SEQ ID NO: 8 and/or SEQ ID NO: 10) is administered at a dosage of about 10 to about 13 mg/day.

[0035] In some examples, a lymphodepletion regimen comprises administering fludarabine and cyclophosphamide. In some examples, a lymphodepletion regimen comprises administering fludarabine, cyclophosphamide, and an anti-CD52 drug (e.g., a CD52 antibody having the sequence of SEQ ID NO: 8 and/or SEQ ID NO: 10). In some examples, a lymphodepletion regimen further comprises administering Mensa (sodium-2-mercaptoethanesulfonate). In some examples, a lymphodepletion regimen further comprises administering at least one corticosteroid. In some examples, the corticosteroid is administered immediately prior to administration of an anti-CD52 antibody (e.g., an antibody comprising the sequence of SEQ ID NO: 8 and/or SEQ ID NO: 10). In some examples, the corticosteroid is methylprednisolone administered at a dose of 1-5 mg/kg (e.g., 2 mg/kg). In some examples, the corticosteroid is administered at least two days prior to administration of CAR-T cells. In some examples, no corticosteroid is administered for at least two days prior to the administration of CAR-T cells.

[0036] In some examples, a patient receives a premedication for infusion-related reaction prior to the lymphodepletion regimen. The premedication may comprise, for example, an antihistamine or acetaminophen.

[0037] In some examples, the components of the lymphodepletion regimen of fludarabine/cyclophosphamide (FC) or fludarabine/cyclophosphamide/anti-CD52 antibody (FCA) are administered simultaneously; in other examples, the components are administered serially. In some examples, the subject receives a FC regimen prior to the first dose of the CAR-T cell therapy; and a FCA regimen prior to a redosing of the CAR-T cell therapy. In some examples, the subject receives a FCA regimen prior to the first dose of the CAR-T cell therapy; and a second FCA regimen prior to a redosing of the CAR-T cell therapy. In some examples, the subject receives a FC regimen prior to the first dose of the CAR-T cell therapy, and a CD52 antibody (e.g., an antibody comprising the sequence of SEQ ID NO: 8 and/or SEQ ID NO: 10) after the first dose of the CAR-T cell therapy. In some examples, the subject receives a FC regimen prior to the first dose of the CAR-T cell therapy, and a CD52 antibody (e.g., an antibody comprising the sequence of SEQ ID NO: 8 and/or SEQ ID NO: 10) after the first dose of the CAR-T cell therapy; and the subject further receives a FC regimen prior to the second/subsequent dose of the CAR-T cell therapy, and a CD52 antibody (e.g., an antibody comprising the sequence of SEQ ID NO: 8 and/or SEQ ID NO: 10) after the second/subsequent dose of the CAR-T cell therapy.

[0038] In some examples, the methods further include administering an agent to debulk the disease prior to administration of the first dose and/or prior to the administration of the second/subsequent dose. In some examples, the methods include administering a chemotherapeutic agent prior to the first dose and/or prior to the administration of the second/subsequent dose. In some examples, the subject has been previously treated with a chemotherapeutic agent prior to the first dose. In some examples, the chemotherapeutic agent is or comprises a conditioning chemotherapy, which reduces burden of the disease in the subject prior to the first dose and/or subsequent dose. In some examples, the administration of the chemotherapeutic agent includes administration of a chemotherapeutic agent prior to the administration of the first dose and optionally not prior to the administration of the second/subsequent dose. In some examples, the chemotherapeutic agent is administered between 2 and 10 days prior to the administration of the first dose. In some examples, the chemotherapeutic agent is administered between 2 and 10 days prior to the administration of the second/subsequent dose. In some examples, the chemotherapeutic agent is administered

between 2 and 10 days prior to the administration first dose and between 2 and 14 days prior to the administration of the second/subsequent dose.

