

a consecutive dose is designed to minimize risk of toxicity and/or host immune response to the cells by the subject, thereby improving persistence and efficacy.

Background

[0004] Various methods are available for adoptive cell therapy using engineered cells expressing recombinant receptors, such as chimeric antigen receptor (CARs). Improved methods are needed, for example, to reduce the risk of toxicity and/or to increase efficacy, for example, by increasing exposure of the subject to the administered cells, for example, by improving expansion and/or persistence of the administered cells. Provided are methods, compositions, and articles of manufacture that meet such needs.

Summary

[0004a] In one aspect, the present invention provides use of a composition comprising cells expressing a chimeric antigen receptor (CAR) for manufacture of a medicament to be administered as a consecutive dose of CAR-expressing cells for treatment of a disease or condition in a subject previously treated with a first dose of CAR-expressing cells, wherein:

the composition comprising the consecutive dose is for use 14 to 28 days after the initiation of administration of the first dose of CAR-expressing cells; and

the composition comprising the consecutive dose is formulated for administration of an increased number of CAR-expressing cells as compared to the first dose and in an amount sufficient for reduction in burden of a disease or condition in the subject having been previously treated with the first dose of CAR-expressing cells.

[0004b] In another aspect, the present invention provides use of cells expressing a chimeric antigen receptor (CAR) in the manufacture of a medicament to be administered as a first dose of CAR-expressing cells and a consecutive dose of CAR-expressing cells for treating a disease or condition in a subject, wherein: the first dose comprises no more than about 1×10^6 of the CAR-expressing cells per kilogram body weight of the subject, no more than about 1×10^8 of the CAR-expressing cells, and/or no more than about 1×10^8 of the CAR-expressing cells cells/m² of the subject; and

the consecutive dose of CAR-expressing cells is for administration at a time point that is at least or more than about 14 days after and less than about 28 days after initiation of

administration of the first dose of cells, and wherein the consecutive dose of CAR-expressing cells comprises an increased number of CAR-expressing cells as compared to the first dose

[0005] Provided are methods of for administering to subjects cells expressing genetically engineered (recombinant) cell surface receptors in adoptive cell therapy, for example, to treat diseases and/or conditions in the subjects. The methods generally involve administering multiple doses of such cells, and/or administering a consecutive dose to a subject having been previously treated with a prior (e.g., first) dose of such cells. In some examples, the doses are a first dose and one or more consecutive doses. In some examples, the first dose is a relatively low dose and/or is a conditioning or debulking dose and/or the consecutive dose(s) is a consolidating dose(s). Also provided are cells, compositions, and articles of manufacture for use in such methods. In some examples, the recombinant receptors are genetically engineered antigen receptors, such as functional non-TCR antigen receptors, e.g., chimeric antigen receptors (CARs) and other recombinant antigen receptors such as transgenic T cell receptors (TCRs). Also among the receptors are other recombinant chimeric receptors, such as those containing an extracellular portion that specifically binds to a ligand or receptor or other binding partner and an intracellular signaling portion, such as the intracellular signaling portion of a CAR. The doses in some examples include a relatively low first dose.

[0006] In some examples, the methods involve (a) administering to a subject with a disease or condition a first dose of cells expressing a recombinant receptor (e.g., chimeric antigen receptor (CAR)), the first dose containing the cells; and (b) administering to the subject a consecutive dose of recombinant receptor-expressing (e.g., CAR-expressing) cells. In other

examples, e.g., for providing consolidating treatment, the methods are carried out by administering to the subject the consecutive dose or doses as in (b), to a subject that has been previously administered the first dose as in (a).

[0007] In some examples, the methods involve (a) administering to a subject having a disease or condition a first dose of cells expressing a recombinant receptor (e.g., chimeric antigen receptor (CAR)). In some examples, the first dose contains no more than about 1×10^6 of the cells per kilogram body weight of the subject, no more than about 1×10^8 of the cells, and/or no more than about 1×10^8 of the cells/m² of the subject. In some examples, the methods further involve (b) administering to the subject a consecutive dose of cells expressing a recombinant receptor (e.g., CAR) at a time point that is at least or more than about 14 days after and less than about 28 days after initiation of the administration in (a).

[0008] In some examples, at the time of the administration in (b) the serum level in the subject of a factor indicative of cytokine release syndrome (CRS) is less than about 10 times, less than about 25 times, and/or less than about 50 times that in the subject immediately prior to said administration in (a). In some examples, the subject does not exhibit grade 3 or higher neurotoxicity. In some examples, a CRS-related outcome or symptom of neurotoxicity in the subject following the administration of the first dose has reached a peak level and begun to decline following the administration in (a). In some examples, the subject does not exhibit a detectable humoral or cell-mediated immune response against the receptor (e.g., CAR) expressed by the cells of said first dose.

