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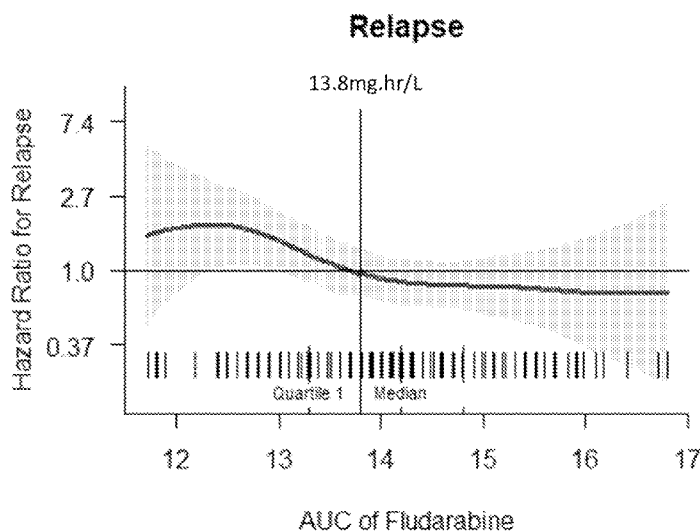


FIG. 1

(57) Abstract: The present disclosure relates to treating a subject comprising administering to the subject a therapy (e.g., a cell therapy, e.g., an adoptive cell therapy, e.g., a CAR-T cell therapy), wherein, prior to the administration, the subject has been preconditioned with a personalized amount of a chemotherapeutic agent. The personalized amount provides an optimal exposure to the chemotherapeutic agent.



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METHODS OF PERSONALIZED PRECONDITIONING FOR CELL THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to United States Provisional Application No.
5 63/161,724 filed March 16, 2021, the content of which is incorporated by reference in its entirety herein.

1. TECHNICAL FIELD

The present disclosure relates to methods for treating a subject comprising
administering to the subject a therapy (e.g., a cell therapy, e.g., an adoptive cell therapy,
10 e.g., a CAR-T cell therapy), wherein, prior to the administration, the subject has been preconditioned with a personalized amount of a chemotherapeutic agent. The personalized amount provides an optimal exposure to the chemotherapeutic agent.

2. BACKGROUND

Chimeric antigen receptor (CAR) T-cells have provided a therapeutic option for
15 patients with relapsed or refractory (R/R) B-cell acute lymphoblastic leukemia (B-ALL) who have failed standard chemotherapy approaches. Under the current medical practice, a lymphodepleting round of chemotherapy prior to CAR T-cell injections (*i.e.*, preconditioning) can increase the efficacy of the CAR T-cell treatment. By reducing tumor burden and the number of normal immune cells, chemotherapy allows CAR-T
20 cells to proliferate and overcome the cancer cells more easily.

However, the current dosing regimen of chemotherapy, which is given based on the body surface area of patients, leads to highly variable fludarabine exposures and failure to consistently improve the efficacy of CAR-T cell therapy and achieve optimal outcomes in all patients. Despite initial response, the incidence of relapse and death still
25 approaches 50% in CAR-T cell treated patients.

Accordingly, there is unmet need for improved preconditioning methods that provides optimal efficacy of CAR-T cell therapy in patients.

3. SUMMARY OF THE INVENTION

The present disclosure relates to methods for treating a subject comprising
30 administering to the subject a therapy (e.g., a cell therapy, e.g., an adoptive cell therapy, e.g., a CAR-T cell therapy), wherein, prior to the administration, the subject has been preconditioned with a personalized amount of a chemotherapeutic agent. The personalized amount provides an optimal exposure to the chemotherapeutic agent. In

certain embodiments, the optimal exposure is indicated by an area under the curve (AUC).

In certain embodiments, the present disclosure provides a method of treating a subject, comprising administering to the subject cells comprising an antigen recognizing receptor, wherein, prior to the administration, the subject has been preconditioned with a personalized amount of a chemotherapeutic agent, wherein the personalized amount provides an area under the curve (AUC) of at least about 10 mg.hr/L of the chemotherapeutic agent. In certain embodiments, the personalized amount is not greater than about 40 mg.hr/L of the chemotherapeutic agent.

In certain embodiments, the present disclosure provides a method of increasing durability of a therapy comprising cells comprising an antigen recognizing receptor in a subject, the method comprising: administering to the subject the therapy, wherein, prior to the administration, the subject has been preconditioned with a personalized amount of a chemotherapeutic agent, wherein the personalized amount provides an area under the curve (AUC) of at least about 10 mg.hr/L of the chemotherapeutic agent. In certain embodiments, the personalized amount is not greater than about 40 mg.hr/L of the chemotherapeutic agent.

In certain embodiments, the present disclosure provides a method of improving efficacy of a therapy comprising cells comprising an antigen recognizing receptor in a subject, the method comprising administering to the subject the therapy, wherein, prior to the administration, the subject has been preconditioned with a personalized amount of a chemotherapeutic agent, wherein the personalized amount provides an area under the curve (AUC) of at least about 10 mg.hr/L of the chemotherapeutic agent. In certain embodiments, the personalized amount is not greater than about 40 mg.hr/L of the chemotherapeutic agent.

In certain embodiments, the present disclosure provides a method of lowering the risk of relapse and/or lengthening survival of a subject receiving a therapy comprising cells comprising an antigen recognizing receptor, the method comprising preconditioning the subject with a personalized amount of a chemotherapeutic agent prior to the therapy, wherein the personalized amount provides an area under the curve (AUC) of at least about 10 mg.hr/L of the chemotherapeutic agent. In certain embodiments, the personalized amount is not greater than about 40 mg.hr/L of the chemotherapeutic agent.

In certain embodiments, the personalized amount of the chemotherapeutic agent provides an AUC of at least about 11 mg.hr/L, at least about 12 mg.hr/L, at least about 13 mg.hr/L, at least about 14 mg.hr/L, at least about 15 mg.hr/L, or at least about 20 mg.hr/L of the chemotherapeutic agent. In certain embodiments, the personalized amount of the chemotherapeutic agent provides an AUC of at least about 13 mg.hr/L of the chemotherapeutic agent. In certain embodiments, the personalized amount of the chemotherapeutic agent provides an AUC of about 14 mg.hr/L of the chemotherapeutic agent.

In certain embodiments, the personalized amount is determined by body weight and renal function of the subject. In certain embodiments, the renal function is determined by a glomerular filtration rate (GFR) of the subject. In certain embodiments, the GFR is determined by creatinine clearance of the subject.

In certain embodiments, the chemotherapeutic agent is an antimetabolite. In certain embodiments, the antimetabolite is selected from the group consisting of folic acid antagonists, pyrimidine analogs, purine analogs, adenosine deaminase inhibitors, lympho- or myeloid depleting antibodies (e.g., monoclonal antibodies, polyclonal antibodies, bispecific antibodies), alkylators, topoisomerase II inhibitors, derivatives thereof, and combinations thereof. In certain embodiments, the chemotherapeutic agent is an adenosine deaminase inhibitor. In certain embodiments, the adenosine deaminase inhibitor is fludarabine or a derivative thereof. In certain embodiments, the chemotherapeutic agent is a purine analog. In certain embodiments, the purine analog is clofarabine or a derivative thereof.

In certain embodiments, the subject suffers a tumor. In certain embodiments, the tumor is cancer. In certain embodiments, the tumor is blood cancer. In certain embodiments, the tumor is B-cell malignancy. In certain embodiments, the tumor is leukemia or lymphoma. In certain embodiments, the tumor is selected from the group consisting of B cell leukemia, B cell lymphoma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), non-Hodgkin's lymphoma, Burkitt lymphoma, acute myeloid leukemia (AML) and Mixed-phenotype acute leukemia (MPAL). In certain embodiments, the tumor is B cell acute-lymphoblastic leukemia (B-ALL).

In certain embodiments, the subject has a high disease burden at the time of or immediately prior to the preconditioning. In certain embodiments, the subject who has high disease burden has more than about 5% lymphoblasts in bone marrow, detectable

peripheral blood lymphoblasts in the subject, a CNS3 status, and/or non-CNS extramedullary (EM) site of disease. In certain embodiments, the CNS3 status is determined by detecting at least about 5 white blood cells/ml cerebrospinal fluid and at least one lymphoblast in the cerebrospinal fluid.

5 In certain embodiments, the antigen-recognizing receptor is a T cell receptor (TCR) or a chimeric antigen receptor (CAR). In certain embodiments, the antigen-recognizing receptor is a CAR. In certain embodiments, the CAR comprises an extracellular antigen-recognition domain and an intracellular signaling domain. In certain embodiments, the extracellular antigen-binding domain binds to an antigen. In
10 certain embodiments, the antigen is a tumor antigen or a pathogen antigen. In certain embodiments, the antigen is CD19. In certain embodiments, the intracellular signaling domain comprises a CD3 ζ polypeptide. In certain embodiments, the intracellular signaling domain of the CAR further comprises at least one co-stimulatory signaling region. In certain embodiments, the costimulatory signaling region comprises an
15 intracellular domain of 4-1BB or an intracellular domain of CD28.

In certain embodiments, the cell is an immunoresponsive cell. In certain embodiments, the cell is a cell of the lymphoid lineage or a cell of the myeloid lineage. In certain embodiments, the cell is selected from the group consisting of T cells, Natural Killer (NK) cells, stem cells from which lymphoid cells may be differentiated. In certain
20 embodiments, the cell is a T cell. In certain embodiments, the T cell is selected from the group consisting of a cytotoxic T lymphocyte (CTL), a $\gamma\delta$ T cell, a tumor-reactive lymphocyte, a tumor-infiltrating lymphocyte (TIL), a regulatory T cell, and a Natural Killer T (NKT) cell.

4. BRIEF DESCRIPTION OF THE DRAWINGS

25 Figure 1 shows the analysis of the impact of fludarabine AUC on cumulative incidence of relapse (CIR).

Figures 2A and 2B show the comparison between patient who had fludarabine AUC of ≥ 13.8 mg.hr/L, and patients who had fludarabine AUC of ≤ 13.8 mg.hr/L. Cumulative Incidence of Relapse (Figure 2A) and Composite Endpoint (Loss of BCA or
30 Relapse) (Figure 2B) were measured.

Figures 3A-3B show the comparison between patient who had a fludarabine AUC of ≥ 13.8 mg.hr/L, and patients who had a fludarabine AUC of ≤ 13.8 mg.hr/L. All patients had high pre-treatment disease burden. Overall Survival (Figure 3A) and

Cumulative Incidence of Relapse (Figure 3B) in patient having high pre-treatment disease burden were measured.

Figure 4 shows the patient flow diagram of the study design described in Example 2.

Figures 5A-5D show graphs reporting the impact of fludarabine exposure and pre-infusion disease burden on outcomes after tisagenlecleucel in responding patients. Patients were cohorted based on fludarabine exposure (optimal vs sub-optimal) and pre-infusion disease burden (high vs low/no). Figure 5A shows cumulative incidence of relapse of the cohorted responders to tisagenlecleucel. Figure 5B shows cumulative incidence of composite endpoint of the cohorted responders to tisagenlecleucel. Figure 5C shows OS of all responding patients after tisagenlecleucel. Figure 5D shows the impact of fludarabine exposure and pre-infusion disease burden on overall survival in all treated patients after tisagenlecleucel infusion.

15 5. DETAILED DESCRIPTION

The present disclosure relates to methods of treating a subject, wherein the method comprises administering to the subject a therapy, and prior to the administration, the subject has been preconditioned with a personalized amount of a chemotherapeutic agent. The preconditioning with the personalized amount of the chemotherapeutic agent can improve the efficacy of the therapy. The therapy can be a cell therapy, *e.g.*, an adoptive cell therapy, *e.g.*, a therapy comprising cells (*e.g.*, T cells) comprising an antigen-recognizing receptor (*e.g.*, a chimeric antigen receptor (CAR) or a T cell receptor (TCR)). In certain embodiments, the chemotherapeutic agent comprises fludarabine. The present disclosure is at least based on the discovery that patients who have been preconditioned with an optimal fludarabine exposure (as indicated by an area under the curve (AUC)) achieved improved efficacy of a CAR-T cell therapy, such as reduced relapse, maintained B cell aplasia, increased durability of the CAR T-cell therapy, and increased survival. The beneficial effects of the optimal fludarabine exposure was also observed in patients who had high disease burden prior to the CAR T-cell therapy.

30 Non-limiting embodiments of the present disclosure are described by the present specification and Examples.

For purposes of clarity of disclosure and not by way of limitation, the detailed description is divided into the following subsections:

- 5.1. Definitions;
- 5.2. Methods of Treatment;
- 5.3. Chemotherapeutic Agents;
- 5.4. Therapy; and
- 5.5 Administration.

5.1. *Definitions*

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art. The following references provide one of skill with a general definition of many of the terms used in the presently disclosed subject matter: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991).

As used herein, the use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” Still further, the terms “having,” “including,” “containing” and “comprising” are interchangeable and one of skill in the art is cognizant that these terms are open ended terms.

The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” can mean within 3 or more than 3 standard deviations, per the practice in the art. Alternatively, “about” can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value.

By “immunoresponsive cell” is meant a cell that functions in an immune response or a progenitor, or progeny thereof. In certain embodiments, the immunoresponsive cell is a cell of lymphoid lineage. Non-limiting examples of cells of

lymphoid lineage include T cells, Natural Killer (NK) cells, B cells, and stem cells from which lymphoid cells may be differentiated. In certain embodiments, the immunoresponsive cell is a cell of myeloid lineage.

5 The term “antigen-recognizing receptor” as used herein refers to a receptor that is capable of activating an immunoresponsive cell (*e.g.*, a T cell) in response to its binding to an antigen.

10 The term “chimeric antigen receptor” or “CAR” as used herein refers to a molecule comprising an extracellular antigen-binding domain that is fused to an intracellular signaling domain that is capable of activating an immunoresponsive cell, and a transmembrane domain. In certain embodiments, the extracellular antigen-binding domain of a CAR comprises a single chain variable fragment (scFv). The scFv can be derived from fusing the variable heavy and light regions of an antibody. Alternatively or additionally, the scFv may be derived from Fab’s (instead of from an antibody, *e.g.*, obtained from Fab libraries). In certain embodiments, the scFv is fused to the
15 transmembrane domain and then to the intracellular signaling domain.

An “effective amount” is an amount sufficient to affect a beneficial or desired clinical result upon treatment. An effective amount can be administered to a subject in one or more doses. In certain embodiments, an effective amount can be an amount that is sufficient to palliate, ameliorate, stabilize, reverse or slow the progression of the
20 disease, or otherwise reduce the pathological consequences of the disease. The effective amount can be determined by a physician on a case-by-case basis and is within the skill of one in the art. Several factors are typically taken into account when determining an appropriate dosage to achieve an effective amount. These factors include age, sex and weight of the subject, the condition being treated, the severity of the condition and the
25 form and effective concentration of the cells administered.

By “increase” is meant to alter positively by at least about 5%. An alteration may be by about 5%, about 10%, about 25%, about 30%, about 50%, about 75%, about 100% or more.

30 By “reduce” is meant to alter negatively by at least about 5%. An alteration may be by about 5%, about 10%, about 25%, about 30%, about 50%, about 75%, or even by about 100%.

The term “antigen-binding domain” as used herein refers to a domain capable of specifically binding a particular antigenic determinant or set of antigenic determinants present on a cell.