[0039] Also provided are cells and compositions for use and uses of allogeneic CAR-T cells and compositions for treating a disease in a subject, such as a tumor or cancer. Also provided are cells and compositions for use and uses of allogeneic CAR-T cells and compositions for the manufacture of a medicament for treatment of a disease in a subject previously treated with allogeneic CAR-T cells. In some examples, the compositions or cells for use or medical uses are for use 4 to 24 weeks after the previous treatment. In some examples, the compositions or cells for use are formulated for administration of a subsequent dose in an amount sufficient for reduction in burden of a disease in the subject having been previously treated with the CAR-T cells. In some examples, the CAR-T cells are CD19-specific CAR-T cells (e.g., UCART19).

[0040] In some examples of such medical uses, the compositions or cells are for use that includes administering to a subject having the disease a first dose of allogeneic CAR-T cells. In some examples, the first dose contains about 1×10^6 total cells, about 6×10^6 total cells, or about 1×10^7 total cells. In some examples, the compositions or cells are for use that includes administering to the subject a subsequent dose of allogeneic CAR-T cells at a time point that is at least or more than about 4 weeks after and less than about 24 weeks after initiation of the administration of the first dose.

[0041] In some examples, allogeneic CAR-T cells are provided for use in methods of treating a disease in a subject previously treated with allogeneic CAR-T cells. In some examples, the cells are for use between about 4 and 24 weeks after the previous treatment. In some examples, the cells for use are formulated for administration of a subsequent dose in an amount sufficient for reduction in burden of a disease in the subject having been previously treated with the allogeneic CAR-T cells. In some examples, the CAR-T cells are CD19-specific CAR-T cells (e.g., UCART19).

[0042] In some examples of any such compositions or cells for use or medical uses, the subject does not exhibit morphologic disease and/or the subject does not exhibit greater than 5% blast cells in the bone marrow.

[0043] In some examples, the compositions or cells are for use in a method including administering to a subject having the disease a first dose of allogeneic CAR-T cells. In some examples, the first dose about 6×10^5 cells, about 6×10^6 cells, about 6×10^7 cells, about 8×10^7

cells, about 1.8×10^8 cells, or about 2.4×10^8 cells. In some examples, the cells are for use in a method that includes administering to the subject a subsequent dose of allogeneic CAR-T cells at a time point that is at least or more than about 5 weeks after and less than about 24 weeks after initiation of said administration of the first dose.

[0044] Also provided herein are uses of allogeneic CAR-T cells for manufacture of a medicament for the treatment of a disease in a subject includes cells that are formulated and/or packaged for administration to the subject in a first and a subsequent dose. In some examples, the treatment includes administering the cells to the subject in a first and a subsequent dose, where the first dose includes about 6×10^5 cells, about 6×10^6 cells, about 6×10^7 cells, about 8×10^7 cells, about 1.8×10^8 cells, or about 2.4×10^8 cells. In some examples, the CAR-T cells are CD19-specific CAR-T cells (e.g., UCART19).

[0045] In some examples, the cells for use are formulated and/or packaged for administration to the subject in a first and a subsequent dose and/or the treatment includes administering the cells to the subject in a first and a subsequent dose. In some examples, the first dose contains about 6×10^5 cells, about 6×10^6 cells, about 6×10^7 cells, about 8×10^7 cells, about 1.8×10^8 cells, or about 2.4×10^8 cells.

[0046] In some examples, the use includes where the first and subsequent administrations include administering the cells in one or more unit dose, each unit dose comprising about 6×10^5 cells, about 6×10^6 cells, about 6×10^7 cells, about 8×10^7 cells, about 1.8×10^8 cells, or about 2.4×10^8 cells.

[0047] In some examples, the cells or use includes where the first administration comprises administering a single unit dose. In some examples, the cells or use includes where the subsequent administration comprises administration of two or more unit doses. In some examples, the cells or use includes where the subsequent administration comprises administration a single unit dose.

[0048] In some examples, a use of the cells is provided for treating a disease, wherein the disease is a tumor or a cancer. In some examples the disease is a lymphoid malignancy such as for example without limitation, acute and chronic leukemias, lymphomas, multiple myelomas, a myeloid malignancy (e.g., leukemias, myelodysplastic syndromes, myelodysplastic/myeloproliferative neoplasms), or a mixed lineage malignancy. In some examples, a composition or cells are provided for use in treating acute lymphoblastic leukemia (ALL). In some

examples, the ALL is relapsed/refractory ALL. In some examples, a composition or cells are provided for use in treating Non-Hodgkin's lymphoma, such relapsed or refractory Non-Hodgkin's lymphoma. In some examples, a composition or cells are for use in treating relapsed or refractory large B-cell or follicular lymphoma.