[0009] In some examples, the methods further involve the administration of additional consecutive or subsequent doses, such that a first and multiple consecutive doses are administered, e.g., in accordance with the dosing amounts and timing schedules as specified for the first and consecutive doses. In some examples, the first of one or more subsequent doses is administered at a time that is at least or greater than 14 days after the initiation of the administration of the consecutive dose. In some examples, the administration of the first, consecutive, and subsequent doses includes administering at least three of the doses within at or about 28 days. In some examples, the consecutive dose is administered at about day 14 following the initiation of administration of the first dose, and an additional consecutive or subsequent dose is administered at day 28 following the initiation of administration of the first

dose. In some examples, additional subsequent doses are administered at day 42 and/or day 56 following the initiation of administration of the first dose.

[0010] In some examples, the first dose is administered in an amount sufficient to reduce burden of the disease or condition in the subject. In some examples, the first dose is a low dose, such as a debulking dose, such as a dose that is lower than that required to eradicate the disease or condition but that may effect a reduction in burden or bulk of such disease or condition. In some examples, the administration of the first dose does not induce severe cytokine release syndrome (CRS) in the subject. 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.

[0011] 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 β), elevated levels of glutamate, and elevated levels of oxygen radicals.

[0012] In some examples, the first dose is lower than a dose that would cause CRS or severe CRS in the subject. In some examples, the first dose comprises no more than about 1×10^6 of the cells per kilogram body weight of the subject, no more than 5×10^5 of the cells per kilogram body weight of the subject, no more than about 1×10^8 of the cells, or no more than about 1×10^8 of the cells/m² of the subject.

[0013] In some examples, the consecutive dose(s) is administered at a time at which a clinical risk for neurotoxicity, cytokine-release syndrome (CRS), macrophage activation syndrome, or tumor lysis syndrome, is not present or has passed or has subsided following the

administration of the first (or prior) dose. In some examples, the consecutive dose is administered at a time at which a biochemical readout evidencing CRS, neurotoxicity, macrophage activation syndrome, or tumor lysis syndrome, is not present or has passed or has subsided following said administration of the first (or prior) dose. In some examples, the consecutive dose(s) is administered at a time at which a serum level of a factor indicative of cytokine-release syndrome (CRS) or neurotoxicity in the subject is less than about 10 times, less than about 25 times, and/or less than about 50 times the serum level of the indicator in the subject immediately prior to said administration of the first dose. In some examples, the consecutive dose(s) is administered at a time after a neurotoxicity and/or CRS-related outcome or serum factor indicating CRS in the subject has reached a peak level and begun to decline following said administration, such as where at the time of the administration of the consecutive dose, the level of a CRS-related outcome or serum factor indicative of CRS is no more than 50 % of the peak level, is no more than 20 % of the peak level, or is no more than 5 % of the peak level, or is at or about the level immediately prior to the administration of the first dose.

[0014] In some examples, the subject does not exhibit cytokine release syndrome (CRS), does not exhibit severe CRS, does not exhibit neurotoxicity, does not exhibit severe neurotoxicity, or does not exhibit neurotoxicity above grade 3 following administration of the first dose and/or following the administration of the consecutive dose.

[0015] Among the CRS-related outcomes are fever, hypotension, hypoxia, neurologic disturbances, or a serum level of an inflammatory cytokines, such as interferon gamma ($\text{IFN}\gamma$), granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor alpha ($\text{TNF}\alpha$), IL-6, IL-10, IL-1 β , IL-8, IL-2, MIP-1, Flt-3L, fractalkine, and IL-5, and C reactive protein (CRP). Among the factors, e.g., serum factors, indicative of CRS are inflammatory cytokines such as $\text{IFN}\gamma$, GM-CSF, $\text{TNF}\alpha$, IL-6, IL-10, IL-1 β , IL-8, IL-2, MIP-1, Flt-3L, fractalkine, and IL-5, and CRP.

[0016] In some examples, the time of administering the consecutive 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 receptor (e.g., CAR) expressed by the cells of said first (or prior) dose.

[0017] In some examples, the time between the administration of the first dose, e.g., the initiation of the administration of the first or prior dose, and the initiation of the administration

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

[0018] In some examples, at the time of the administration of the consecutive dose, the serum level in the subject of a factor indicative of CRS is less than about 10 times, less than about 25 times, and/or less than about 50 times that in the subject immediately prior to said administration of the first dose; and/or a CRS-related outcome in the subject following said administration of said first dose has reached a peak level and begun to decline following the administration in (a); and/or the subject does not exhibit a detectable humoral or cell-mediated immune response against the receptor (e.g., CAR) expressed by the cells of said first dose. In some examples, at the time of the administration in (b) or of the consecutive dose, the serum level of said factor indicative of CRS is no more than ten times the level immediately prior to the administration in (a) or of the first dose.