5 The terms “comprises”, “comprising”, and are intended to have the broad meaning ascribed to them in U.S. Patent Law and can mean “includes”, “including” and the like.

As used herein, “treatment” refers to clinical intervention in an attempt to alter the disease course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Therapeutic effects of treatment
10 include, without limitation, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastases, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. By preventing progression of a disease or disorder, a treatment can prevent deterioration due to a
15 disorder in an affected or diagnosed subject or a subject suspected of having the disorder, but also a treatment may prevent the onset of the disorder or a symptom of the disorder in a subject at risk for the disorder or suspected of having the disorder.

An “individual” or “subject” herein is a vertebrate, such as a human or non-human animal, for example, a mammal. Mammals include, but are not limited to,
20 humans, primates, farm animals, sport animals, rodents and pets. Non-limiting examples of non-human animal subjects include rodents such as mice, rats, hamsters, and guinea pigs; rabbits; dogs; cats; sheep; pigs; goats; cattle; horses; and non-human primates such as apes and monkeys. The term “immunocompromised” as used herein refers to a subject who has an immunodeficiency. The subject is very vulnerable to opportunistic infections,
25 infections caused by organisms that usually do not cause disease in a person with a healthy immune system but can affect people with a poorly functioning or suppressed immune system.

5.2. *Methods of Treatment*

The present disclosure provides methods for treating a subject, for improving the
30 efficacy of a therapy, for improving the durability of a therapy, and for maintaining B-cell aplasia in a subject receiving a therapy. In certain embodiments, the therapy is an

adoptive cell therapy. In certain embodiments, the methods disclosed herein comprise administering to the subject a therapy (e.g., an adoptive cell therapy), wherein, prior to the administration, the subject has been preconditioned with a personalized amount of a chemotherapeutic agent.

5 B-cell aplasia, defined as “low numbers of B cells or absent B cells”, can be measured for its ability to predict treatment efficacy and persistence for an adoptive cell therapy (in setting of CD19 targeted therapy), e.g., a CAR-T cell therapy. In CD19 CAR T cell trials, B cell aplasia has been shown to be a treatable and tolerable toxicity. Furthermore, B cell aplasia can be a marker of CAR T cell efficacy and persistence, and
10 thus an indicator of treatment success and risk of relapse (Maude et al., Blood (2015);125(26):4017–23). In certain embodiments, the B-cell aplasia indicates the existence of durable functional CAR T-cells in the subject. In certain embodiments, the B-cell aplasia indicates less than about 1% B cells of peripheral blood mononuclear cells of a subject. In certain embodiments, the B-cell aplasia indicates less than about 1%
15 CD19⁺CD20⁺ B cells of peripheral blood mononuclear cells of a subject.

In certain embodiments, the presence and/or persistence of CAR-T cells is measured by antibodies that binds to the antigen to which the CAR binds.

In certain embodiments, the present disclosure provides methods for treating and/or preventing a tumor in a subject. In certain embodiments, the present disclosure
20 provides methods for reducing disease burden in a subject having a tumor. In certain embodiments, the methods disclosed herein comprise administering to the subject a therapy, wherein, prior to the administration, the subject has been preconditioned with a personalized amount of a chemotherapeutic agent. In certain embodiments, the therapy is an adoptive cell therapy.

25 In certain embodiments, reducing the disease burden comprises reducing a total number of tumor cells in an organ, tissue, or bodily fluid of the subject, such as the organ or tissue of the tumor or another location, e.g., which would indicate metastasis. In certain embodiments, reducing the disease burden comprises reducing the total number of tumor cells detected and/or quantified in the blood or bone marrow in the context of
30 certain hematological malignancies. In certain embodiments, reducing the disease burden comprises reducing the number of tumor cells, reducing tumor size, reducing tumor mass, and/or reducing the number or extent of metastases. In certain embodiments, the subject has leukemia, and reducing the disease burden comprises

reducing residual leukemia in blood or bone marrow (and cerebral spinal fluid) and/or reducing the percentage of lymphoblast cells present in bone marrow.

In certain embodiments, the present disclosure provides methods for lowering the risk of relapse, lengthening survival, and/or promoting event (e.g., cancer)-free survival in a subject receiving a therapy. In certain embodiments, the methods disclosed herein comprise administering to the subject the therapy, wherein, prior to the administration, the subject has been preconditioned with a chemotherapeutic agent in a personalized amount. In certain embodiments, the therapy is an adoptive cell therapy.

Non-limiting examples of tumors include blood cancer, B cell leukemia, B cell lymphoma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), multiple myeloma, lymphoma (Hodgkin's lymphoma, non-Hodgkin's lymphoma), glioblastoma, myelodysplastic syndrome (MDS), and chronic myelogenous leukemia (CML), bone cancer, intestinal cancer, liver cancer, skin cancer, cancer of the head or neck, melanoma (cutaneous or intraocular malignant melanoma), renal cancer (*e.g.* clear cell carcinoma), throat cancer, prostate cancer (*e.g.* hormone refractory prostate adenocarcinoma), blood cancers (*e.g.* leukemias, lymphomas, and myelomas), uterine cancer, rectal cancer, cancer of the anal region, bladder cancer, brain cancer, stomach cancer, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, leukemias (*e.g.*, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, , polycythemia vera, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, include Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as sarcomas and carcinomas (*e.g.*, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma,

lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, hepatoma, nile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, salivary gland cancer, uterine cancer, testicular cancer, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma). In certain embodiments, the tumor is a cancer.

In certain embodiments, the tumor is blood cancer. In certain embodiments, the tumor is leukemia or lymphoma. In certain embodiments, the tumor is selected from the group consisting of B cell leukemia, B cell lymphoma, acute lymphoblastic leukemia (ALL) (*e.g.*, T cell acute lymphoblastic leukemia (T-ALL), B cell acute lymphoblastic leukemia (B-ALL)), chronic lymphocytic leukemia (CLL) (*e.g.*, T cell chronic lymphocytic leukemia (T-CLL), B cell chronic lymphocytic leukemia (B-CLL)), multiple myeloma, non-Hodgkin's lymphoma, Burkitt lymphoma, acute myeloid leukemia (AML), and Mixed-phenotype acute leukemia (MPAL). In certain embodiments, the tumor is B cell acute lymphoblastic leukemia (B-ALL).

In certain embodiments, the present disclosure provides methods for treating or preventing an autoimmune disease. Non-limiting examples of autoimmune disease include Systemic lupus erythematosus (SLE) Rheumatoid arthritis (RA), Juvenile Rheumatoid Arthritis (JIA). In certain embodiments, the present disclosure provides methods for treating or preventing an allo-immune disease, *e.g.*, Graft Versus Host Disease. In certain embodiments, the present disclosure provides methods for treating or preventing an infectious disease.

In certain embodiments, the subject has a high disease burden at the time of or immediately prior to the preconditioning. In certain embodiments, the subject who has a high disease burden has more than about 5% lymphoblasts in bone marrow. In certain embodiments, the subject who has a high disease burden has detectable peripheral blood lymphoblasts. In certain embodiments, the subject who has a high disease burden has a CNS3 status. In certain embodiments, the CNS3 status refers to the detection of at least

about five (5) white blood cells/ml cerebrospinal fluid and at least one lymphoblast in the cerebrospinal fluid. In certain embodiments, the subject who has a high disease burden has non-CNS extramedullary (EM) site of disease.

5.2.1. Preconditioning with a Chemotherapeutic Agent

5 In certain embodiments, the subject, prior to the administration of the therapy, has been preconditioned with a personalized amount of a chemotherapeutic agent. The presently disclosed personalized amount of the chemotherapeutic agent provides an optimal exposure of the chemotherapeutic agent to the subject. In certain embodiments, the optimal exposure of the chemotherapeutic agent is indicated by an area under the
10 curve (AUC) of the chemotherapeutic agent in the subject.

In certain embodiments, the personalized amount of the chemotherapeutic agent provides an AUC of at least about 10 mg.hr/L. In certain embodiments, the personalized amount of the chemotherapeutic agent provides an AUC of at least about 11 mg.hr/L, at least about 12 mg.hr/L, at least about 13 mg.hr/L, at least about 14 mg.hr/L,
15 at least about 15 mg.hr/L, at least about 16 mg.hr/L, at least about 17 mg.hr/L, at least about 18 mg.hr/L, at least about 19 mg.hr/L, at least about 20 mg.hr/L, at least about 25 mg.hr/L, at least about 30 mg.hr/L, at least about 35 mg.hr/L, or at least about 40 mg.hr/L,. In certain embodiments, the personalized amount of the chemotherapeutic agent provides an AUC of no greater than about 40 mg.hr/L, no greater than about 35
20 mg.hr/L, no greater than about 30 mg.hr/L, no greater than about 25 mg.hr/L, or no greater than about 20 mg.hr/L. In certain embodiments, the personalized amount of the chemotherapeutic agent provides an AUC of between about 10 mg.hr/L and about 40 mg.hr/L.

In certain embodiments, the personalized amount of the chemotherapeutic agent
25 provides an AUC of at least about 12.0 mg.hr/L, at least about 12.5 mg.hr/L, at least about 13.0 mg.hr/L, at least about 13.5 mg.hr/L, at least about 14.0 mg.hr/L, at least about 14.5 mg.hr/L, or at least about 15.0 mg.hr/L. In certain embodiments, the personalized amount of the chemotherapeutic agent provides an AUC of between about 12.0 mg.hr/L and about 15.0 mg.hr/L, e.g., between about 12.0 mg.hr/L and about 14.0
30 mg.hr/L, between about 13.0 mg.hr/L and about 15.0 mg.hr/L, between about 13.0 mg.hr/L and about 14.0 mg.hr/L, or between about 13.5 mg.hr/L and about 14.0 mg.hr/L. In certain embodiments, the personalized amount of the chemotherapeutic agent provides an AUC of at least about 14 mg.hr/L. In certain embodiments, the personalized amount

of the chemotherapeutic agent provides an AUC of about 14 mg.hr/L. In certain
embodiments, the personalized amount of the chemotherapeutic agent provides an AUC
of at least about 13.5 mg.hr/L. In certain embodiments, the personalized amount of the
chemotherapeutic agent provides an AUC of about 13.5 mg.hr/L. In certain
5 embodiments, the personalized amount of the chemotherapeutic agent provides an AUC
of at least 13.8 mg.hr/L. In certain embodiments, the personalized amount of the
chemotherapeutic agent provides an AUC of 13.8 mg.hr/L.

In certain embodiments, the chemotherapeutic agent is fludarabine. In certain
embodiments, the personalized amount of fludarabine provides an AUC of at least about
10 14 mg.hr/L. In certain embodiments, the personalized amount of fludarabine provides an
AUC of about 14 mg.hr/L. In certain embodiments, the personalized amount of
fludarabine provides an AUC of at least about 13.5 mg.hr/L. In certain embodiments,
the personalized amount of fludarabine provides an AUC of about 13.5 mg.hr/L. In
certain embodiments, the personalized amount of fludarabine provides an AUC of at
15 least 13.8 mg.hr/L. In certain embodiments, the personalized amount of fludarabine
provides an AUC of 13.8 mg.hr/L.

In certain embodiments, the personalized amount of the chemotherapeutic agent
is determined by body weight and renal function of the subject. In certain embodiments,
the renal function is indicated by a glomerular filtration rate (GFR). In certain
20 embodiments, the GFR is an estimated glomerular filtration rate (GFR). In certain
embodiments, the GFR or eGFR is indicated by creatinine clearance of the subject. In
certain embodiments, the creatinine clearance of the subject is calculated using
Cockcroft–Gault equation as disclosed in Cockcroft *et al.*, *Nephron*. 1976;16(1):31–41,
the contents of which are incorporated herein by reference in its entirety. In certain
25 embodiments, the creatinine clearance of the subject is calculated using the following
formula:

$$\text{Creatine Clearance (male)} = ([140 - \text{age}] \times \text{weight in kg}) / (\text{serum creatinine} \times 72)$$

$$\text{Creatine Clearance (female)} = \text{Creatine Clearance (male)} \times 0.85$$

In certain embodiments, the GFR is estimated using Cystatin-C. In certain
30 embodiments, the GFR is estimated by performing a nuclear scan.

In certain embodiments, the personalized amount of the chemotherapeutic agent
is determined by a population pharmacokinetics model of the chemotherapeutic agent.
In certain embodiments, the population pharmacokinetics model is a three-compartment

model. In certain embodiments, the population pharmacokinetics model is a one-compartment model. In certain embodiments, the population pharmacokinetics model is a two-compartment model.

In certain embodiments, the population pharmacokinetics model is a three-compartment model, and the chemotherapeutic agent is fludarabine.

In certain embodiments, the three-compartment model is parameterized in terms of volume of distribution of the central (V1), peripheral (V2), and second peripheral (V3) compartment, and clearance from the central compartment as well as intercompartmental clearance between V1 and V2 (Q2) and V1 and V3 (Q3).

In certain embodiments, the chemotherapeutic agent is fludarabine. In certain embodiments, the personalized amount of the chemotherapeutic agent is determined by a population pharmacokinetics model disclosed in Langenhorst et al., *Clinical Pharmacokinetics* (2019) 58:627-637.

Structural model

$$Cl = (Cl_{70kg-non-renal} + eGFR \times Slope_{pop}) \times \left(\frac{BW}{70kg} \right)^{0.75}$$

Preconditioning the subject with the personalized amount of the chemotherapeutic agent, prior to the administration of the therapy, can improve the efficacy of the therapy, improve the responsiveness of the subject to the therapy, and improve the durability of the therapy. In certain embodiments, the subject has B cell malignancy, and the preconditioning maintains B-cell aplasia of the subject.

5.3. Chemotherapeutic Agents

Any suitable chemotherapeutic agents known in the art can be used in the precondition regimen of the presently disclosed methods. Non-limiting suitable examples of chemotherapeutic agents include antimetabolite, alkylating agents, anthracyclines (*e.g.*, doxorubicin and liposomal doxorubicin), vinca alkaloids (*e.g.*, vinblastine, vincristine, vindesine, and vinorelbine), immune cell antibodies (*e.g.*, alemtuzumab, gemtuzumab, rituximab, ofatumumab, tocitumomab, brentuximab: see below also ATG), mTOR inhibitors (*e.g.*, rapamycin, rapamycin analogs, sirolimus, temsirolimus, everolimus, ridaforolimus, umirolimus, zotarolimus), TNFR glucocorticoid induced TNFR related protein (GITR) agonists (*e.g.*, TRX518), proteasome inhibitors (*e.g.*, aclacinomycin A, gliotoxin or bortezomib),

immunomodulators such as thalidomide or thalidomide derivatives (*e.g.*, lenalidomide), cytokine antagonists (*e.g.* IL1 blockade such as Anakinra), derivatives thereof, and combinations thereof.

5 In certain embodiments, the chemotherapeutic agent is an antimetabolite. In certain embodiments, the antimetabolite is selected from the group consisting of folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors, lympho- or myeloid depleting antibodies (*e.g.*, monoclonal antibodies, polyclonal antibodies, or bispecific antibodies), alkylators, topoisomerase II inhibitors, derivatives thereof, and combinations thereof.

