involving stimulation of an immune response, such as targeted T cell therapies. Accordingly, novel therapeutic strategies for treating neoplasia are urgently required.

SUMMARY

In one aspect, the present disclosure refers to an immunoresponsive cell comprising:

1) a chimeric antigen receptor (CAR) that binds to ROR1 and comprises an intracellular signaling domain of a CD3ζ polypeptide and an intracellular signaling domain of a co-stimulatory receptor; and

2) a nucleic acid encoding a recombinant CD40L.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising an effective amount of an immunoresponsive cell as described herein and a pharmaceutically acceptable excipient.

In another aspect, the present disclosure refers to a kit for treating a neoplasm, the kit comprising an immunoresponsive cell comprising: a) a chimeric antigen receptor (CAR) that binds to ROR1 and comprises an intracellular signaling domain of a CD3ζ polypeptide and an intracellular signaling domain of a co-stimulatory receptor; and b) a nucleic acid encoding a recombinant CD40L.

The present disclosure generally provides immunoresponsive cells (e.g., T cells, Natural Killer (NK) cells, cytotoxic T lymphocytes (CTLs), and regulatory T cells), expressing an antigen binding receptor (e.g., CAR or TCR) having immune cell activating activity and a single-chain variable fragment (scFv) that binds an antigen having immunosuppressive activity (e.g., CD47, PD-1, CTLA-4, and ligands thereof), thereby reducing or eliminating the immunosuppressive activity of the antigen.

The disclosure further provides immunoresponsive cells (e.g., T cells, Natural Killer (NK) cells, cytotoxic T lymphocytes (CTLs), and regulatory T cells), expressing an antigen binding receptor (e.g., CAR or TCR) having immune cell activating activity and a single-chain variable fragment (scFv) that binds an antigen having immunostimulatory or proinflammatory activity (e.g., CD28, OX-40, 4-1BB, CD40 and ligands thereof), thereby enhancing the immunostimulatory activity of the antigen.

The disclosure further provides immunoresponsive cells (e.g., T cells, Natural Killer (NK) cells, cytotoxic T lymphocytes (CTLs), and regulatory T

cells), expressing an antigen binding receptor (e.g., CAR or TCR) having immune cell activating activity and CD40L, for example, exogenous CD40L (CD40L that has been introduced directly or indirectly into the cell (for example, via a vector of naked nucleic acid comprising a nucleic acid sequence encoding CD40L), as compared to endogenous CD40L arising in the cell itself), thereby enhancing the immunostimulatory activity of the antigen.

Accordingly, the disclosure provides methods of using such immunoresponsive cells for the treatment of neoplasia, infectious disease, and other pathologies.

In one example, the disclosure provides an isolated immunoresponsive cell having an antigen recognizing receptor that binds an antigen, where the binding activates the immunoreponsive cell, and a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity.

In another example, the disclosure provides a method of treating or preventing neoplasia in a subject, the method comprising administering, to the subject, an

effective amount of an immunoresponsive cell having an antigen recognizing receptor that binds a first antigen, where the binding activates the immunoreponsive cell, and a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, thereby treating or preventing neoplasia in the subject. In non-limiting examples, the antigen recognizing receptor is a CAR.

In another example, the disclosure provides a method of reducing tumor burden in a subject, the method involving administering, to the subject, an effective amount of an immunoresponsive cell having an antigen recognizing receptor that binds a first antigen, where the binding activates the immunoreponsive cell, and a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, thereby inducing tumor cell death in the subject. In still another example, the disclosure provides a method of lengthening survival of a subject having neoplasia, the method involving administering, to the subject, an effective amount of an immunoresponsive cell having an antigen recognizing receptor that binds a first antigen, where the binding activates the immunoreponsive cell, and a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, thereby lengthening survival of the subject. In non-limiting examples, the antigen recognizing receptor is a CAR.

In various non-limiting examples, the disclosure provides a method of increasing immune-activating cytokine production in response to a cancer cell in a subject, comprising administering, to the subject, an immunoresponsive cell having an antigen recognizing receptor that binds an antigen of the cancer cell and further expressing exogenous CD40L. In a particular non-limiting example, the immune-activating cytokine is IL-12. In non-limiting examples, the antigen recognizing receptor is a CAR.