[0019] In some examples, the subject has not received a dose of cells expressing the receptor (e.g., CAR) that is expressed by the cells in the first dose prior to the administration in (a). In some examples, the receptor (e.g., the CAR) expressed by the cells in the consecutive dose contains at least one immunoreactive epitope present in the receptor (e.g., CAR) expressed by the cells in the first dose. In some examples, the receptor in the cells of the consecutive dose is identical or substantially identical to the receptor (e.g. the CAR) expressed by the cells in the first dose. In some examples, the receptor expressed by the cells of the first dose specifically binds to an antigen expressed by a cell or tissue of the disease or condition or

associated with the disease or condition. In some examples, the receptor expressed by the cells of the consecutive dose binds to the same antigen, e.g., to the same epitope, and/or contains the same antigen-binding domain as that in the first dose.

[0020] In some examples, the disease or condition 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), ALL, non-Hodgkin's lymphoma, acute myeloid leukemia, multiple myeloma, refractory follicular lymphoma, mantle cell lymphoma, indolent B cell lymphoma, B cell malignancies, cancers of the colon, lung, 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, and/or mesothelioma. In some examples, the disease or condition is a leukemia or lymphoma. In some examples, the disease or condition is acute lymphoblastic leukemia. In some examples, the disease or condition is non-Hodgkin lymphoma (NHL).

[0021] In some examples, the disease is a cancer and the subject does not exhibit morphologic disease at the time of initiation of the administration of the consecutive 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 consecutive dose. In some examples, the subject exhibits detectable molecular disease and/or minimum residual disease at the time of the administration of the consecutive dose.

[0022] In some examples, administration of the first dose leads to a reduction in burden of the disease or condition in the subject, for example, as indicated by a reduction in one or more factors indicative of disease burden following said administration of the first dose, e.g., following the administration in (a). In some examples, at the time of the administration in (b) or of the consecutive dose, the subject has not relapsed and/or the one or more factors indicative of disease burden have not increased following the initial reduction experienced after the first dose. In some examples, the consecutive dose of cells contains cells in an amount sufficient for reduction in burden of a disease or condition in the subject. In some examples, the administration of the consecutive dose leads to a further reduction in burden of the disease or condition in the subject. In some examples, administration of the consecutive dose leads to a reduction in burden of the disease or condition in the subject as compared with immediately

prior to initiation of the administration of the consecutive dose. In some examples, the method reduces burden of the disease or condition 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 consecutive dose in a single dose. The reduction in burden and/or further reduction in burden 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 reduction 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 reduction comprises improvement in survival of the subject, e.g., increased time of survival or incident-free, progression-free, or relapse-free survival.

[0023] In some examples, the disease or condition persists following the administration of the first dose and/or the administration of the first dose is not sufficient to eradicate the disease or condition in the subject. In some examples, the administration of said consecutive dose leads to a reduction in burden of the disease or condition in the subject as compared with immediately prior to initiation of the administration of the consecutive dose.

[0024] In some examples, the methods reduce burden of the disease or condition to a greater degree and/or for a greater period of time as compared to a method comprising an alternative dosing regimen wherein the subject is administered the cells in (a) (or of the first dose) and the cells in (b) (or of the consecutive dose) in a single dose. In some examples, the area under the curve (AUC) of recombinant receptor-expressing (e.g., CAR-expressing) cells over time, and/or the duration of detectable receptor-expressing cells in the subject following the administration of the consecutive dose (or the administration in (b)) is greater as compared to that achieved via a method comprising an alternative dosing regimen wherein the subject is administered the cells in (a) (or the first dose) and the cells in (b) (or the consecutive dose) as a single dose. In some examples, the method results in a maximum concentration or number of receptor-expressing (e.g., CAR-expressing) cells in the blood of the subject of at least at or about 10 receptor-expressing cells per microliter, at least at or about 100 receptor-expressing cells per microliter, at least 50 % of the total number of peripheral blood mononuclear cells (PBMCs), at least at least about 1×10^5 receptor-expressing cells, or at least 5,000 copies of recombinant receptor-encoding (e.g., CAR-encoding) DNA per micrograms DNA, or at least

10,000 copies of recombinant receptor-encoding DNA per micrograms of DNA. In some examples, at day 90 following the initiation of the administration in (a) or of the first dose, recombinant receptor-expressing (e.g., CAR-expressing) cells are detectable in the blood or serum of the subject, e.g., the blood of the subject contains at least 10 %, at least 20 %, at least 40 %, or at least 50 %, receptor (e.g., CAR)-expressing cells, at least 10 receptor (e.g., CAR)-expressing cells per microliter or at least 1×10^4 receptor (e.g., CAR)-expressing cells.

[0025] In some examples, the AUC for blood concentration of receptor-expressing (e.g., CAR-expressing) cells over time following the administration in (a) or of the first dose is greater as compared to that achieved via a method comprising an alternative dosing regimen wherein the subject is administered the cells in (a) or of the first dose and the cells in (b) or of the second dose as a single dose.