10 In certain embodiments, the chemotherapeutic agent is a purine analog. In certain embodiments, the purine analog is selected from the group consisting of clofarabine, 6-mercaptopurine and 6-thioguanine, derivatives thereof, and combinations thereof. In certain embodiments, the chemotherapeutic agent is clofarabine or a derivative thereof.

15 In certain embodiments, the chemotherapeutic agent is an adenosine deaminase inhibitor. In certain embodiments, the adenosine deaminase inhibitor is selected from the group consisting of fludarabine, cladribine, pentostatin, derivatives thereof, and combinations thereof. In certain embodiments, the chemotherapeutic agent is fludarabine or a derivative thereof.

20 In certain embodiments, the chemotherapeutic agent is a folic acid antagonist. In certain embodiments, the folic acid antagonist is selected from the group consisting of methotrexate, derivatives thereof, and combinations thereof.

In certain embodiments, the chemotherapeutic agent is a pyrimidine analog. In certain embodiments, the pyrimidine analog is selected from the group consisting of 5-
25 fluorouracil, floxuridine, cytarabine, capecitabine, and gemcitabine, derivatives thereof, and combinations thereof.

In certain embodiments, the chemotherapeutic agent is an alkylating agent. In certain embodiments, the alkylating agent is selected from the group consisting of cyclophosphamide, decarbazine, melphalan, ifosfamide, temozolomide, treosulfan,
30 thiothepa, busulfan, derivatives thereof, and combinations thereof. In certain embodiments, the chemotherapeutic agent is cyclophosphamide or a derivative thereof.

In certain embodiments, the chemotherapeutic agent is an antibody. Non-limiting examples of antibodies include Alemtuzumab (Anti-CD52), rabbit ATG

(Thymoglobuline), rabbit ATLG (anti-thymocyte lymphoglobuline), anti-CD30 (Brentuximab).

5.4. Therapy

In certain embodiments, the therapy is an immunotherapy. In certain
 5 embodiments, the therapy is a cell therapy. In certain embodiments, the therapy is an
 adoptive cell therapy. In certain embodiments, the therapy cells comprising an antigen-
 recognizing receptor. In certain embodiments, the antigen-recognizing receptors binds to
 an antigen. In certain embodiments, the antigen-recognizing receptor is a chimeric
 antigen receptor (CAR). In certain embodiments, the antigen-recognizing receptor is a
 10 T-cell receptor (TCR). The antigen-recognizing receptor can bind to a tumor antigen or
 a pathogen antigen. In certain embodiments, the antigen-recognizing receptor binds to a
 tumor antigen. In certain embodiments, the tumor antigen is a tumor-specific antigen or a
 tumor-associated antigen.

5.4.1 Antigens

15 In certain embodiments, the antigen-recognizing receptor binds to a tumor
 antigen. Any tumor antigen (antigenic peptide) can be used in the tumor-related
 embodiments described herein. Sources of antigen include, but are not limited to, cancer
 proteins. The antigen can be expressed as a peptide or as an intact protein or portion
 thereof. The intact protein or a portion thereof can be native or mutagenized. In certain
 20 embodiments, the tumor antigen is a tumor specific antigen (TSA). In certain
 embodiment, the tumor antigen is a tumor-associated antigen (TAA).

Non-limiting examples of tumor antigens include CD19, MUC16, MUC1, CAIX,
 CEA, CD8, CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41,
 CD44, CD49f, CD56, CD74, CD123, CD133, CD138, EGP-2, EGP-40, EpCAM, Erb-
 25 B2, Erb-B3, Erb-B4, FBP, Fetal acetylcholine receptor, folate receptor-a, GD2, GD3,
 HER-2, hTERT, IL-13R- α 2, κ -light chain, KDR, mutant KRAS (including, but not
 limited to, G12V, G12D, G12C), mutant HRAS, mutant PIK3CA (including, but not
 limited to, E52K, E545K, H1047R, H1047L), mutant IDH (including, but not limited to,
 R132H), mutant p53 (including, but not limited to, R175H, Y220C, G245D, G245S,
 30 R248L, R248Q, R248W, R249S, R273C, R273L, R273H and R282W), mutant NRAS
 (including, but not limited to, Q61R, Q61K, and Q61L), LeY, L1 cell adhesion molecule,

MAGE-A1, Mesothelin, ERBB2, MAGEA3, CT83 (also known as KK-LC-1), p53, MART1, GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ESO-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, CD99, CD70, ADGRE2, CCR1, LILRB2, PRAME, HPV E6 oncoprotein, HPV E7 oncoprotein, and ERBB. In certain embodiments, the tumor antigen is CD19.

In certain embodiments, the antigen-recognizing receptor binds to a human CD19 polypeptide. In certain embodiments, the antigen-recognizing receptor binds to the extracellular domain of a human CD19 protein.

10 5.4.2 T-cell receptor (TCR)

In certain embodiments, the antigen-recognizing receptor is a TCR. A TCR is a disulfide-linked heterodimeric protein consisting of two variable chains expressed as part of a complex with the invariant CD3 chain molecules. A TCR is found on the surface of T cells, and is responsible for recognizing antigens as peptides bound to major histocompatibility complex (MHC) molecules. In certain embodiments, a TCR comprises an alpha chain and a beta chain (encoded by TRA and TRB, respectively). In certain embodiments, a TCR comprises a gamma chain and a delta chain (encoded by TRG and TRD, respectively).

Each chain of a TCR is composed of two extracellular domains comprising a Variable (V) region and a Constant (C) region. The Constant region is proximal to the cell membrane, followed by a transmembrane region and a short cytoplasmic tail that lacks the ability to transduce a signal. The Variable region binds to the peptide/MHC complex. The variable domain of each pair (alpha/beta or gamma/delta) of TCR polypeptides comprises three complementarity determining regions (CDRs).

25 In certain embodiments, a TCR can form a receptor complex with three dimeric signaling modules CD3 δ / ϵ , CD3 γ / ϵ and CD3 ζ / ζ or ζ / η . When a TCR complex engages with its antigen and MHC (peptide/MHC), the T cell expressing the TCR complex is activated.

In certain embodiments, the TCR recognizes a tumor antigen (including a TAA or TSA). In certain embodiments, the TCR is expressed in a tumor-specific T cell. In certain embodiments, the tumor-specific T cell is a tumor-infiltrating T cell generated by culturing T cells with explants of a tumor, e.g., melanoma or an epithelial cancer. In certain embodiments, the tumor-specific T cell is a T cell disclosed in Stevanovic *et al*,

Science, 356, 200-205, 2017; Dudley *et al.* *Journal of Immunotherapy*, 26(4): 332–342, 2003; or Goff *et al.* *Journal of Clinical Oncology*, Vol. 34, No. 20, 2016, each of which is incorporated by reference in its entirety.

In certain embodiments, the antigen-recognizing receptor is a recombinant TCR.

5 In certain embodiments, the recombinant TCR differs from any naturally occurring TCR by at least one amino acid residue. In certain embodiments, the recombinant TCR differs from any naturally occurring TCR by at least about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 20, about 25, about 30, about 40, about 50, about 60, about 70, about 80, about 90,
10 about 100 or more amino acid residues. In certain embodiments, the recombinant TCR is modified from a naturally occurring TCR by at least one amino acid residue. In certain embodiments, the recombinant TCR is modified from a naturally occurring TCR by at least about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 20, about 25, about 30, about 40,
15 about 50, about 60, about 70, about 80, about 90, about 100 or more amino acid residues.

5.4.3 Chimeric Antigen Receptor (CAR)

In certain embodiments, the antigen-recognizing receptor is a CAR. CARs are engineered receptors, which graft or confer a specificity of interest onto an immune
20 effector cell or immunoresponsive cell. CARs can be used to confer non-MHC-restricted antigen specificity onto a T cell. Transfer of their coding sequence facilitated by retroviral vectors.

CARs have developed via a series of significant improvements referred to as “generations”. So-called “first generation” CARs are typically composed of an
25 extracellular antigen-binding domain (*e.g.*, a single chain variable fragment (scFv)), which is fused to a transmembrane domain, which is fused to a cytoplasmic/intracellular signaling domain. The cytoplasmic/intracellular signaling domain can comprise a single activating domain – usually an ITAM derived from CD3zeta. “First generation” CARs can provide *de novo* antigen recognition and cause activation of both CD4⁺ and CD8⁺ T
30 cells through their CD3ζ chain signaling domain in a single fusion molecule, independent of HLA-mediated antigen presentation. “Second generation” CARs add an intracellular signaling domain derived from any one of the various co-stimulatory molecules (*e.g.*, CD28, 4-1BB, ICOS, OX40) to the cytoplasmic tail of the CAR to

provide additional signals to the T cell. “Second generation” CARs comprise those that provide both co-stimulation (*e.g.*, CD28 or 4-1BB) and activation (CD3 ζ). “Third generation” CARs comprise those that provide multiple co-stimulation (*e.g.*, CD28 and 4-1BB) and activation (CD3 ζ). In certain embodiments, the antigen-recognizing receptor is a first-generation CAR. In certain embodiments, the antigen-recognizing receptor is a second-generation CAR. In certain embodiments, the antigen-recognizing receptor is a third-generation CAR.

In accordance with the presently disclosed subject matter, a CAR comprises an extracellular antigen-binding domain, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen-binding domain specifically binds to an antigen, which can be a tumor antigen (TAA or TSA).

In certain embodiments, the CAR comprises an extracellular antigen-binding domain that binds to CD19. In certain embodiments, the CAR is CTL019 (also known as tisagenlecleucel).

5.4.3.1 Extracellular Antigen-Binding Domain of A CAR

In certain embodiments, the extracellular antigen-binding domain specifically binds to an antigen. In certain embodiments, the antigen is a tumor antigen. In certain embodiments, the tumor antigen is a tumor specific antigen (TSA). In certain embodiments, the tumor antigen is a tumor-associated antigen (TAA). In certain embodiments, the tumor antigen is CD19.

In certain embodiments, the extracellular antigen-binding domain is an scFv. In certain embodiments, the scFv is a human scFv. In certain embodiments, the scFv is a humanized scFv. In certain embodiments, the scFv is a murine scFv. In certain embodiments, the extracellular antigen-binding domain is a Fab, which is optionally crosslinked. In certain embodiments, the extracellular antigen-binding domain is a F(ab)₂. In certain embodiments, any of the foregoing molecules may be comprised in a fusion protein with a heterologous sequence to form the extracellular antigen-binding domain. In certain embodiments, the scFv is identified by screening scFv phage library with an antigen-Fc fusion protein.

5.4.3.2 Transmembrane Domain of a CAR

In certain embodiments, the transmembrane domain of the CAR comprises a hydrophobic alpha helix that spans at least a portion of the membrane. Different transmembrane domains result in different receptor stability. After antigen recognition,

receptors cluster and a signal are transduced to the cell. In accordance with the presently disclosed subject matter, the transmembrane domain of the CAR can comprise a CD8 polypeptide (e.g., a transmembrane domain of CD8), a CD28 polypeptide (e.g., a transmembrane domain of CD28), a CD3 ζ polypeptide (e.g., a transmembrane domain of CD3 ζ), a CD4 polypeptide (e.g., a transmembrane domain of CD4), a 4-1BB polypeptide (e.g., a transmembrane domain of 4-1BB), an OX40 polypeptide (e.g., a transmembrane domain of OX40), an ICOS polypeptide (e.g., a transmembrane domain of ICOS), a synthetic peptide, or a combination thereof. In certain embodiments, the transmembrane domain of the CAR comprises a CD8 polypeptide (e.g., a transmembrane domain of CD8).

In certain embodiments, the CAR further comprises a spacer region that links the extracellular antigen-binding domain to the transmembrane domain. The spacer region can be flexible enough to allow the antigen binding domain to orient in different directions to facilitate antigen recognition. The spacer region can be the hinge region from IgG1, or the CH₂CH₃ region of immunoglobulin and portions of CD3, a portion of a CD28 polypeptide, a portion of a CD8 polypeptide, or a synthetic spacer sequence. In certain embodiments, the spacer region is derived from a CD8 polypeptide.

5.4.3.3 Intracellular Signaling Domain of a CAR

In certain embodiments, the intracellular signaling domain of the CAR comprises a CD3 ζ polypeptide, which can activate or stimulate a cell (e.g., a cell of the lymphoid lineage, e.g., a T cell). Wild type ("native") CD3 ζ comprises three immunoreceptor tyrosine-based activation motifs ("ITAMs") (e.g., ITAM1, ITAM2 and ITAM3), and transduces an activation signal to the cell (e.g., a cell of the lymphoid lineage, e.g., a T cell) after antigen is bound. The intracellular signaling domain of the native CD3 ζ polypeptide is the primary transmitter of signals from endogenous TCRs.

In certain embodiments, the intracellular signaling domain of the CAR comprises a native CD3 ζ polypeptide. In certain embodiments, the intracellular signaling domain of the CAR comprises a human CD3 ζ polypeptide. In certain embodiments, the intracellular signaling domain of the CAR comprises a murine CD3 ζ polypeptide. In certain embodiments, the intracellular signaling domain of the CAR comprises a modified CD3 ζ polypeptide. In certain embodiments, the intracellular signaling domain of the CAR comprises a modified human or modified murine CD3 ζ polypeptide.

In certain embodiments, the intracellular signaling domain of the CAR does not comprise a co-stimulatory signaling region, *i.e.*, the CAR is a first-generation CAR.

In certain embodiments, the intracellular signaling domain of the CAR further comprises at least one co-stimulatory signaling region. In certain embodiments, the co-stimulatory signaling region comprises an intracellular domain of a co-stimulatory molecule or a portion thereof. As used herein, “co-stimulatory molecules” refer to cell surface molecules other than antigen receptors or their ligands that are required for an efficient response of lymphocytes to antigen. Co-stimulatory molecules can provide optimal lymphocyte activation. In certain embodiments, the at least one co-stimulatory signaling region comprises a CD28 polypeptide (e.g., an intracellular domain of CD28 or a portion thereof), a 4-1BB polypeptide (e.g., an intracellular domain of 4-1BB or a portion thereof), an OX40 polypeptide (e.g., an intracellular domain of OX40 or a portion thereof), an ICOS polypeptide (e.g., an intracellular domain of ICOS or a portion thereof), a DAP-10 polypeptide (e.g., an intracellular domain of DAP-10 or a portion thereof), or a combination thereof. In certain embodiments, the at least one co-stimulatory signaling region comprises a CD28 polypeptide (e.g., an intracellular domain of CD28 or a portion thereof). In certain embodiments, the at least one co-stimulatory signaling region comprises a 4-1BB polypeptide (e.g., an intracellular domain of 4-1BB or a portion thereof). The co-stimulatory molecule can bind to a co-stimulatory ligand, which is a protein expressed on cell surface that upon binding to its receptor produces a co-stimulatory response, *i.e.*, an intracellular response that effects the stimulation provided when an antigen binds to its CAR molecule. Co-stimulatory ligands, include, but are not limited to CD80, CD86, CD70, OX40L, and 4-1BBL. As one example, a 4-1BB ligand (*i.e.*, 4-1BBL) may bind to 4-1BB (also known as “CD137”) for providing an intracellular signal that in combination with a CAR signal induces an effector cell function of the CAR⁺ T cell.