In various non-limiting examples, the disclosure provides a method of increasing immune-activating cytokine production in response to a pathogen in a subject, comprising administering, to the subject, an immunoresponsive cell having an antigen recognizing receptor that binds an antigen of the pathogen and further expressing exogenous CD40L. In a particular non-limiting example, the immune-activating cytokine is IL-12. In non-limiting examples, the antigen recognizing receptor is a CAR.

In various non-limiting examples, the disclosure provides a method of increasing a $CD8^+$ cytotoxic T cell response to a cancer cell in a subject, comprising administering, to the subject, an immunoresponsive cell having an antigen recognizing receptor that binds an antigen of the cancer cell and further expressing exogenous CD40L. In non-limiting examples, the antigen recognizing receptor is a CAR.

In various non-limiting examples, the disclosure provides a method of increasing a $CD8^+$ cytotoxic T cell response to a pathogen in a subject, comprising administering, to the subject, an immunoresponsive cell having an antigen recognizing receptor that binds an antigen of the pathogen and further expressing exogenous CD40L. In non-limiting examples, the antigen recognizing receptor is a CAR.

In various non-limiting examples, the disclosure provides a method of promoting dendritic cell maturation in a subject having a cancer, comprising administering, to the subject, an immunoresponsive cell having an antigen recognizing receptor that binds an antigen of a cell of the cancer and further expressing exogenous CD40L. In non-limiting examples, the antigen recognizing receptor is a CAR.

In various non-limiting examples, the disclosure provides a method of promoting dendritic cell maturation in a subject having a disease caused by a pathogen, comprising administering, to the subject, an immunoresponsive cell having an antigen recognizing receptor that binds an antigen of the pathogen and further expressing exogenous CD40L. In non-limiting examples, the antigen recognizing receptor is a CAR.

In still another example, the disclosure provides a method of treating or preventing neoplasia in a subject, the method comprising administering, to the subject, an effective amount of an immunoresponsive cell having an antigen recognizing receptor that binds a first antigen, where the binding activates the immunoreponsive cell, and expressing exogenous CD40L, thereby treating or preventing a neoplasia in the subject. In non-limiting examples, the antigen recognizing receptor is a CAR.

In another example, the disclosure provides a method of reducing tumor burden in a subject, the method involving administering, to the subject, an effective amount of an immunoresponsive cell having an antigen recognizing receptor that binds a first antigen, where the binding activates the immunoreponsive cell, and expressing exogenous CD40L, thereby inducing tumor cell death in the subject. In still another example, the disclosure provides a method of lengthening survival of a subject having neoplasia, the method involving administering, to the subject, an effective amount of an immunoresponsive cell having an antigen recognizing receptor that binds a first antigen, where the binding activates the immunoreponsive cell, and expressing exogenous CD40L, thereby lengthening survival of the subject.

In yet another example, the disclosure provides a method of treating blood cancer in a subject in need thereof, the method involving administering to the subject a therapeutically effective amount of a T cell having an antigen recognizing receptor that binds CD19, where the binding activates the immunoreponsive cell, and a soluble single-chain variable fragment (scFv) that binds one or more of CD47, PD-1, CTLA-4, and ligands thereof, thereby treating blood cancer in the subject. In non-limiting examples, the antigen recognizing receptor is a CAR.

In yet another example, the disclosure provides a method of treating blood cancer in a subject in need thereof, the method involving administering to the subject a therapeutically effective amount of a T cell having an antigen recognizing receptor that binds CD19, where the binding activates the immunoreponsive cell, and expressing exogenous CD40L, thereby treating blood cancer in the subject. In non-limiting examples, the antigen recognizing receptor is a CAR.

In one example, the disclosure provides a method for producing an antigen-specific immunoresponsive cell, the method involving introducing into the immunoresponsive cell a nucleic acid sequence that encodes a single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, where the immunoresponsive cell has an antigen recognizing receptor that binds an antigen. In non-limiting examples, the antigen recognizing receptor is a CAR.