[0026] In some examples, a CRS-related outcome in the subject at day 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 following the administration in (b) or of the consecutive dose is not detectable or is reduced as compared to a method comprising an alternative dosing regimen wherein the subject is administered the cells in (b) or the consecutive dose without having been administered the first dose.

[0027] In some examples, the AUC for a serum level of a factor indicative of CRS over time in the subject following the administration in (b) or the consecutive dose is lower as compared to that of a method comprising an alternative dosing regimen wherein the subject is administered the cells in (b) or the consecutive dose without having been administered the first dose.

[0028] 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 consecutive dose. In some examples, subsequent to administration in (a) or the first dose and before said administration in (b) or the consecutive dose, or prior to administration in (a) or the first dose, the methods further include assessing a serum level of a factor indicative of CRS, a factor indicative of disease burden, 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 factor indicative of disease burden 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.

[0029] In some examples, the methods include assessing a factor indicative of disease burden prior to administration of the consecutive dose, and based on the result of the assessment, determining the consecutive 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 consecutive dose containing less than or about the same number of recombinant receptor- (e.g., CAR-) expressing cells as the number of recombinant receptor- (e.g., CAR-) expressing cells in the first dose. In some examples, if the assessment determines that the subject has minimal residual disease, the subject is administered a consecutive dose containing an increased number of receptor- (e.g., CAR-) expressing cells as compared to the first dose. In some examples, the consecutive dose comprises about the same number of recombinant receptor-expressing (e.g., CAR-expressing) cells as the number in the first dose. In some examples, the number of receptor- (e.g., CAR-) expressing cells per kilogram administered in the consecutive dose is less than or about less than or is the same or about the same as the number of receptor- (e.g., CAR-) expressing cells per kilogram administered in the first dose. In other examples, the number of receptor-expressing (e.g., CAR-expressing) cells per kilogram administered in the consecutive dose is greater than the number of receptor-expressing (e.g., CAR-expressing) cells per kilogram administered in the first dose. In some examples, the consecutive 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 receptor-expressing (e.g., CAR-expressing) cells per kilogram administered in the consecutive 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.

[0030] In some examples, the receptor-expressing (e.g., CAR-expressing) cells in the first dose expand in the subject following administration of the first dose and/or following the administration of the consecutive dose. In some examples, the expansion is evidenced by an increase in serum CRP level following the administration of the first dose and/or consecutive dose as compared to just prior to the administration. In some examples, the expansion is evidenced by an increase in a level of receptor- (e.g., CAR-) encoding nucleic acid in the serum,

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

[0031] In some examples, the first and/or consecutive 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 consecutive dose are administered in a single pharmaceutical composition comprising the cells of the consecutive dose. In other examples, the first and/or consecutive 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 consecutive dose is a split dose, where the cells of the consecutive dose are administered in a plurality of compositions, collectively comprising the cells of the consecutive dose, over a period of no more than three days.

[0032] In some examples, the methods include administering a consecutive dose of cells expressing a recombinant receptor (e.g., a chimeric antigen receptor (CAR)) to a subject previously administered a first dose of cells expressing a CAR. In some examples, the consecutive dose of cells is administered at a time point that is at least or more than about 14 days after and less than about 28 days after initiation of the first dose. In some examples, the number of receptor- (e.g., CAR-) expressing cells administered in the consecutive dose is increased as compared to the first dose.

[0033] In some examples, the number of cells administered in the first dose is between about 0.5×10^6 cells/kg body weight of the subject and 3×10^6 cells/kg, between about 0.75×10^6 cells/kg and 2.5×10^6 cells/kg or between about 1×10^6 cells/kg and 2×10^6 cells/kg, each inclusive.

[0034] In some examples, the number of cells administered in the consecutive dose of receptor- (e.g., CAR-) expressing cells is between about 2×10^6 cells per kilogram (cells/kg) body weight and about 6×10^6 cells/kg, between about 2.5×10^6 cells/kg and about 5.0×10^6 cells/kg, or between about 3.0×10^6 cells/kg and about 4.0×10^6 cells/kg, each inclusive.

[0035] In some examples, the number of receptor- (e.g., CAR-) expressing cells administered in the first dose is at or about or no more than at or about 1×10^6 per kilogram of

the subject and/or the number of receptor- (e.g., CAR-) expressing cells administered in the consecutive dose is at or about 3×10^6 per kilogram of the subject.

[0036] In some examples, the methods further include administering a chemotherapeutic agent prior to the administration in (a) or prior to the first dose and/or prior to the administration in (b) or prior to the consecutive dose. In some examples, the subject has been previously treated with a chemotherapeutic agent prior to the administration in (a). In some examples, the chemotherapeutic agent is or comprises a conditioning chemotherapy, which reduces burden of the disease or condition in the subject prior to the first dose and/or consecutive dose. In some examples, the chemotherapeutic agent is cyclophosphamide, fludarabine, and/or a combination thereof. 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 consecutive dose. In some examples, the chemotherapeutic agent is administered between 2 and 5 days prior to the administration of the first dose. In some examples, the chemotherapeutic agent is administered between 2 and 5 days prior to the administration of the second dose. In some examples, the chemotherapeutic agent is administered between 2 and 5 days prior to the administration first dose and between 2 and 5 days prior to the administration of the second dose. In some examples, the chemotherapeutic agent is administered at a dose of between at or about 1 g/m^2 of the subject and at or about 3 g/m^2 of the subject.