5.4.4. Cells

In certain embodiments, the cell that comprises an antigen-recognizing receptor is an unresponsive cell. In certain embodiments, the cell is a cell of the lymphoid lineage. Cells of the lymphoid lineage produce antibodies, regulate cellular immune system, and detect foreign agents in the blood and cells foreign to the host and the like. Non-limiting examples of cells of the lymphoid lineage include T cells, Natural Killer (NK) cells, B cells, dendritic cells, and stem cells from which lymphoid cells may be

differentiated. In certain embodiments, the stem cell is a pluripotent stem cell (*e.g.*, embryonic stem cell or induced pluripotent stem cell). In certain embodiments, the cell is an antigen presenting cell.

In certain embodiments, the cell is a T cell. T cells can be lymphocytes that mature in the thymus and are chiefly responsible for cell-mediated immunity. T cells are part of the adaptive immune system. In certain embodiments, the T cells provided herein comprise any type of T cells, including, but not limited to, helper T cells, cytotoxic T cells, memory T cells (including central memory T cells, stem-cell-like memory T cells (or stem-like memory T cells), and two types of effector memory T cells: *e.g.*, T_{EM} cells and T_{EMRA} cells, regulatory T cells (also known as suppressor T cells or T_{regs}), tumor-infiltrating lymphocytes (TILs), natural killer T cells, mucosal associated invariant T cells, and $\gamma\delta$ T cells. Cytotoxic T cells (CTLs or killer T cells) are a subset of T lymphocytes capable of inducing the death of infected somatic or tumor cells. A patient's own T cells (*i.e.*, autologous T cells) may be genetically modified to target specific antigens through the introduction of an antigen-recognizing receptor, *e.g.*, a CAR or a TCR. In certain embodiments, the cell is a T cell. The T cell can be a CD4⁺ T cell or a CD8⁺ T cell. In certain embodiments, the T cell is a CD4⁺ T cell. In certain embodiments, the T cell is a CD8⁺ T cell.

In certain embodiments, the cell is a tumor-specific T cell. In certain embodiments, the tumor-specific T cell comprises an endogenous TCR that recognizes a tumor antigen (TSA or TAA).

In certain embodiments, the cell is an NK cell. Natural killer (NK) cells can be lymphocytes that are part of cell-mediated immunity and act during the innate immune response. NK cells do not require prior activation in order to perform their cytotoxic effect on target cells.

Types of human lymphocytes of the presently disclosed subject matter include, without limitation, peripheral donor lymphocytes, *e.g.*, those disclosed in Sadelain, M., *et al.* 2003 *Nat Rev Cancer* 3:35-45 (disclosing peripheral donor lymphocytes genetically modified to express CARs), in Morgan, R.A., *et al.* 2006 *Science* 314:126-129 (disclosing peripheral donor lymphocytes genetically modified to express a full-length tumor antigen-recognizing T cell receptor complex comprising the α and β heterodimer), in Panelli, M.C., *et al.* 2000 *J Immunol* 164:495-504; Panelli, M.C., *et al.* 2000 *J Immunol* 164:4382-4392 (disclosing lymphocyte cultures derived from tumor infiltrating

lymphocytes (TILs) in tumor biopsies), and in Dupont, J., *et al.* 2005 *Cancer Res* 65:5417-5427; Papanicolaou, G.A., *et al.* 2003 *Blood* 102:2498-2505 (disclosing selectively *in vitro*-expanded antigen-specific peripheral blood leukocytes employing artificial antigen-presenting cells (AAPCs) or pulsed dendritic cells). The
 5 immunoresponsive cells (*e.g.*, T cells) can be autologous, non-autologous (*e.g.*, allogeneic), or derived *in vitro* from engineered progenitor or stem cells.

In certain embodiments, the cell is a cell of the myeloid lineage. Non-limiting examples of cells of the myeloid lineage include monocytes, macrophages, basophils, neutrophils, eosinophils, mast cell, erythrocytes, megakaryocytes, thrombocytes, and
 10 stem cells from which myeloid cells may be differentiated. In certain embodiments, the stem cell is a pluripotent stem cell (*e.g.*, embryonic stem cell or induced pluripotent stem cell).

5.5. Administration

In certain embodiments, the personalized amount of the chemotherapeutic agent
 15 can be administered by parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intrathecal, intra-Ommaya, intraocular, intravitreous, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural,
 20 intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal routes. In certain embodiments, the methods disclosed herein comprise administer the personalized amount of chemotherapeutic agent intravenously (IV).

25 The chemotherapeutic agent can be administered in a single dose, or in multiple doses. In certain embodiments, the chemotherapeutic agent is administered to the subject in multiple doses. The multiple doses can be administered sequentially, *e.g.*, at daily, weekly, or monthly intervals, or in response to a specific need of the subject. In certain embodiments, the chemotherapeutic agent is administered to the subject daily in
 30 2, 3, 4, or 5 consecutive days.

In certain embodiments, the cells comprising the antigen-recognizing receptor are administered to the subject by directly injecting into an organ of interest (*e.g.*, an organ affected by a tumor). In certain embodiments, the cells comprising the antigen-recognizing receptor are administered to the subject by indirectly injecting to organ of interest, for example, by administration into the circulatory system (*e.g.*, the tumor vasculature).

In certain embodiments, the cells can be administered in any physiologically acceptable vehicle, normally intravascularly, although they may also be introduced into bone or other convenient site where the cells may find an appropriate site for regeneration and differentiation (*e.g.*, the thymus). Usually, at least about 1×10^5 cells will be administered, eventually reaching about 1×10^{10} or more. In certain embodiments, the cells can comprise a purified population of cells. Those skilled in the art can readily determine the percentage of the presently disclosed cells in a population using various well-known methods, such as fluorescence activated cell sorting (FACS). The cells can be introduced by injection, catheter, or the like.

In certain embodiments, the cells can be autologous or heterologous. For example, cells or progenitors can be obtained from one subject, and administered to the same subject or a different, compatible subject. Peripheral blood derived cells or their progeny (*e.g.*, *in vivo*, *ex vivo* or *in vitro* derived) can be administered via localized injection, including catheter administration, systemic injection, localized injection, intravenous injection, or parenteral administration.

6. EXAMPLES

The present disclosure will be better understood by reference to the following Example, which is provided as exemplary of the present disclosure, and not by way of limitation.

Example 1: Fludarabine-Exposure Predicts Disease Control Following CD19-specific CAR T cell (tisagenlecleucel)

A retrospective analysis was performed on data abstracted from the Pediatric Real-World CAR Consortium (PRWCC) consisting 15 different US institutions. Patients who received standard fludarabine dosing ($30 \text{ mg/m}^2/\text{daily} \times 4 \text{ days}$) and had ≤ 3 days between chemotherapy and infusion were included. The area under the curve (AUC) of

fludarabine exposure was calculated using a previously published population-PK model (Langenhorst et al., *Blood Advances* (2019);3:2179-2187). Main outcomes of interest included: response rate, relapse-free (RFS) and overall survival (OS), a composite endpoint of loss of B-cell aplasia (BCA) and/or relapse.

5 Total 152 patients were included in the study. The median age was 13 years old (range between <1 and 26 years old). The estimated median fludarabine AUC prior to tisagenlecleucel (CD19-specific CAR T cell) infusion was 14.4 mg.hr/L (range between 11.1 and 22.4). Complete remission was achieved in 86% (n=131) of patients, the 12-month cumulative incidence of relapse in responders was 39.2% (95% CI: 30.2-48.2%),
10 and the 12-month OS was 75% (95% CI: 67.6-82.6%). Fludarabine exposure was analyzed as a continuous variable. An AUC of 13.8 mg.hr/L was demonstrated to be a threshold of interest (Figure 1) for disease relapse. Patients who received a fludarabine AUC of ≥ 13.8 mg.hr/L had a lower risk of relapse ($p=0.03$; Figure 2A) and loss of BCA/relapse ((composite endpoint) $p=0.03$; Figure 2B). The PRWCC previously
15 reported pre-treatment disease burden negatively impacts OS and disease control (Schultz et al., *Blood* (2020) 136:14–15). Analysis of this sub-cohort of patients with high pre-treatment disease burden demonstrated that fludarabine AUC ≥ 13.8 mg.hr/L was associated with improved OS ($p=0.03$) and a lower incidence of relapse ($p=0.03$; Figures 3A-3B).

20 Fludarabine-exposure impacted outcomes after CAR-T cell treatment. Higher fludarabine exposure (AUC ≥ 13.8 mg.hr/L) was associated with lower relapse rates and protected BCA regardless of pre-treatment disease burden. Higher fludarabine exposure was also associated with higher OS. Also, in patients with high-disease burden, optimizing fludarabine exposure by personalized dosing can improve the durability of
25 CD19-specific CAR T cell therapy and subsequent survival.

Example 2: Fludarabine Lymphodepletion Is Associated With Improved Outcomes Following CAR T Cell Therapy

 Acute lymphoblastic leukemia (ALL) is the most common malignancy occurring in children, and for patients with relapsed or refractory (R/R) B-cell ALL (B-ALL) the
30 prognosis is dismal (Nguyen et al., *Leukemia*. Dec 2008;22(12):2142-50; Gaynon PS, *Br J Haematol*. Dec 2005;131(5):579-87; and von Stackelberg et al., *Eur J Cancer*. Jan

2011;47(1):90-7). It has been reported that the use of CD19-specific CAR T cells as a therapeutic option for patients with R/R B-ALL (Davila et al., *Sci Transl Med.* Feb 2014;6(224):224ra25; Maude et al., *N Engl J Med.* Oct 2014;371(16):1507-17; Lee et al., *Lancet.* Feb 2015;385(9967):517-528; Maude, *Clin Adv Hematol Oncol.* Oct 2018;16(10):664-666; Gardner et al., *Blood.* 06 2017;129(25):3322-3331; Curran et al., *Blood.* Oct 17 2019; Park et al., *N Engl J Med.* 02 2018;378(5):449-459; Frey et al., *J Clin Oncol.* Dec 9 2019; and Maude et al., *N Engl J Med.* 2018;378(5):439-448).

Accessibility of this cellular therapy was broadened with the U.S. Food and Drug Administration's approval of tisagenlecleucel, a CD19-specific CAR T cell for R/R B-ALL in patients <26 years old (Maude et al., *N Engl J Med.* 2018;378(5):439-448).

Despite initial response to CD19-specific CAR T cell therapy in the setting of R/R B-ALL, the incidence of relapse among responders is estimated at 40-50%, demonstrating a need to improve this treatment strategy (Maude et al., *N Engl J Med.* Oct 2014;371(16):1507-17; Lee et al., *Lancet.* Feb 2015;385(9967):517-528; Maude, *Clin Adv Hematol Oncol.* Oct 2018;16(10):664-666; and Park et al., *N Engl J Med.* 02 2018;378(5):449-459). The current successful application of CAR T cell therapy has required pre-treatment of patients with lymphodepleting chemotherapy (LDC). Most commonly LDC utilizes a combination of cyclophosphamide and fludarabine; however, dosing, duration, and intensity of LDC have varied across institutions and trials (Davila et al., *Sci Transl Med.* Feb 2014;6(224):224ra25; Maude et al., *N Engl J Med.* Oct 2014;371(16):1507-17; Lee et al., *Lancet.* Feb 2015;385(9967):517-528; Maude, *Clin Adv Hematol Oncol.* Oct 2018;16(10):664-666; Gardner et al., *Blood.* 06 2017;129(25):3322-3331; Curran et al., *Blood.* Oct 17 2019; Park et al., *N Engl J Med.* 02 2018;378(5):449-459; Frey et al., *J Clin Oncol.* Dec 9 2019; and Maude et al., *N Engl J Med.* 2018;378(5):439-448). The addition of fludarabine to cyclophosphamide has been shown to improve CAR T cell kinetics, initial response, and decrease rejection in both B-ALL and non-Hodgkin lymphoma (NHL) (Gardner et al., *Blood.* 06 2017;129(25):3322-3331; Curran et al., *Blood.* Oct 17 2019; Turtle et al., *Sci Transl Med.* Sep 7 2016;8(355):355ra116; and Turtle et al., *J Clin Invest.* 06 2016;126(6):2123-38). Dose intensity of cyclophosphamide has also been shown to improve response and peak CAR T cell expansion in pediatric and young adults with R/R B-ALL and progression free survival and cytokine milieu in adult patients with R/R NHL (Curran et al., *Blood.* Oct 17 2019; Hirayama et al., *Blood.* Apr 25 2019;133(17):1876-1887). In

patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT), optimal fludarabine exposure has been found to decrease non-relapse mortality due to improved immune reconstitution and subsequently improve survival (Langenhorst et al., *Blood Adv.* Jul 23 2019). Fludarabine exposure has not yet been studied in patients undergoing CAR T cell therapy. The presently disclosed subject matter reports the results of a cohort of pediatric and young adult patients with R/R B-ALL who received tisagenlecleucel and defines the optimal fludarabine exposure associated with improved outcomes.

Methods

Study Design. A retrospective analysis of pediatric and young adult patients with R/R B-ALL as part of the Pediatric Real-World CAR Consortium (PRWCC; n=15 centers) was conducted. Two hundred (n=200) patients underwent collection/manufacture for planned standard-of-care CD19-specific CAR T cell therapy (tisagenlecleucel). Patients in this analysis were excluded if they did not receive their tisagenlecleucel infusion (n=15), they did not have evaluable response data at the cutoff date (n=1), they received lymphodepletion other than the tisagenlecleucel package insert LDC (fludarabine 30mg/m²/day x 4 days and cyclophosphamide 500mg/m²/day x 2 days; n=7), their weight was not supported by the population pharmacokinetic (PK) model (n=2), or the number of days between lymphodepletion and infusion was >3 days (n=23; Figure 4).

Outcomes of interest. The main outcomes of interest were overall survival (OS), cumulative incidence of relapse (CIR), and the cumulative incidence of a composite endpoint (relapse or loss of BCA). Other outcomes of interest were response rates, cytokine release syndrome (CRS), and immune effector cell associated neurotoxicity syndrome (ICANS). Complete remission (CR) was defined as ≤5% bone marrow blasts by morphology, absence of circulating lymphoblasts, and no evidence of extramedullary disease. MRD negativity was defined as <0.01% abnormal B cells assessed by flow cytometry. Relapse was defined as any (medullary or extramedullary) evidence of hematologic, cytogenetic, and/or molecular recurrence of primary disease. CD19-positive B cell recovery was defined as any detectable CD19-positive B cells (>1 cell/mL) on a peripheral blood lymphocyte flow cytometry panel. Toxicity grading was described according to ASTCT (Lee et al., *Biol Blood Marrow Transplant.* 04 2019;25(4):625-638) for CRS and ICANS. Additionally, patients were further

delineated based on their disease burden: high disease burden ($\geq 5\%$ lymphoblasts, CNS3 disease and/or isolated EM disease), and no (undetectable) or low disease burden ($< 5\%$ lymphoblasts, $\leq \text{CNS2}$ disease, and/or no detectable EM disease) based off the previous PRWCC analysis (Schultz et al., *Blood*. 2020;136(Supplement 1):14-15). The
5 fludarabine exposure, area under the curve (AUC), for each patient was calculated using a population PK model as previously described in Langenhorst et al., *Clin Pharmacokinet*. May 2019;58(5):627-637. The patient variables required to calculate an estimated fludarabine exposure include estimated GFR (using either the Cockcroft-Gault or Schwartz equation depending on age), actual body weight, height, and dosage of
10 fludarabine utilized where actual body weight and estimated GFR are the best predictors of fludarabine PK (Langenhorst et al., *Clin Pharmacokinet*. May 2019;58(5):627-637).