[0049] In some examples, the cells, composition, or use includes where the subsequent dose is formulated for administration of about the same number of allogeneic CAR-T cells as the allogeneic CD19-specific CAR-T cells in the previous dose. In some examples, the composition containing the cells of the subsequent dose is formulated for administration of an increased number of allogeneic CAR-T cells as compared to the first dose or previous dose. In some examples, the cells, composition, or use includes where the subsequent dose is formulated for administration of less than the number of allogeneic CAR-T cells as the allogeneic CD19-specific CAR-T cells in the previous dose.

[0050] Also provided are pharmaceutical kits for treating a patient suffering from a disease such as cancer, the kit comprising CAR-T cells and a CD52 antibody. In some examples, the CAR-T cells are CD19 specific CAR-T cells, such as UCART19 cells. In some examples, the CAR-T cells express the CAR of SEQ ID NO: 1. In some examples, the CD52 antibody comprises the sequence of SEQ ID NO: 8 and/or SEQ ID NO: 10. In some examples, the kit comprises a first container comprising the CAR-T cells, and a second container comprising the CD52 antibody. In some examples, at least one of the first and the second container is a flexible cell infusion bag. In some examples, the kit further comprises a label or package insert comprising instructions for administering the CAR-T cells and the CD52 antibody to the subject.

[0051] Also provided are articles of manufacture for carrying out the methods. In some examples, the article of manufacture includes a plurality of containers, e.g., sealable containers, each individually comprising a unit dose of allogeneic CAR-T cells, for administration to the subject, packaging material, and/or a label or package insert. In some examples, the CAR-T cells are CD19-specific CAR-T cells (e.g., UCART19).

[0052] In some examples, the unit dose comprises the amount of cells to be given in the lowest dose in the methods, such as the size of the first dose. In some examples, the unit dose includes about 6×10^5 cells, about 6×10^6 cells, about 6×10^7 cells, about 8×10^7 cells, about 1.8×10^8 cells, or about 2.4×10^8 cells.

[0053] In some examples, the label or package insert includes instructions for administering a plurality of the unit doses to the subject, for example, by administering a certain number of such unit doses, e.g., one unit dose, in administration of a first dose, and then administering a subsequent dose including one or a plurality of the unit doses. In some examples, the instructions specify carrying out a first administration, said first administration comprising delivering one of said unit doses to the subject, and carrying out a subsequent administration, said subsequent administration comprising administering one or a plurality of said unit doses to the subject. In some examples, they specify that the subsequent administration is to be carried out at a time between about 5 and about 24 weeks following said first administration. In some examples, the containers are or comprise flexible cell infusion bags. In some examples, the methods are for allogeneic administration. In some examples, the label and/or packaging material further includes an identifier specific to the subject, indicating that the cells were derived from the subject and/or should be administered to the subject specifically. In some examples, the CAR-T cells are CD19-specific CAR-T cells (e.g., UCART19).

[0054] Also provided is a method of producing a population of allogeneic chimeric antigen receptor (CAR)-T cells (CAR-T cells) directed to a target of interest comprising: (a) isolating peripheral blood mononuclear cells (PBMCs) from a healthy donor; (b) activating the T-cells in the PBMCs; (c) transducing the activated T-cells with a lentiviral vector, wherein the lentiviral vector is a self-inactivating recombinant vector expressing a CAR of interest; (d) disrupting TCR $\alpha\beta$ and CD-52 gene expression in a subset of the T-cells; (e) expanding the population of T-cells; and (f) enriching the population of T-cells for TCR $\alpha\beta$ -negative cells; whereby generating a population of “off the shelf” allogeneic chimeric antigen receptor (CAR)-T cells (CAR-T cells). In some examples, the CAR-T cells comprise allogeneic CD19CAR/RQR8+₊TCR $\alpha\beta$ -₋T-cells.