[0037] In some examples, the subject has received cryoreductive chemotherapy prior to the administration or the first dose. In some examples, the method further includes the administration of cryoreductive chemotherapy prior to the administration of the first dose.

[0038] Also provided are cells and compositions for use and uses of cells and compositions for treating a disease or condition in a subject, such as a tumor or cancer, where said cells contain recombinant receptor- (e.g., chimeric antigen receptor (CAR)-) expressing cells for treatment of a disease or condition in a subject or for the manufacture of a medicament for treatment of a disease or condition in a subject previously treated with recombinant receptor-expressing (e.g., CAR-expressing) cells. In some examples, the compositions or cells for use or medical uses are for use 14 to 28 days after the previous treatment. In some examples, the compositions or cells for use are formulated for administration of a consecutive dose in an amount sufficient for reduction in burden of a disease or condition in the

subject having been previously treated with the recombinant receptor-expressing (e.g., CAR-expressing) cells.

[0039] In some examples of such medical uses, the compositions or cells are for use that includes administering to a subject having the disease or condition a first dose of cells expressing the receptor (e.g., CAR). In some examples, the first dose contains no more than about 1×10^6 of the cells per kilogram body weight of the subject, no more than about 1×10^8 of the cells, and/or no more than about 1×10^8 of the cells/m² of the subject. In some examples, the compositions or cells are for use that includes administering to the subject a consecutive dose of cells expressing a receptor (e.g., CAR) at a time point that is at least or more than about 14 days after and less than about 28 days after initiation of the administration of the first dose.

[0040] In some examples, the cells for use are cells expressing a recombinant receptor (e.g., chimeric antigen receptor (CAR)) are for use in methods of treating a disease in a subject previously treated with receptor- (e.g., CAR-) expressing cells. In some examples, the cells are for use between about 14 and 28 days after the previous treatment. In some examples, the cells for use are formulated for administration of a consecutive dose in an amount sufficient for reduction in burden of a disease or condition in the subject having been previously treated with the receptor- (e.g., CAR-) expressing cells.

[0041] 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.

[0042] In some examples, the compositions or cells are for use in a method including administering to a subject having the disease or condition a first dose of cells expressing the receptor (e.g., CAR). In some examples, the first dose contains no more than about 1×10^6 of the cells per kilogram body weight of the subject, no more than about 1×10^8 of the cells, and/or no more than about 1×10^8 of the cells/m² of the subject. In some examples, the cells are for use in a method that includes administering to the subject a consecutive dose of cells expressing a receptor (e.g., CAR) at a time point that is at least or more than about 14 days after and less than about 28 days after initiation of said administration of the first dose.

[0043] In some examples, the compositions or cells for use are formulated for administration in an amount that does not induce severe CRS in the subject or does not induce

CRS in the subject. In some examples, the cells for use are formulated for administration in an amount that does not induce grade 3 or higher neurotoxicity in the subject. In some examples, the cells for use are formulated for administration in an amount that, based on clinical data, does not induce severe CRS in a majority of subjects so-treated. In some examples, the cells for use are formulated for administration in an amount that, based on clinical data, does not induce grade 3 or higher neurotoxicity in a majority of subjects so-treated.

[0044] In some examples, the use of cells expressing a receptor (e.g., chimeric antigen receptor (CAR)) are for manufacture of a medicament for the treatment of a disease or condition in a subject includes cells that are formulated and/or packaged for administration to the subject in a first and a consecutive dose. In some examples, the treatment includes administering the cells to the subject in a first and a consecutive dose, where the first dose includes no more than about 1×10^6 of the cells per kilogram body weight of the subject, no more than about 1×10^8 of the cells, and/or no more than about 1×10^8 of the cells/m² of the subject.

[0045] In some examples of any such compositions or cells for use or medical uses, the consecutive dose is for administration at a time point that is at least or more than about 14 days after and less than about 28 days after initiation of the first administration. In some examples, the consecutive dose is for administration at a time point at which the serum level in the subject of a factor indicative of cytokine release syndrome (CRS) is less than about 10 times, less than about 25 times, and/or less than about 50 times that in the subject immediately prior to said first administration. In some examples, the consecutive dose is for administration at a time point at which the subject does not exhibit grade 3 or higher neurotoxicity. In some examples, the consecutive dose is for administration at a time point at which a CRS-related outcome or symptom of neurotoxicity in the subject following said administration of said first dose has reached a peak level and begun to decline following the first administration. In some examples, the consecutive dose is for administration at a time point at which the subject does not exhibit a detectable humoral or cell-mediated immune response against the receptor (e.g., CAR) expressed by the cells of said first dose.