Statistical Analysis. For all analyses, tisagenlecleucel infusion date was considered time zero (0). The impact of different factors on the chance of response was assessed through Mann-Whitney-Wilcoxon test for continuous variables, Chi-squared or
15 Fisher's exact test for categorical variables (univariable analysis), and logistic regression (multivariable analysis). OS for all treated patients was defined as the time from infusion to death from all causes. OS in responding patients was defined as time from day of response (day 28 from CAR infusion) to death from all causes. Patients alive were censored at their last follow-up date. Time-to-relapse was defined as the time from
20 the date of infusion to the date of disease relapse, while time-to-composite endpoint was defined as the time from the date of infusion to the date of either disease relapse or loss of BCA, whichever came first. For both endpoints, patients alive without disease relapse (and loss of BCA for composite endpoint) were censored at their date of last follow-up. Death and allo-HCT without disease relapse (and loss of BCA) were considered
25 competing events. Patients who died prior to day 28 or did not achieve a CR after tisagenlecleucel were not at risk of relapse and therefore were excluded from the cumulative incidence of relapse and composite endpoint analyses. Kaplan-Meier analysis was used to estimate OS while an Aalen-Johansen estimator was used for the cumulative incidence of relapse and the composite endpoint (prodlm: Product-Limit Estimation for
30 Censored Event History Analysis. Version 2018.04.18. CRAN.R-project.org . The potential follow-up was estimated using reverse Kaplan-Meier estimate. Differences in survival curves between groups were tested using a log-rank test or Gray's test for cumulative incidences. The impact of clinical factors associated with response and

fludarabine exposure on survival endpoints was assessed using Cox models (cause-specific hazard models in presence of competing risks). The estimated fludarabine (cumulative) AUC for each patient was plotted as a continuous variable against hazard ratios for relapse and optimal exposure was explored using Hazard Ratio plots (Greg: Regression Helper Functions. Version 1.3.1. 2019. <https://CRAN.R-project.org/package=Greg>). In both logistic and Cox models, variables with p-value <0.1 in univariable analysis were included in multivariable analysis. A p-value <0.05 was considered statistically significant.

Results

Patient Characteristics. One-hundred fifty-two (n=152) patients met inclusion criteria for analysis (Figure 4). Baseline patient characteristics are shown in Table 1.

Table 1. Patient characteristics and outcomes.

Patient characteristics	n=152
Median age at infusion (years; (range))	12.5 (<1-26)
Median age at diagnosis (years; (range))	8 (<1-25)
Gender (male/female)	93/59
Race	
Asian	6 (4%)
Black	7 (5%)
Hispanic	58 (39%)
More than 1 race	2 (1%)
White	76 (51%)
Unknown	3
Initial cytogenetic risk	
Favorable	22 (19%)
Intermediate	42 (36%)
Unfavorable	53 (45%)
Unknown	35
Pre-infusion disease burden	
No detectable disease	41 (28%)
Low disease	33 (22%)
High disease	74 (50%)
Unknown	4
Mean time diagnosis to infusion (months)	43 (3-164)
Mean number of prior lines of therapy	3.5 (1-10)
Prior allo-HCT	8 (5%)
Prior CD19 directed therapy	33 (22%)

Disease status	
Refractory disease	24 (16%)
≥1 relapse	128 (84%)
Overall day 28 response	
No CR	16 (11%)
CR	131 (86%)
Died prior to day28	5 (3%)
CRS grade	
none	55 (36%)
1	36 (24%)
2	28 (18%)
3	17 (11%)
4	15 (10%)
5	1 (1%)
<i>Unknown</i>	1
Neurotoxicity grade	
none	116 (76%)
1	17 (11%)
2	7 (5%)
3	8 (5%)
4	3 (2%)
5	1 (1%)
Relapsed post CAR	55 (36%)
CD19-positive relapse	28 (55%)
CD19-negative relapse	23 (45%)
<i>unknown</i>	4
Loss of BCA post CAR	52 (34%)
Survival status (alive/dead)	112/40
Cause of death	
Leukemia	30 (74%)
Infection	4 (10%)
CRS	1 (2%)
Neurotoxicity	1 (2%)
Transplant related	3 (7%)
ARDS/cardiac arrest	1 (2%)

allo-HCT, allogeneic hematopoietic cell transplantation; CR, complete remission; CRS, cytokine release syndrome; CAR, chimeric antigen receptor; BCA, B cell aplasia; ARDS, acute respiratory distress syndrome.

- 5 The median age at diagnosis was 8 years (<1-25 years) and the median age at tisagenlecleucel infusion was 12.5 years (range <1-26 years). The median time from initial diagnosis to infusion was 2.9 years (range 0.3-13.7). Complete remission was achieved in 86% (131/152) of patients. Death prior to day 28 disease assessment

occurred in 3% (5/152) of patients and 11% (16/152) of patients did not achieve CR.

Univariable analysis of baseline patient characteristics found pre-infusion disease burden (p=0.001), prior CD19 directed therapy (p=0.01), and race (p=0.025) as factors

associated with response (Table 2). The PRWCC previously reported that pre-infusion

5 disease burden and race were prognostic for response (Schultz et al., *Blood*.

2020;136(Supplement 1):14-15; Baggott et al., 2021 TCT¹ *Transplantation & Cellular Therapy Meetings of ASTCT and CIBMTR*. TCT Meetings, 2021).

Table 2: Univariable analysis of disease characteristics and response.

Characteristics	Non-responders n=16	Responders n=131	p-value
Age at diagnosis			>0.99
<1 year or >10 years	8 (12%)	61 (88%)	
1 to 10 years	8 (10%)	70 (90%)	
Gender (male/female)	10/6	81/50	>0.99
Race			0.025
Black/Hispanic	12 (19%)	50 (81%)	
Others	0 (0%)	8 (100%)	
White	4 (5%)	70 (95%)	
Unknown	0	3	
Initial cytogenetic risk			0.44
Favorable	4 (18%)	18 (82%)	
Intermediate	4 (10%)	37 (90%)	
Unfavorable	4 (8%)	46 (92%)	
Unknown	4	30	
Pre-infusion disease burden			0.001
No or low disease	1 (1%)	73 (99%)	
High disease	13 (19%)	56 (81%)	
Unknown	2	2	
Time diagnosis to infusion (months)	36 (6-117)	45 (3-164)	0.44
Number of prior lines of therapy	3.9 (2-7)	3.4 (1-10)	0.14
Prior allo-HCT			0.56
no	15 (11%)	125 (89%)	
yes	1 (14%)	6 (86%)	
Prior CD19 directed therapy			0.01
no	8 (7%)	106 (93%)	
yes	8 (24%)	25 (76%)	
Disease status			>0.99
Refractory disease	2 (8%)	22 (92%)	
≥1 relapse	14 (11%)	109 (89%)	

10 allo-HCT, allogeneic hematopoietic cell transplantation

Fludarabine Exposure and Response. The mean dose of fludarabine received was 41 mg per dose (range 11-70mg) and the estimated mean fludarabine cumulative AUC prior to tisagenlecleucel infusion was 14.4mg*hr/L (sd=1.6, range 11.1-22.4 mg*hr/L). The impact of fludarabine AUC on response was explored in the entire cohort. In context of a limited sample of non-responding patients (n=16), fludarabine AUC was found to be higher in non-responders than responders, with a mean of 14.7mg*hr/L (sd=1.6, range 11.1-16.7) and 14.4mg*hr/L (sd=1.5, range 11.7-22.4) respectively (Mann-Whitney-Wilcoxon, p=0.046). Univariable analysis of baseline characteristics and response is shown in Table 2. Multivariable analysis of baseline characteristics prognostic for response in this cohort (race, pre-infusion disease burden, and prior CD19 directed therapy) revealed that fludarabine AUC was not predictive of response after adjustment (p=0.26; Table 3).

Table 3. Multivariable analysis of response by patient's characteristics significant from univariable analysis.

Characteristics	Odds Ratio	95% CI	p-value
Cumulative Flu AUC (mg*hr/L)	0.81	0.56-1.21	0.26
Race*			0.019
Black/Hispanic	Ref		
White	5.28	1.37-25.7	
Pre-infusion disease burden			<0.001
No or low disease	Ref		
High disease	0.06	0.00-0.32	
Prior CD19 directed therapy			0.007
no	Ref		
yes	0.16	0.04-0.60	

Flu, fludarabine; AUC, area under the curve; ref, reference; CI, confidence intervals

*No events were observed in the other race groups

Fludarabine Exposure and Outcomes. The median potential follow-up was 1.1 year (Q1=0.8, Q3=1.7 years). Following response, 52 patients relapsed and the 12-month CIR in responding patients was 36.4% (95% CI: 27.5 – 45.2%). The cumulative incidence of a clinically important composite endpoint (relapse or the loss of BCA) was 55.2% (95% CI: 46.0-64.4) at 12-months with relapse or loss of BCA occurring in 76 patients and only loss of BCA occurring in 52 patients post tisagenlecleucel. A total of

40 patients died in this cohort. The 12-month and 24-month OS for the entire cohort was 75.1% (95% CI: 67.6-82.6%) and 56.5% (95% CI: 41.8-71.2%), respectively.

Estimated fludarabine AUC for each patient was plotted as a continuous variable against cumulative risk of relapse (Figure 1). Optimal fludarabine exposure was defined as an AUC $\geq 13.8\text{mg}\cdot\text{hr}/\text{L}$ as this is where, graphically, the hazard ratio curve for CIR crosses hazard ratio (HR)=1 (Figure 1). This threshold was utilized as the optimal fludarabine exposure for all outcomes of interest (OS, CIR, and composite endpoint), as the threshold for other endpoints was similar (data not shown). In this cohort, 67% (102/152) had optimal fludarabine exposure. Univariable and multivariable analyses incorporating baseline patient factors and covariate of interest, optimal/sub-optimal fludarabine exposure, were analyzed with outcomes of interest (Tables 4A-4C). After multivariable adjustment, both high pre-infusion disease burden and sub-optimal fludarabine exposure were associated with an increased risk of relapse (HR=2.66; $p=0.001$ and HR=2.45; $p=0.005$) and increased composite endpoint (HR=2.02; $p=0.005$ and HR= 1.96; $p=0.01$; Tables 4A-4C). OS was not associated with sub-optimal fludarabine exposure in the entire cohort (HR=1.53; $p=0.26$) or when analyzing responding patients only (HR=1.97; $p=0.14$), whereas pre-infusion disease burden (HR=4.77; $p<0.001$ and HR=2.90; $p=0.013$) and age (HR=0.41; $p=0.008$ and HR=0.34; $p=0.010$) were associated with survival as previously reported by the PRWCC (Tables 4A-4C and 5; Schultz et al., *Blood*. 2020;136(Supplement 1):14-15). Analysis of patients with high pre-infusion disease burden did associate sub-optimal fludarabine exposure with reduced overall survival for all treated patients (HR=2.27; $p=0.03$) and in responding patients only (HR=3.50; $p=0.02$). Outcomes of interest for cohorts of patients based off fludarabine exposure and pre-treatment disease burden are shown in Figures 5A-5D. Patients who received optimal or sub-optimal fludarabine exposure had no difference in pre-treatment disease burden or other factors associated with response in this cohort (Table 6).

Table 4A. Univariable and multivariable analysis of outcomes of interest based on patient characteristics in responding patients: cumulative incidence of relapse.

Characteristics	Univariable			Multivariable		
	HR	95% CI	P-value	HR	95% CI	p-value
Sex			0.13			
Female	Ref	0.36-				
Male	0.64	1.13				
Number of prior lines of therapy	1.09	0.92-1.28	0.33			
Time dx to infusion (months)	0.99	0.99-1.00	0.20			
Age at diagnosis			0.005			0.075
<1 or >10 years	Ref			Ref		
1 to 10 years	0.43	0.24-0.78		0.55	0.29-1.06	
Pre-infusion disease burden			0.006			0.001
No or low disease	Ref	1.25-		Ref	1.45-	
High disease	2.26	4.10		2.66	4.87	
Initial cytogenetic risk			0.43			
Favorable	Ref	0.68-5.15				
Intermediate	1.87	0.62-				
Unfavorable	1.68	4.53				
Prior HCT			0.92			
No	Ref	0.23-				
Yes	0.93	3.85				
Prior CD19 directed therapy			0.43			
No	Ref	0.67-				
Yes	1.32	2.61				
Prior relapse			0.062			0.14
1 or more relapse	Ref	0.15-		Ref	0.18-	
Refractory	0.42	1.17		0.49	1.38	
CNS or EM disease			0.17			
No	Ref	0.84-				
Yes	1.50	2.68				
Race			0.98			
Black/Hispanic	Ref	0.34-3.89				
Others	1.14	0.55-				
White	1.02	1.88				
Cumulative Flu AUC (mg*hr/L)			0.029			0.005
≥13.8	Ref	1.08-		Ref	1.34-	
<13.8	1.95	3.52		2.45	4.48	

Table 4B. Univariable and multivariable analysis of outcomes of interest based on patient characteristics in responding patients: cumulative incidence of composite endpoint (relapse or loss of B-cell aplasia).

Characteristics	Univariable			Multivariable		
	HR	95% CI	P-value	HR	95% CI	P-value
Sex			0.40			
Female	Ref	0.50-				
Male	0.81	1.31				
Number of prior lines of therapy	1.04	0.90-1.19	0.63			
Time dx to infusion (months)	1.00	0.99-1.00	0.22			
Age at diagnosis			0.052			0.21
<1 or >10 years	Ref	0.39-		Ref	0.43-	
1 to 10 years	0.62	1.00		0.72	1.21	
Pre-infusion disease burden			0.014			0.005
No or low disease	Ref	1.13-		Ref	1.24-	
High disease	1.82	2.95		2.02	3.29	
Initial cytogenetic risk			0.54			
Favorable	Ref	0.58-				
Intermediate	1.27	2.80				
Unfavorable	1.51	0.71-3.24				
Prior HCT			0.64			
No	Ref	0.24-				
Yes	0.77	2.44				
Prior CD19 directed therapy			0.82			
No	Ref	0.60-				
Yes	1.07	1.90				
Prior relapse			0.55			
1 or more relapse	Ref	0.64-				
Refractory	1.22	2.33				
CNS or EM disease			0.93			
No	Ref	0.64-				
Yes	1.02	1.64				
Race			0.71			
Black/Hispanic	Ref	0.19-				
Others	0.63	2.07				
White	0.99	0.60-1.62				
Cumulative Flu AUC (mg*hr/L)			0.025			0.010
≥13.8	Ref	1.09-		Ref	1.19-	
<13.8	1.77	2.88		1.96	3.23	

Table 4C. Univariable and multivariable analysis of outcomes of interest based on patient characteristics in responding patients: overall survival in responding patients

Characteristics	Univariable			Multivariable		
	HR	95% CI	p-value	HR	95% CI	p-value
Sex			0.10			0.69
Female	Ref	0.23-		Ref	0.35-	
Male	0.51	1.13		0.83	2.01	
Number of prior lines of therapy	1.16	0.93-1.45	0.20			
Time dx to infusion (months)	0.99	0.98-1.00	0.31			
Age at diagnosis			0.011			0.010
<1 or >10 years	Ref	0.14-		Ref	0.14-	
1 to 10 years	0.34	0.80		0.34	0.80	
Pre-infusion disease burden			0.013			0.013
No or low disease	Ref	1.19-		Ref	1.20-	
High disease	2.88	6.96		2.90	7.00	
Initial cytogenetic risk			0.41			
Favorable	Ref	0.56-				
Intermediate	2.67	12.7				
Unfavorable	1.96	0.42-9.11				
Prior HCT			0.88			
No	Ref	0.12-				
Yes	0.86	6.37				
Prior CD19 directed therapy			0.98			
No	Ref	0.38-				
Yes	1.01	2.71				
Prior relapse			0.19			
1 or more relapse	Ref	0.10-				
Refractory	0.42	1.80				
CNS or EM disease			0.68			
No	Ref	0.37-				
Yes	0.84	1.90				
Race			0.73			
Black/Hispanic	Ref	0.26-				
Others	1.20	5.44				
White	0.75	0.33-1.73				
Cumulative Flu AUC (mg*hr/L)			0.056			0.14
≥13.8	Ref	1.00-		Ref	0.80-	
<13.8	2.25	5.06		1.97	4.85	

HR, hazard ratio; CI, confidence interval; dx, diagnosis; HCT, hematopoietic cell transplantation; CNS, central nervous system; EM, extramedullary disease; flu, fludarabine; AUC, area under the curve; ref, reference

Table 5. Univariable and Multivariable analysis of overall survival in all treated patients based on patient characteristics.