[0055] Also provided are allogeneic chimeric antigen receptor (CAR)-T cells (CAR-T cells) comprising an anti-human CD19 4-1BB/CD3zeta CAR, for use in a method of treating a subject who has refractory and/or relapsed Non-Hodgkin’s Lymphoma, the method comprising administering to the subject at least one dose of the CAR-T cells, wherein the at least one dose is about 20×10^6 cells/dose to about 360×10^6 cells/dose.

[0056] Also provided are chimeric antigen receptor (CAR)-T cells (CAR-T cells) for use in a method of treatment comprising: (a) administering to a subject a first dose of the CAR-T cells,

administration of a subsequent dose in an amount sufficient for amelioration of a disease in the subject having been previously treated with the CAR-T cells.

[0062] In some examples, the methods of treatment disclosed herein are applied to subjects who have been treated with an antibody for the depletion of CD52⁺ cells (e.g. an antibody comprising SEQ ID NO: 8 and SEQ ID NO: 10), or who will be treated with said antibody as part of said methods of treatment. These methods of treatment may comprise the use of a CAR-T cell (e.g. UCART19) deficient in CD52.

[0063] Also provided are methods of producing a population of allogeneic chimeric antigen receptor (CAR)-T cells (CAR-T cells) directed to a target of interest comprising:

- (a) providing isolated peripheral blood mononuclear cells (PBMCs) from a healthy donor;
- (b) activating the T-cells in the PBMCs;
- (c) transducing the activated T-cells with a lentiviral vector, wherein the lentiviral vector is a self-inactivating recombinant vector expressing a CAR of interest;
- (d) disrupting TCR $\alpha\beta$ and CD-52 gene expression in a subset of the T-cells;
- (e) expanding the population of T-cells; and
- (f) enriching the population of T-cells for TCR $\alpha\beta$ -negative cells;

whereby generating a population of allogeneic chimeric antigen receptor (CAR)-T cells (CAR-T cells).

BRIEF DESCRIPTION OF THE DRAWINGS

[0064] **FIG. 1** depicts an exemplary schematic showing possible steps for a multiple dosing regimen for allogeneic CAR-T cell treatment.

[0065] **FIG. 2A** and **FIG. 2B** depict kinetic data from two patients receiving a second UCART19 infusion (redosing study).

[0066] **FIG. 3A** depicts an exemplary schematic showing the steps of a dosing regimen for allogeneic CAR-T cell treatment.

[0067] **FIG. 3B** depicts an exemplary schematic showing the steps of a dosing regimen for allogeneic CAR-T cell treatment.

[0068] **FIG. 4A** and **FIG. 4B** depict exemplary flow diagrams for allogeneic CAR- T cell manufacturing.

CLAIMS

1. Use of at least one dose of allogeneic chimeric antigen receptor (CAR)-T cells (CAR-T cells) comprising an anti-human CD19 4-1BB/CD3zeta CAR in the manufacture of a medicament for treating a subject who has refractory and/or relapsed Non-Hodgkin's Lymphoma, wherein the at least one dose is selected from the group consisting of about 120×10^6 cells/dose, about 240×10^6 cells/dose, and about 360×10^6 cells/dose.
2. The use of claim 1, wherein the Non-Hodgkin's Lymphoma is large B-cell lymphoma.
3. The use of claim 1, wherein the Non-Hodgkin's Lymphoma is follicular lymphoma.
4. The use of any one of claims 1 to 3, wherein the at least one dose is about 240×10^6 cells/dose.
5. The use of any one of claims 1 to 3 wherein the at least one dose is selected from the group consisting of about 120×10^6 cells/dose, and about 360×10^6 cells/dose.
6. The use of any one of claims 1 to 5, wherein the CAR-T cells are CD52 deficient.
7. The use of any one of claims 1 to 5, wherein the CAR-T cells comprise a mixture of CD52-deficient and CD-52 positive cells.
8. The use of any one of claims 1 to 7, wherein the CAR-T cells comprise the CAR of SEQ ID NO: 1.
9. The use of any one of claims 1 to 8, wherein the CAR-T cells comprise UCART19(CD19)CAR/RQR8+_TCR $\alpha\beta$ -_T-cells.
10. The use of any one of claims 1 to 8, wherein the CAR-T cells do not express a safety switch.
11. The use of any one of claims 1 to 10, wherein CAR expression is detectable in the subject for up to at least 14 days after administration of the CAR-T cells.
12. The use of any one of claims 1 to 11, wherein CAR expression is detectable in the subject for up to at least 28 days after administration of the CAR-T cells.
13. The use of any one of claims 1 to 12, wherein the subject exhibits a complete response (CR) or complete response with incomplete recovery of blood count (Cri) state for at least 1 months after CAR-T administration.