[0046] In some examples, the cells for use are formulated and/or packaged for administration to the subject in a first and a consecutive dose and/or the treatment includes administering the cells to the subject in a first and a consecutive dose. In some examples, the first dose contains no more than about 1×10^6 of the cells per kilogram body weight of the

subject, no more than about 1×10^8 of the cells, and/or no more than about 1×10^8 of the cells/ m^2 of the subject.

[0047] In some examples, the use includes where the first and consecutive administrations include administering the cells in one or more unit dose, each unit dose comprising about between 5×10^7 of the cells and about 5×10^8 cells, about between 5×10^7 of the cells and about 2.5×10^8 cells or about between 2.5×10^8 cells and 4×10^8 cells. In some examples, the cells are formulated in a unit dose comprising no more than about 5×10^7 cell, no more than about 1×10^8 cells, no more than about 2×10^8 of the cells, no more than about 2.5×10^8 of the cells, no more than about 3.0×10^8 of the cells or no more than about 4×10^8 of the cells.

[0048] 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 consecutive administration comprises administration of two or more unit doses. In some examples, the cells or use includes where the consecutive administration comprises administration a single unit dose.

[0049] In some examples, the cells composition for use or use includes where the disease or condition is a tumor or a cancer. In some examples, the cells or composition for use or use includes where the tumor or cancer is leukemia or lymphoma. In some examples, the composition or cells are for use in treating acute lymphoblastic leukemia. In some examples, the composition or cells are for use in treating non-Hodgkin lymphoma (NHL).

[0050] In some examples, the cells, composition, or use includes where the consecutive dose is formulated for administration of less than or about the same number of recombinant receptor-expressing (e.g., CAR-expressing) cells as the number of recombinant receptor-expressing (e.g., CAR-expressing) cells in the previous dose. In some examples, the composition containing the cells of the consecutive dose is formulated for administration of an increased number of recombinant receptor-expressing (e.g., CAR-expressing) cells as compared to the first dose or previous dose.

[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 cells expressing the recombinant

receptor, e.g., chimeric antigen receptor (CAR), for administration to the subject, packaging material, and/or a label or package insert.

[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 no more than about 1×10^8 of the cells, no more than about 5×10^7 of the cells, no more than about 1×10^6 of the cells per kg of the subject, or no more than about 5×10^5 of the cells per kg of the subject.

[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 consecutive 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 consecutive administration, said consecutive administration comprising administering one or a plurality of said unit doses to the subject. In some examples, they specify that the consecutive administration is to be carried out at a time between about 15 and about 27 days following said first administration. In some examples, the instructions specify that the consecutive administration is to be carried out at a time after which it has been determined that a serum level of a factor indicative of cytokine-release syndrome (CRS) in the subject is less than about 10 times, less than about 25 times, and/or less than about 50 times the serum level of said indicator in the subject immediately prior to said previous dose, or at a time after which it has been determined that an indicator of CRS has peaked and is declining, such as is at less than at or about 40 %, 30 %, 20 %, or 10 % of the peak value, and/or that the subject does not exhibit a detectable adaptive host immune response specific for the receptor (e.g., CAR) expressed by the cells of the previous dose. In some examples, the containers are or comprise flexible cell infusion bags. In some examples, the cells to be administered and/or in the containers or unit doses are for autologous transfer. Thus, in some examples, the cells have been derived from the subject to which they are to be administered. 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.

[0054] In some examples, the cells are primary cells, such as primary human immune cells, e.g., PBMCs, T cells, and/or NK cells. In some examples, the cells comprise CD8+ and/or CD4+ T cells. In some examples, the T cells are autologous to the subject.

Brief Description of the Drawings

[0055] **Figure 1A** shows disease responses of subjects with morphological or molecular disease (disease burden at time of treatment initiation) treated with a single infusion of varying doses of CAR-expressing T cells. MRD-CR = no minimal residual disease, complete remission; MRD+CR = minimal residual disease, complete remission; NR = not responsive

[0056] **Figure 1B** shows the presence (Y) or absence (N) of severe cytokine release syndrome (CRS) in subjects with morphological or molecular disease (disease burden at time of treatment initiation) treated with a single infusion of varying doses of CAR-expressing T cells.

[0057] **Figure 1C** shows the presence (Y) or absence (N) of severe neurotoxicity in subjects with morphological or molecular disease (disease burden at time of treatment initiation) treated with a single infusion of varying doses of CAR-expressing T cells.

[0058] **Figure 2** shows peak C reactive protein (CRP) levels in subjects exhibiting morphological or molecular disease at the time of administration of a first dose (Infusion = #1) (left-hand panel) and in subjects exhibiting morphological or molecular disease at the time of administration of a consecutive dose (Infusion = #2) (right-hand panel) of CAR-expressing T cells. Numbers and text/abbreviations (e.g., “MRD- CR”, “MRD+CR,” etc.) shown next to individual data points reflect patient numbers and disease burden following the indicated infusion. MRD+ = minimum residual disease; MRD- = no minimum residual disease; CR = complete remission.