Characteristics	Univariable			Multivariable		
	HR	95% CI	P-value	HR	95% CI	p-value
Sex			0.092			0.96
Female	Ref	0.31-		<i>Ref</i>	<i>0.50-</i>	
Male	0.59	1.09		<i>0.98</i>	<i>1.97</i>	
Number of prior lines of therapy		1.01-	0.050	<i>1.16</i>	<i>0.97-</i>	<i>0.11</i>
	1.19	1.41			<i>1.38</i>	
Time dx to infusion (months)		0.98-	0.14			
	0.99	1.00				
Age at diagnosis			0.004		0.21-	0.008
<1 or >10 years	Ref	0.20-		Ref	0.81	
1 to 10 years	0.39	0.75		0.41		
Pre-infusion disease burden			<0.001			<0.001
No or low disease	Ref	2.10-		Ref	2.10-	
High disease	4.76	10.8		4.77	10.9	
Initial cytogenetic risk		0.87-	0.15			
Favorable	Ref	10.8				
Intermediate	3.06	0.69-				
Unfavorable	2.40	8.32				
Prior HCT			0.90			
No	Ref	0.26-				
Yes	1.09	4.54				
Prior CD19 directed therapy			0.69			
No	Ref	0.57-				
Yes	1.16	2.38				
Prior relapse			0.065			0.055
1 or more relapse				<i>Ref</i>		
Refractory	Ref	0.12-		<i>0.36</i>	<i>0.11-</i>	
	0.38	1.24			<i>1.18</i>	
CNS or EM disease			0.64			
No	Ref	0.46-				
Yes	0.86	1.62				
Race		0.16-	0.32			
Black/Hispanic	Ref	2.88				
Others	0.68	0.32-				
White	0.62	1.17				
Cumulative Flu AUC			0.22			0.26
(mg*hr/L)						
≥13.8	Ref	0.79-		<i>Ref</i>	<i>0.73-</i>	
<13.8	1.51	2.87		<i>1.53</i>	<i>3.21</i>	

HR, hazard ratio; CI, confidence interval; dx, diagnosis; HCT, hematopoietic cell transplantation; CNS, central nervous system; EM, extramedullary disease; flu, fludarabine; AUC, area under the curve; ref, reference

Table 6. Univariable analysis of disease characteristics associated with response and fludarabine exposure.

Characteristics	Sub-optimal ($<13.8\text{mg*hr/L}$) n=50	Optimal ($\geq 13.8\text{mg*hr/L}$) n=102	p-value
Race			0.92
Black/Hispanic	21 (32%)	44 (68%)	
Others	2 (25%)	6 (75%)	
White	26 (34%)	50 (66%)	
Unknown	1	2	
Pre-infusion disease burden			0.16
No or low disease	29 (39%)	45 (61%)	
High disease	20 (27%)	54 (73%)	
Unknown	1	3	
Prior CD19 directed therapy			0.79
no	38 (32%)	81 (68%)	
yes	12 (36%)	21 (64%)	

5 *Fludarabine Exposure and Toxicity.* CRS and severe CRS (sCRS; defined as grade 3 or higher) were observed in 64% (97/152) of patients and in 22% (33/152) of patients, respectively. Neurotoxicity was seen in 24% (36/152) of patients and severe neurotoxicity (defined as grade 3 or higher) in 8% (12/152) of patients. The neurotoxicity grading was heterogeneous with ICANS (n=98), CRES (n=21), and other grading

10 systems (n=22). Optimal fludarabine exposure was not associated with increased CRS or neurotoxicity (limited to ICANS graded patients) (Table 7). Patients who received optimal fludarabine exposure had sCRS of 18% (18/102) compared to 30% (15/50) of patients in the sub-optimal fludarabine exposure group (p=0.10). There was also no difference between optimal and sub-optimal fludarabine exposure and overall ICANS

15 (22% (15/67) vs 29% (9/31), p=0.61) or sICANS (7% (5/67) vs 13% (4/31), p=0.46) respectively (Table 7). Infection post CAR T cell therapy occurred in 38% of patients (57/152) including 35% (36/102) and 42% (21/50) in patients who received optimal versus suboptimal fludarabine exposure respectively (p=0.42). In this cohort, four patients died from infection, all of whom received suboptimal fludarabine. Grade 4

20 neutropenia was seen in 66% (97/148) of the patients, 67% of patients receiving suboptimal fludarabine, and 65% of patients receiving optimal fludarabine (p=0.84). In

addition, there was no correlation between receiving an optimal fludarabine exposure and resolution of neutropenia ($p=0.75$) or with the time to recovery (in patients who recovered, $p=0.61$).

5 **Table 7. Toxicity rates according to optimal and sub-optimal fludarabine exposure.**

Characteristics	Sub-optimal flu ($<13.8\text{mg*hr/L}$)	Optimal flu ($\geq 13.8\text{mg*hr/L}$)	p-value
CRS grade ≥ 3 (sCRS)	15/50 (30%)	18/102 (18%)	0.09
ICANS grade ≥ 1	9/31 (29%)	15/67 (22%)	0.61
ICANS grade ≥ 3 (sICANS)	4/31 (13%)	5/67 (7%)	0.46

Flu, fludarabine; CRS, cytokine release syndrome; sCRS, severe CRS.

Discussion

Lymphodepleting chemotherapy prior to CAR T cell therapy has been shown to be beneficial for efficacy with the current generation of investigational or commercial CAR T cells (Gardner et al., *Blood*. 06 2017;129(25):3322-3331; Curran et al., *Blood*. Oct 17 2019; Turtle et al., *J Clin Invest*. 06 2016;126(6):2123-38; and Hirayama et al., *Blood*. Apr 25 2019;133(17):1876-1887). Using a validated population PK model, it was found that fludarabine exposure is a major driver of outcomes in the standard cyclophosphamide and fludarabine LDC prior to tisagenlecleucel for R/R B-ALL in a real-world setting. The presently disclosed subject matter defines an optimal fludarabine exposure for CAR T cell therapy. Optimal fludarabine exposure was found to be $\geq 13.8\text{mg*hr/L}$ and was associated with reduced disease relapse and a clinically relevant composite endpoint of relapse or loss of BCA. Fludarabine exposure was noted to impact OS in patients with high pre-infusion tumor burden, a cohort of patients previously reported to have dismal outcomes with CAR T cell therapy (Schultz et al., *Blood*. 2020;136(Supplement 1):14-15). Fludarabine exposure as part of LDC is an easily modifiable factor that could improve the durability and efficacy of CAR T cell therapy.

Higher intensity LDC has been shown to improve lymphodepletion and has been associated with improved response following CD19-specific CAR T cells (Gardner et al., *Blood*. 06 2017;129(25):3322-3331). Other potential mechanisms include increased

availability of cytokines and/or improvement of cytokine milieu which has also been shown following more intense lymphodepletion (Hirayama et al., *Blood*. Apr 25 2019;133(17):1876-1887; and Gattinoni et al., *J Exp Med*. Oct 3 2005;202(7):907-12). A consideration in personalizing fludarabine dosing is to determine if any increase in toxicity is seen with increasing fludarabine LDC. Previous studies have noted the association of delayed neurotoxicity in adult leukemia patients treated with fludarabine (Warrell et al., *J Clin Oncol*. Jan 1986;4(1):74-9; Spriggs et al., *Cancer Res*. Nov 1986;46(11):5953-8; and Chun et al., *Cancer Treat Rep*. Oct 1986;70(10):1225-8). No increase in toxicity was noted in the current analysis. In this cohort, no apparent increased infection risk was noted in patients who received increased fludarabine, e.g., $\geq 13.8\text{mg*hr/L}$, exposure.

The use of a fludarabine population PK model is clinically feasible, and unique data on the association of fludarabine exposure and outcomes following CAR T cell therapy were generated. Patients also received cyclophosphamide as part of their LDC, however cyclophosphamide pharmacokinetics are not included in this analysis as the number of active metabolites of cyclophosphamide make estimation of exposure not feasible. This analysis can be applied in alternative LDC agents that have more predictable PK thereby creating a more favorable environment for CAR T cell expansion, persistent, and durability.

In summary, increased, e.g., $\geq 13.8\text{mg*hr/L}$, fludarabine exposure prior to CD19-specific CAR T cell therapy (tisagenlecleucel) in pediatric and young adult patients with R/R B-ALL was associated with lower relapse.

Although the presently disclosed subject matter and certain of its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the disclosure. Moreover, the scope of the present application is not intended to be limited to the particular embodiments of the process, machine, manufacture, and composition of matter, and methods described in the specification. As one of ordinary skill in the art will readily appreciate from the disclosure of the presently disclosed subject matter, processes, machines, manufacture, compositions of matter, or methods, presently existing or later to be developed that perform substantially the same function or achieve substantially the same result as the corresponding embodiments described herein may be utilized according to the presently disclosed subject matter. Accordingly, the appended

claims are intended to include within their scope such processes, machines, manufacture, compositions of matter, or methods.

Various patents, patent applications, publications, product descriptions, protocols, and sequence accession numbers are cited throughout this application, the disclosure of
5 which are incorporated herein by reference in their entireties for all purposes.

WHAT IS CLAIMED IS:

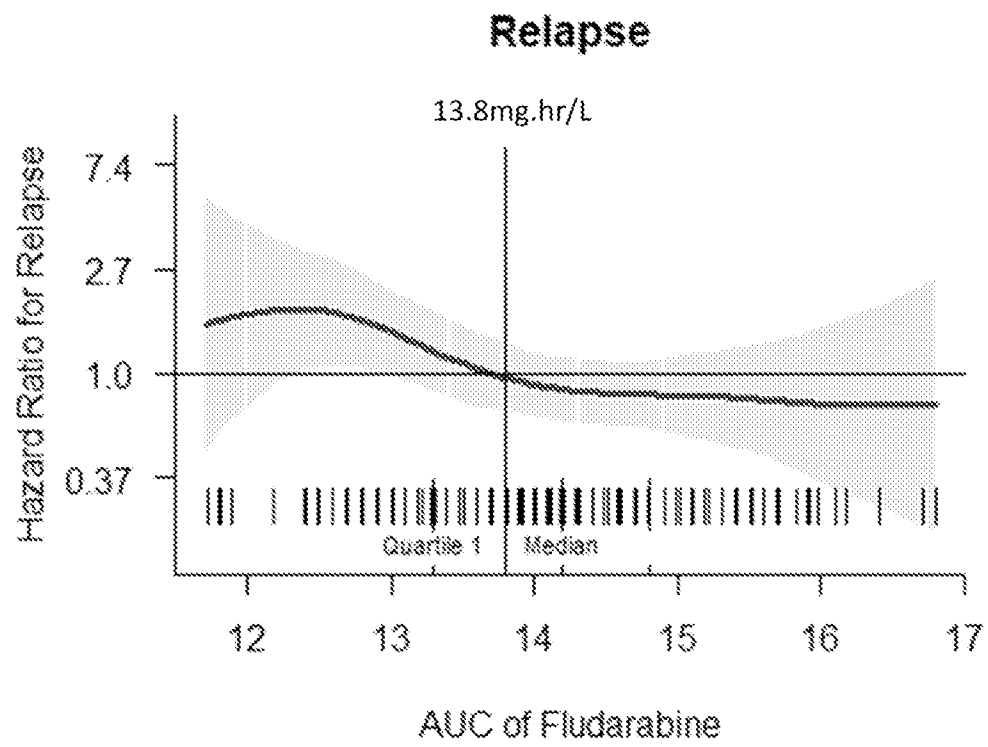
1. A method of treating a subject, comprising administering to the subject cells comprising an antigen recognizing receptor, wherein, prior to the administration, the subject has been preconditioned with a personalized amount of a chemotherapeutic agent, wherein the personalized amount provides an area under the curve (AUC) of at least about 10 mg.hr/L of the chemotherapeutic agent.
2. A method of increasing durability of a therapy comprising cells comprising an antigen recognizing receptor in a subject, the method comprising: administering to the subject the therapy, wherein, prior to the administration, the subject has been preconditioned with a personalized amount of a chemotherapeutic agent, wherein the personalized amount provides an area under the curve (AUC) of at least about 10 mg.hr/L of the chemotherapeutic agent.
3. A method of improving efficacy of a therapy comprising cells comprising an antigen recognizing receptor in a subject, the method comprising administering to the subject the therapy, wherein, prior to the administration, the subject has been preconditioned with a personalized amount of a chemotherapeutic agent, wherein the personalized amount provides an area under the curve (AUC) of at least about 10 mg.hr/L of the chemotherapeutic agent.
4. A method of lowering the risk of relapse and/or lengthening survival of a subject receiving a therapy comprising cells comprising an antigen recognizing receptor, the method comprising preconditioning the subject with a personalized amount of a chemotherapeutic agent prior to the therapy, wherein the personalized amount provides an area under the curve (AUC) of at least about 10 mg.hr/L of the chemotherapeutic agent.
5. The method of any one of claims 1-4, wherein the personalized amount of the chemotherapeutic agent provides an AUC of at least about 11 mg.hr/L, at least about 12 mg.hr/L, at least about 13 mg.hr/L, at least about 14 mg.hr/L, or at least about 15 mg.hr/L of the chemotherapeutic agent.
6. The method of any one of claims 1-5, wherein the personalized amount of the chemotherapeutic agent provides an AUC of at least about 13 mg.hr/L of the chemotherapeutic agent.