14. The use of any one of claims 1 to 13, wherein the subject exhibits a CR or Cri state for at least 2 months after CAR-T administration.
15. The use of any one of claims 1 to 14, wherein the subject exhibits a CR or Cri state for at least 6 months after CAR-T administration.
16. The use of any one of claims 1 to 15, wherein the subject exhibits a CR or Cri state for at least 12 months after CAR-T administration.
17. The use of any one of claims 1 to 16, wherein the subject receives a first lymphodepletion regimen prior to administration of the at least one dose.
18. The use of claim 17, wherein the first lymphodepletion regimen comprises administration of fludarabine and cyclophosphamide.
19. The use of claim 17, wherein the first lymphodepletion regimen comprises administration of fludarabine, cyclophosphamide, and an anti-CD52 antibody.
20. The use of claim 18 or 19, wherein the first lymphodepletion regimen further comprises administration of Mensa (sodium-2-mercaptoethanesulfonate).
21. The use of any one of claims 18 to 20, wherein the first lymphodepletion regimen further comprises administration of at least one corticosteroid.
22. The use of claim 21, wherein the corticosteroid is to be administered immediately prior to administration of the anti-CD52 antibody.
23. The use of claim 21 or 22, wherein the corticosteroid is methylprednisolone.
24. The use of claim 23, wherein the methylprednisolone is to be administered at a dose of 2 mg/kg.
25. The use of any one of claims 1 to 24, wherein the subject is one that receives a premedication for infusion-related reaction prior to the lymphodepletion regimen.
26. The use of claim 25, wherein the premedication comprises at least one antihistamine.
27. The use of claim 25 or 26, wherein the premedication comprises acetaminophen.
28. The use of any one of claims 18 to 27, wherein fludarabine is to be administered at a dosage of about 30 mg/m²/day; cyclophosphamide is to be administered at a dosage of about 300

mg/m²/day; or CD52 antibody is to be administered at a dosage of 10 to 13 mg/day.

29. The use of any one of claims 19 to 27, wherein fludarabine is to be administered at a dosage of about 30 mg/m²/day; cyclophosphamide is to be administered at a dosage of about 300 mg/m²/day; and CD52 antibody is to be administered at a dosage of 10 to 13 mg/day.
30. The use of any one of claims 17 to 29, wherein the first lymphodepletion regimen is initiated between 1 to 15 days prior to administration of the at least one dose.
31. The use of any one of claims 17 to 30, wherein the first lymphodepletion regimen is to be administered over the course of 1, 2, 3, 4, or 5 days.
32. The use of any one of claims 1 to 31, wherein the subject is one that receives a subsequent dose of the CAR-T cells.
33. The use of claim 32, wherein the subject exhibits any one of the following at the time of the administration of the subsequent dose:
 - (a) complete response (CR), complete response with incomplete recovery of blood count (CRi), with detectable minimal residual disease (leukemic patients) and absence of cytogenetic response;
 - (b) marrow complete response;
 - (c) partial response; or
 - (d) stable response.
34. The use of claim 32 or 33, wherein the subsequent dose comprises about the same number of cells as the number of cells in the first dose.
35. The use of claim 32 or 33, wherein the subsequent dose comprises an increased number of cells as compared to the first dose.
36. The use of claim 32 or 33, wherein the subsequent dose comprises a decreased number of cells as compared to the first dose.
37. The use of any one of claims 32 to 36, wherein the subsequent dose is to be administered at least 14, at least 28, at least 42, or at least 56 days after the first dose.