[0059] **Figure 3A** depicts surface expression, as detected by flow cytometry, of PD-1, PD-L1, and PD-L2 on a population of T cells gated for positive surface expression of CD4 and an anti-CD19 chimeric antigen receptor (CAR) (gating strategy shown in top panel), following incubation for 24 hours under various conditions (media, K562-tCD19, K562-tROR1, aCD3/aCD28), as described in Example 8.

[0060] **Figure 3B** depicts surface expression, as detected by flow cytometry, of PD-1, PD-L1, and PD-L2 on a population of T cells gated for positive surface expression of CD4 and negative surface expression of an anti-CD19 chimeric antigen receptor (CAR) (gating strategy

CLAIMS

1. Use of a composition comprising cells expressing a chimeric antigen receptor (CAR) for manufacture of a medicament to be administered as a consecutive dose of CAR-expressing cells for treatment of a disease or condition in a subject previously treated with a first dose of CAR-expressing cells, wherein:

the composition comprising the consecutive dose is for use 14 to 28 days after the initiation of administration of the first dose of CAR-expressing cells; and

the composition comprising the consecutive dose is formulated for administration of an increased number of CAR-expressing cells as compared to the first dose and in an amount sufficient for reduction in burden of a disease or condition in the subject having been previously treated with the first dose of CAR-expressing cells.

2. The use of claim 1, wherein the first dose of CAR-expressing cells reduced tumor burden in the subject prior to use of the consecutive dose and/or the subject does not exhibit morphologic disease prior to use of the consecutive dose and/or the subject does not exhibit greater than 5% blast cells in the bone marrow prior to use of the consecutive dose.

3. The use of claim 1 or claim 2, wherein:

the first dose of CAR-expressing cells comprises no more than about 1×10^6 of the CAR-expressing cells per kilogram body weight of the subject, no more than about 1×10^8 of the CAR-expressing cells, and/or no more than about 1×10^8 of the CAR-expressing cells cells/m² of the subject; or

the first dose of CAR-expressing cells comprises between 1×10^6 and 1×10^8 CAR-expressing cells, inclusive; or no more than 2×10^6 CAR-expressing cells, no more than 5×10^6 CAR-expressing cells, no more than 1×10^7 CAR-expressing cells, no more than 5×10^7 CAR-expressing cells, or no more than 1×10^8 CAR-expressing cells.

4. Use of cells expressing a chimeric antigen receptor (CAR) in the manufacture of a medicament to be administered as a first dose of CAR-expressing cells and a consecutive dose of CAR-expressing cells for treating a disease or condition in a subject, wherein: the first dose comprises no more than about 1×10^6 of the CAR-expressing cells per kilogram body weight of

the subject, no more than about 1×10^8 of the CAR-expressing cells, and/or no more than about 1×10^8 of the CAR-expressing cells cells/m^2 of the subject; and

the consecutive dose of CAR-expressing cells is for administration at a time point that is at least or more than about 14 days after and less than about 28 days after initiation of administration of the first dose of cells, and wherein the consecutive dose of CAR-expressing cells comprises an increased number of CAR-expressing cells as compared to the first dose.

5. The use of any of claims 1-4, wherein the composition comprising cells of the consecutive dose is formulated for administration in an amount that (i) does not induce severe CRS in the subject or does not induce CRS in the subject; (ii) does not induce grade 3 or higher neurotoxicity in the subject; (iii) based on clinical data, does not induce severe CRS in a majority of subjects so-treated; and/or (iv) based on clinical data, does not induce grade 3 or higher neurotoxicity in a majority of subjects so-treated.

6. The use of any of claims 1-5, wherein the increased number is at least 2-fold, 5-fold, or 10-fold greater than the number in the first dose.

7. The use of any of claims 1-6, wherein the composition is for use in a subject in which:

- (i) the serum level in the subject of a factor indicative of cytokine release syndrome (CRS) is less than about 10 times, less than about 25 times, and/or less than about 50 times that in the subject immediately prior to administration of the first dose of CAR-expressing cells;
- (ii) the subject does not exhibit grade 3 or higher neurotoxicity;
- (iii) a CRS-related outcome or symptom of neurotoxicity in the subject following said administration of said first dose of CAR-expressing cells has reached a peak level and begun to decline following administration of the first dose of CAR-expressing cells, and/or
- (iv) the subject does not exhibit a detectable humoral or cell-mediated immune response against the CAR expressed by the cells of said first dose of cells.