7. The method of any one of claims 1-6, wherein the personalized amount of the chemotherapeutic agent provides an AUC of about 14 mg.hr/L of the chemotherapeutic agent.
8. The method of any one of claims 1-7, wherein the personalized amount is
5 determined by body weight and renal function of the subject.
9. The method of claim 8, wherein the renal function is determined by a glomerular filtration rate (GFR) of the subject.
10. The method of claim 9, wherein the GFR is determined by creatinine clearance of the subject.
- 10 11. The method of any one of claims 1-10, wherein the chemotherapeutic agent is an antimetabolite.
12. The method of claim 11, wherein the antimetabolite is selected from the group consisting of folic acid antagonists, pyrimidine analogs, purine analogs, adenosine deaminase inhibitors, lympho- or myeloid depleting antibodies, alkylators, topoisomerase
15 II inhibitors, derivatives thereof, and combinations thereof.
13. The method of claim 12, wherein the chemotherapeutic agent is an adenosine deaminase inhibitor.
14. The method of claim 13, wherein the adenosine deaminase inhibitor is fludarabine or a derivative thereof.
- 20 15. The method of claim 12, wherein the chemotherapeutic agent is a purine analog.
16. The method of claim 15, wherein the purine analog is clofarabine or a derivative thereof.
17. The method of any one of claims 1-16, wherein the subject suffers a tumor.
18. The method of claim 17, wherein the tumor is cancer.
- 25 19. The method of claim 17 or 18, wherein the tumor is blood cancer.
20. The method of claim 17, wherein the tumor is B-cell malignancy.
21. The method of any one of claims 17-20, wherein the tumor is leukemia or lymphoma.

22. The method of any one of claims 17-21, wherein the tumor is selected from the group consisting of B cell leukemia, B cell lymphoma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), non-Hodgkin's lymphoma, Burkitt lymphoma, acute myeloid leukemia (AML) and Mixed-phenotype acute leukemia (MPAL).
23. The method of claim 22, wherein the tumor is B cell acute-lymphoblastic leukemia (B-ALL).
24. The method of any one of claims 1-23, wherein the subject has a high disease burden at the time of or immediately prior to the preconditioning.
25. The method of claim 24, wherein the subject who has high disease burden has more than about 5% lymphoblasts in bone marrow, detectable peripheral blood lymphoblasts in the subject, a CNS3 status, and/or non-CNS extramedullary (EM) site of disease
26. The method of claim 25, wherein the CNS3 status is determined by detecting at least about 5 white blood cells/ml cerebrospinal fluid and at least one lymphoblast in the cerebrospinal fluid.
27. The method of any one of claims 1-26, wherein the antigen-recognizing receptor is a T cell receptor (TCR) or a chimeric antigen receptor (CAR).
28. The method of any one of claims 1-27, wherein the antigen-recognizing receptor is a CAR.
29. The method of claim 27 or 28, wherein the CAR comprises an extracellular antigen-recognition domain and an intracellular signaling domain.
30. The method of claim 29, wherein the extracellular antigen-binding domain binds to an antigen.
31. The method of claim 30, wherein the antigen is a tumor antigen or a pathogen antigen.
32. The method of claim 30, wherein the antigen is CD19.
33. The method of any one of claims 29-32, wherein intracellular signaling domain comprises a CD3 ζ polypeptide.

34. The method of any one of claims 29-33, wherein the intracellular signaling domain of the CAR further comprises at least one co-stimulatory signaling region.
35. The method of claim 34, wherein the costimulatory signaling region comprises an intracellular domain of 4-1BB or an intracellular domain of CD28.
- 5 36. The method of any one of claims 1-35, wherein the cell is an immunoresponsive cell.
37. The method of any one of claims 1-36, wherein the cell is a cell of the lymphoid lineage or a cell of the myeloid lineage.
38. The method of any one of claims 1-37, wherein the cell is selected from the
10 group consisting of T cells, Natural Killer (NK) cells, stem cells from which lymphoid cells may be differentiated.
39. The method of any one of claims 1-38, wherein the cell is a T cell.
40. The method of claim 39, wherein the T cell is selected from the group consisting of a cytotoxic T lymphocyte (CTL), a $\gamma\delta$ T cell, a tumor-reactive lymphocyte, a tumor-
15 infiltrating lymphocyte (TIL), a regulatory T cell, and a Natural Killer T (NKT) cell.

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**FIG. 1**

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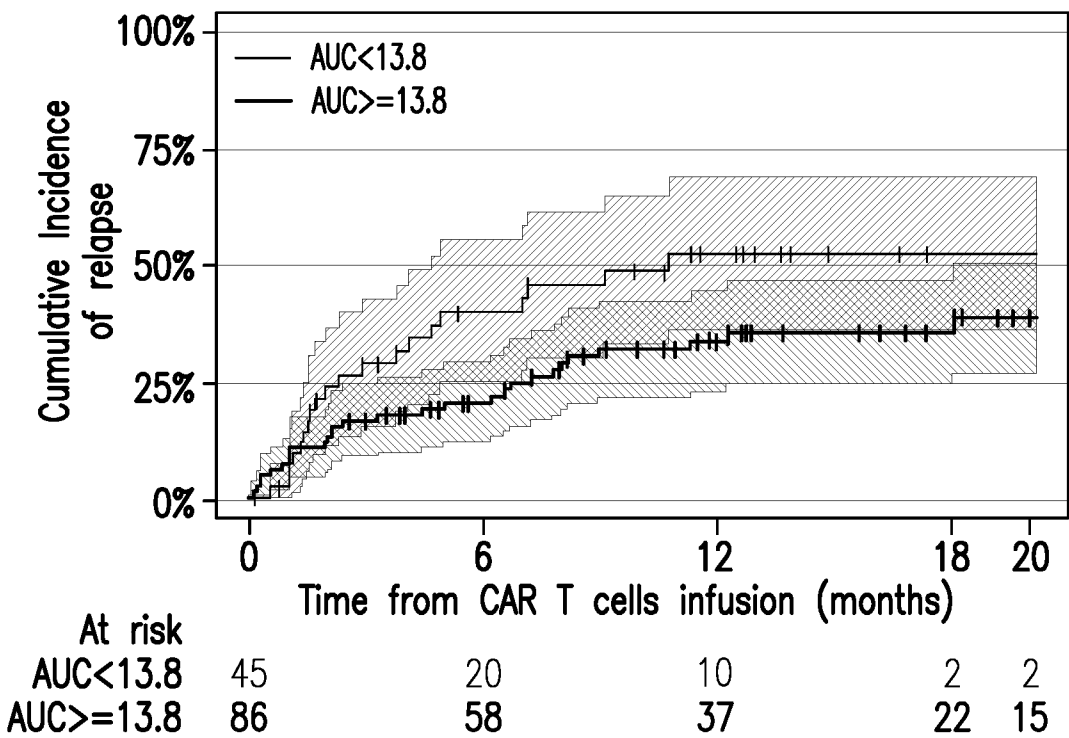


Fig. 2A

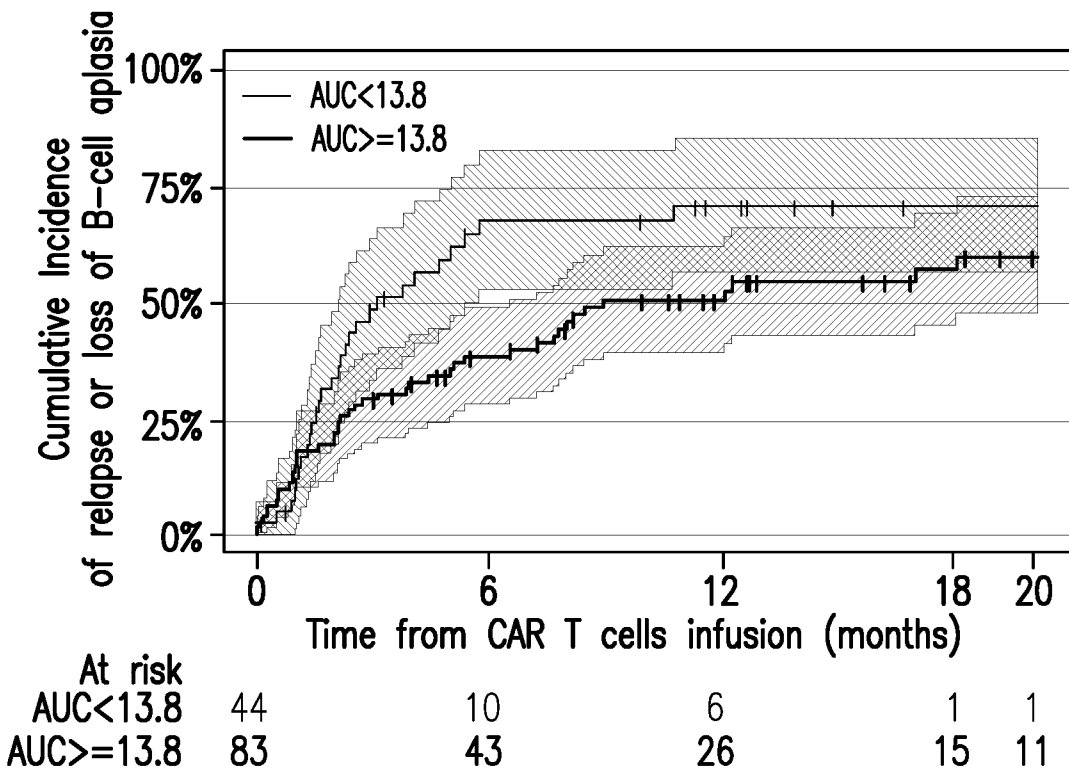


Fig. 2B

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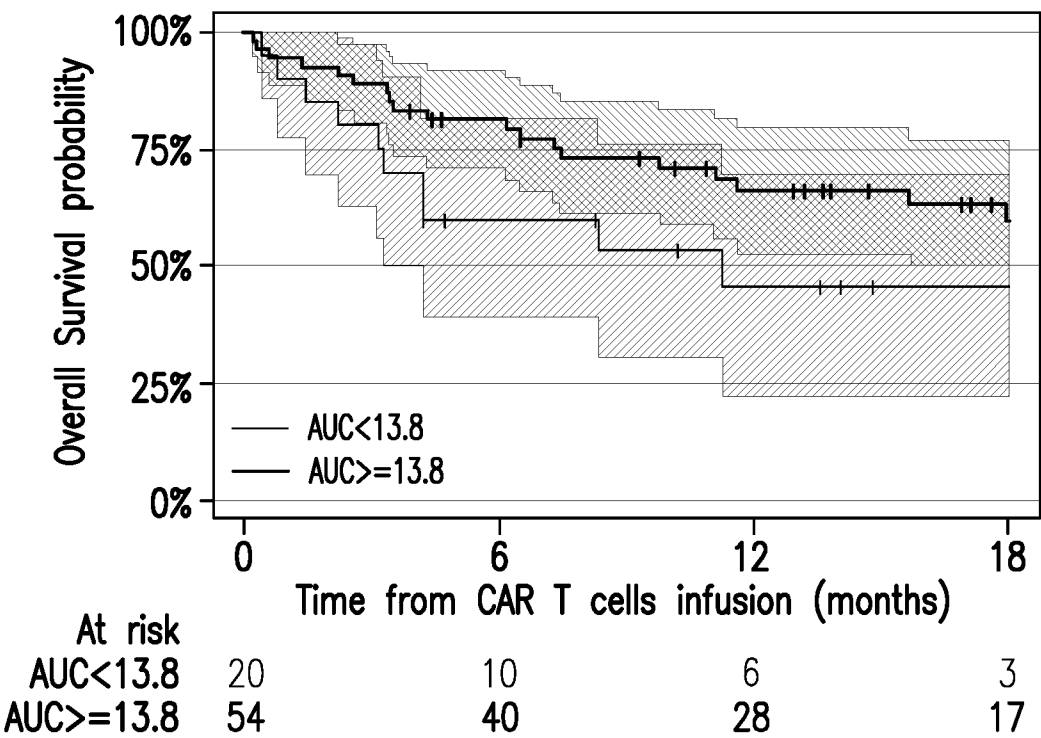