8. The use of any of claims 1-7, wherein the consecutive dose is formulated for administration of a number of cells that is:

between about 2×10^6 cells per kilogram (cells/kg) body weight and about 6×10^6 cells/kg , between about 2.5×10^6 cells/kg and about 5.0×10^6 cells/kg , or between about 3.0×10^6 cells/kg and about 4.0×10^6 cells/kg , each inclusive;

between about 1.5×10^8 cells and 4.5×10^8 cells, between about 1.5×10^8 cells and 3.5×10^8 cells or between about 2×10^8 cells and 3×10^8 cells, each inclusive; or

between about 1.5×10^8 cells/m² and 4.5×10^8 cells/m², between about 1.5×10^8 cells/m² and 3.5×10^8 cells/m² or between about 2×10^8 cells/m² and 3×10^8 cells/m², each inclusive.

9. The use of any of claims 4-8, wherein: the composition comprising the first and/or consecutive dose is formulated for administration in one or more unit dose, each unit dose comprising about between 5×10^7 of the cells and about 5×10^8 cells, about between 5×10^7 of the cells and about 2.5×10^8 cells or about between 2.5×10^8 cells and 4×10^8 cells; or

the composition comprising cells of the first and/or consecutive dose is formulated in a unit dose comprising no more than about 5×10^7 cell, no more than about 1×10^8 cells, no more than about 2×10^8 of the cells, no more than about 2.5×10^8 of the cells, no more than about 3.0×10^8 of the cells or no more than about 4×10^8 of the cells;

the composition comprising the consecutive dose is formulated for administration in one or more unit dose, each unit dose comprising at least 1×10^6 CAR-expressing cells, at least 2×10^6 CAR-expressing cells; at least 3×10^6 CAR-expressing cells, at least 5×10^6 CAR-expressing cells, at least 1×10^7 CAR-expressing cells, at least 1×10^8 CAR-expressing cells or at least 1×10^9 CAR-expressing cells;

the composition comprising the first dose is formulated for administration in one or more unit dose, each unit dose comprising is between 1×10^6 and 1×10^8 CAR-expressing cells, inclusive; or the number of cells administered in the first dose is no more than 2×10^6 CAR-expressing cells, no more than 5×10^6 CAR-expressing cells, no more than 1×10^7 CAR-expressing cells, no more than 5×10^7 CAR-expressing cells, or no more than 1×10^8 CAR-expressing cells.

10. The use of any of claims 4-9, wherein the composition comprising the first and/or consecutive dose of cells is formulated for administration of a single unit dose.

11. The use of any of claims 4-9, wherein the composition comprising the first and/or consecutive dose of cells is formulated for administration of two or more unit doses.

12. The use of any of claims 1-11, wherein the disease or condition is a tumor or a cancer.

13. The use of claim 12, wherein the tumor or cancer is leukemia or lymphoma.
14. The use of claim 13, wherein the composition is for use in treating acute lymphoblastic leukemia.
15. The use of any of claims 1-14, wherein the composition is for use in treating non-Hodgkin lymphoma (NHL).
16. The use of any of claims 1-15, wherein the antigen bound by the CAR expressed by the cells in the first dose and the antigen bound by the CAR expressed by the cells in the consecutive dose is the same antigen and/or the CAR expressed by the cells in the first dose and the CAR expressed by the cells in the consecutive dose contain the same antigen-binding domain.
17. The use of any of claims 1-16, wherein the CAR expressed by the cells in the consecutive dose contains at least one immunoreactive epitope present in the CAR expressed by the cells in the first dose.
18. The use of any of claims 1-17, wherein the CAR expressed by the cells in the consecutive dose is identical to the CAR expressed by the cells in the first dose or is substantially identical to the CAR expressed by the cells in the first dose.
19. The use of any of claims 1-18, wherein the CAR expressed by the cells in the consecutive dose specifically binds to an antigen expressed by a cell or tissue of the disease or condition or associated with the disease or condition.
20. The use of any of claims 1-19, wherein the subject has been previously treated with a chemotherapeutic agent prior to administration of the first dose and/or prior to the administration of the consecutive dose.
21. The use of claim 20, wherein the chemotherapeutic agent comprises conditioning chemotherapy, which reduces burden of the disease or condition in the subject.

22. The use of claim 20 or claim 21, wherein the chemotherapeutic agent comprises an agent selected from the group consisting of cyclophosphamide and fludarabine.

23. The use of any of claims 20-22, wherein the chemotherapeutic agent comprises a combination of cyclophosphamide and fludarabine.

24. The use of any of claims 1-23, wherein the cells are or comprise T cells.

25. The use of claim 24, wherein the T cells are CD4⁺ and/or CD8⁺ T cells

26. The use of any of claims 1-25, wherein the cells of the first and/or consecutive dose are autologous to the subject to whom they are to be administered.

27. The use of any of claims 1-26, wherein the consecutive dose of CAR-expressing cells is for administration at a time point that is between 15 and 27 days after initiation of administration of the first dose of cells.

28. The use of any of claims 1-26, wherein the consecutive dose of CAR- expressing cells is for administration at a time point that is between 17 and 21 days after initiation of administration of the first dose of cells.