Fig. 3A

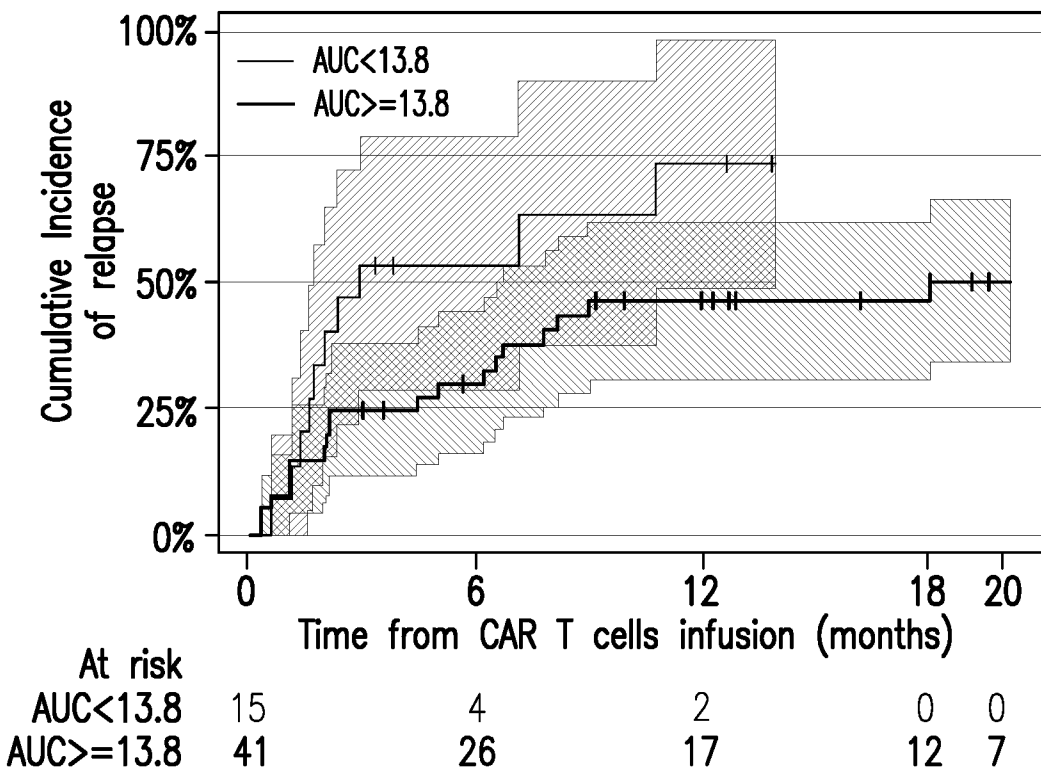


Fig. 3B

Patient Flow Diagram

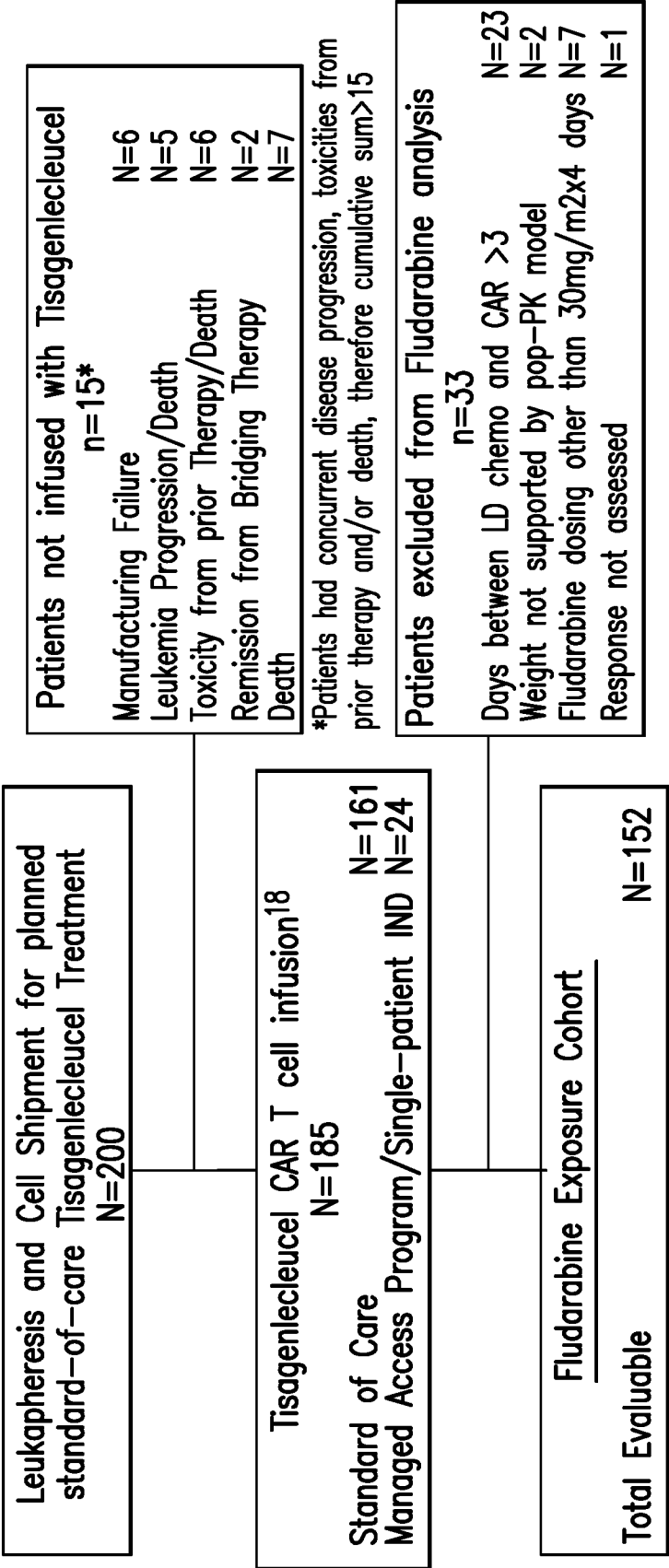


FIG. 4

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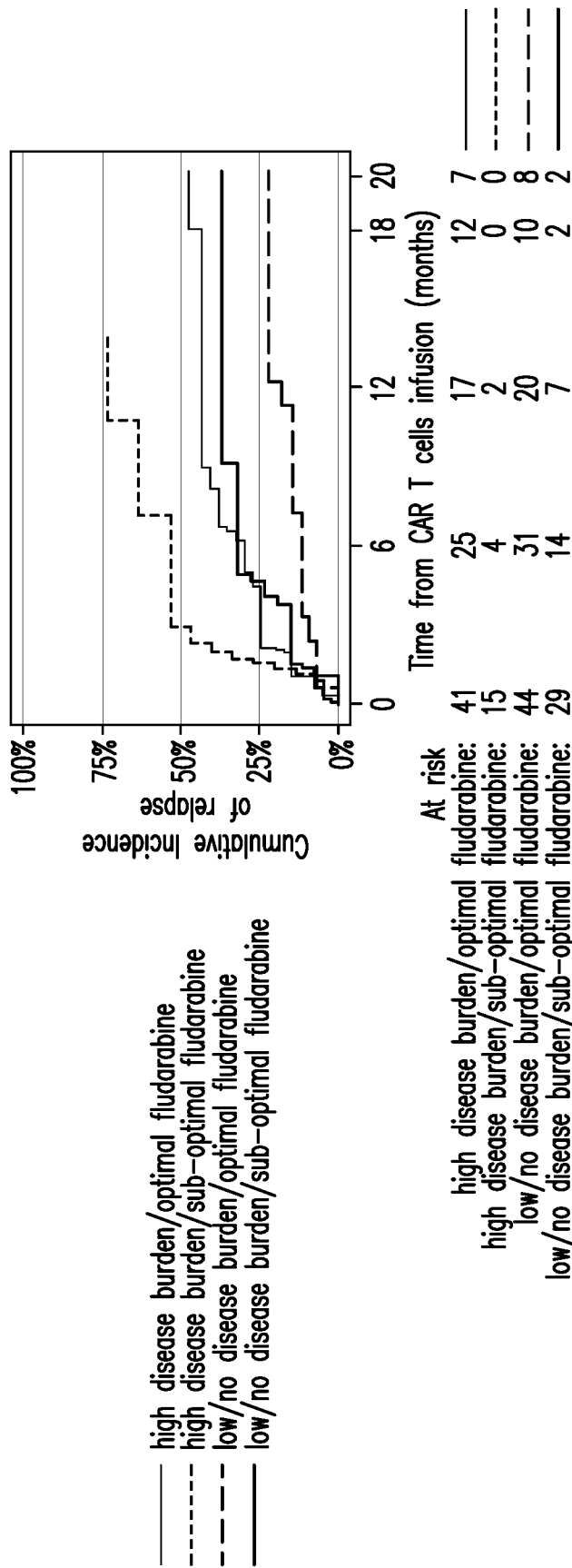


Fig. 5A

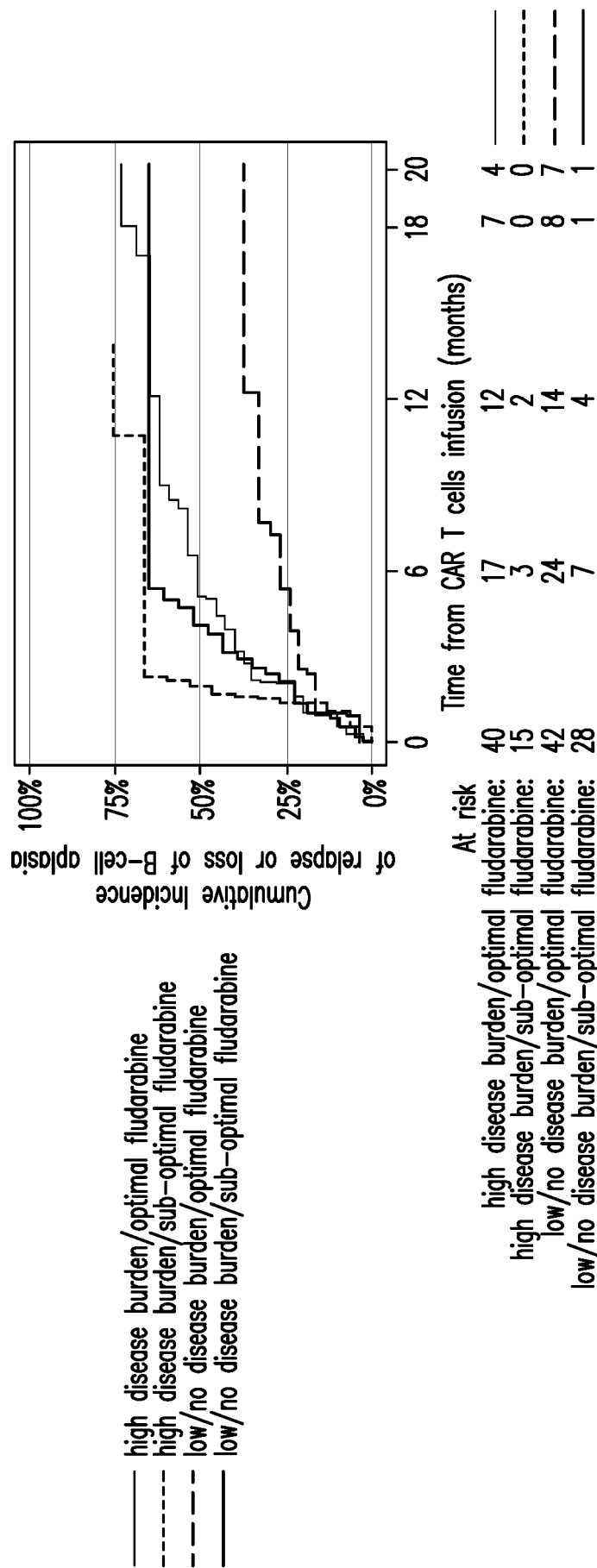


Fig. 5B

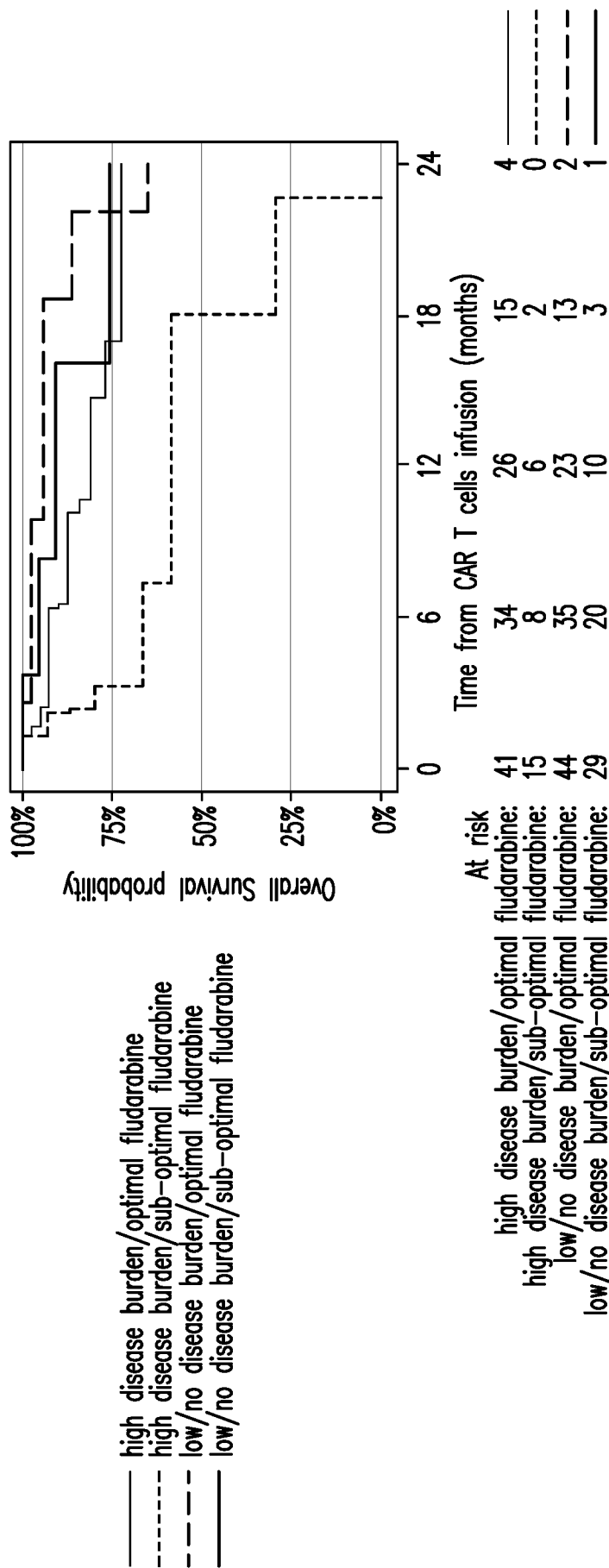


Fig. 5C

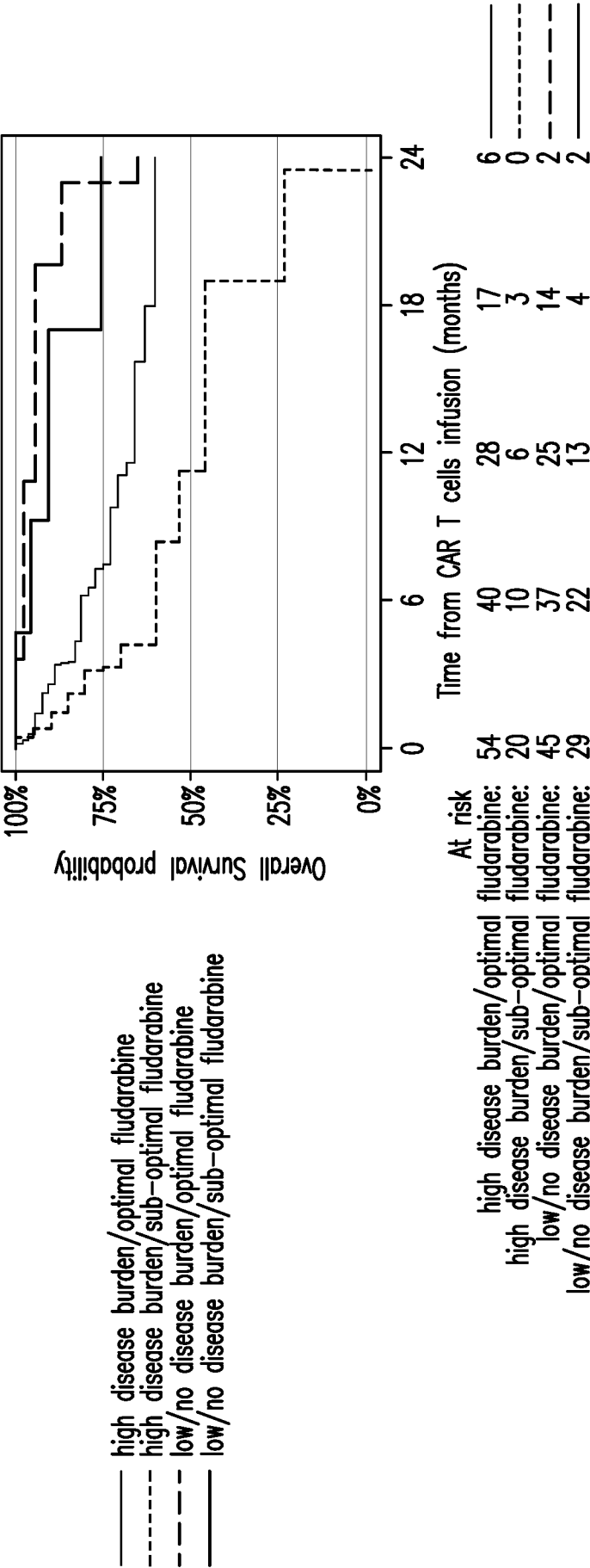


Fig. 5D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/20489

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61P 35/00; C07K 16/28; C12N 5/0783 (2022.01)

CPC - A61K 35/17; A61K 2035/122; C07K 2319/33; C12N 5/0636; A61P 37/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	(LANGENHORST, JB ET AL.). "Fludarabine exposure in the conditioning prior to allogeneic hematopoietic cell transplantation predicts outcomes" pages 2179-2187. Blood Advances. Vol. 3, No. 14. 23 July 2019; Abstract; Page 2184, Column 1 Paragraph 2 – Column 2 Paragraph 1; Page 2186, Column 1, Paragraph 2; DOI: 10.1182/bloodadvances.2018029421	1-5
A	WO 2020/033585 A1 (THE BROAD INSTITUTE, INC.) 13 February 2020; entire document	1-5
A	WO 2019/227003 A1 (NOVARTIS AG) 28 November 2019; entire document	1-5
A	WO 2016/132366 A1 (ENLIVEX THERAPEUTICS LTD.) 25 August 2016; entire document	1-5

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

31 May 2022 (31.05.2022)

Date of mailing of the international search report

JUN 27 2022

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/20489

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-40
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.