In one example, the disclosure provides a method for producing an antigen-specific immunoresponsive cell, the method involving introducing into the immunoresponsive cell a nucleic acid sequence that encodes CD40L, where the

immunoresponsive cell has an antigen recognizing receptor that binds an antigen. In non-limiting examples, the nucleic acid sequence that encodes CD40L is operably linked to a promoter element constitutively or inducibly expressed in the immunoresponsive cell, optionally comprised in a vector. In non-limiting examples, the antigen recognizing receptor is a CAR. In non-limiting examples, the disclosure provides for a nucleic acid comprising sequence encoding a CAR and encoding CD40L, each optionally operably linked to a promoter element constitutively or inducibly expressed in the immunoresponsive cell, and said nucleic acid may optionally be comprised in a vector.In one example, the disclosure provides a vector having a nucleic acid sequence encoding an antigen recognizing receptor that binds an antigen, and a nucleic acid sequence encoding a soluble single-chain variable fragment (scFv) that binds a polypeptide having immunosuppressive activity or immunostimulatory activity. In non-limiting examples, the antigen recognizing receptor is a CAR.

In one example, the disclosure provides a vector having a nucleic acid sequence encoding an antigen recognizing receptor that binds an antigen, and a nucleic acid sequence encoding CD40L. In non-limiting examples, the antigen recognizing receptor is a CAR. In one specific non-limiting example, the disclosure provides for a retroviral vector containing an anti-CD19 CAR (1928z)- and a CD40L-encoding nucleic acid.

In a related example, the disclosure provides a pharmaceutical composition containing an effective amount of an immunoresponsive cell of any example of the disclosure delineated herein in a pharmaceutically acceptable excipient. In another related example, the disclosure provides a pharmaceutical composition for the treatment of a neoplasia containing an effective amount of a tumor antigen- specific T cell of any example of the disclosure delineated herein in a pharmaceutically acceptable excipient.

In an additional example, the disclosure provides a kit for treatment of a neoplasia, pathogen infection, an autoimmune disorder, or an allogeneic transplant, the kit containing an immunoresponsive cell having an antigen recognizing receptor that binds an antigen and activates the immunoreponsive cell, and a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity. In non-limiting examples, the antigen recognizing receptor is a CAR. In particular examples, the kit further contains

written instructions for using the cell for the treatment of a subject having a neoplasia, a pathogen infection, an autoimmune disorder, or an allogeneic transplant.

In an additional example, the disclosure provides a kit for treatment of a neoplasia, pathogen infection, an autoimmune disorder, or an allogeneic transplant, the kit containing an immunoresponsive cell having an antigen recognizing receptor that binds an antigen and activates the immunoreponsive cell, and expressing exogenous CD40L. In non-limiting examples, the antigen recognizing receptor is a CAR. In particular examples, the kit further contains written instructions for using the cell for the treatment of a subject having a neoplasia, a pathogen infection, an autoimmune disorder, or an allogeneic transplant.

In an additional example, the disclosure provides a kit for treatment of a neoplasia, pathogen infection, an autoimmune disorder, or an allogeneic transplant, the kit comprising a nucleic acid encoding a CAR which recognizes an antigen of the neoplasia, pathogen, autoimmune disorder, or transplant to be treated, and a nucleic acid encoding a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity. Optionally one or both nucleic acids may be comprised in a vector, which may be the same vector (bicistronic) or separate vectors. The nucleic acid encoding the CAR and/or the nucleic acid encoding the scFv may each be operably linked to a promoter which may be the same or different promoters. In particular examples, the kit further contains written instructions for using the cell for the treatment of a subject having a neoplasia, a pathogen infection, an autoimmune disorder, or an allogeneic transplant.

In an additional example, the disclosure provides a kit for treatment of a cancer, the kit comprising a nucleic acid encoding a CAR which recognizes an antigen of the cancer, and a nucleic acid encoding a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity. Optionally one or both nucleic acids may be comprised in a vector, which may be the same vector (bicistronic) or separate vectors. The nucleic acid encoding the CAR and/or the nucleic acid encoding the scFv may each be operably linked to a promoter which may be the same or different promoters. In particular examples, the kit further contains written instructions for using the cell for the treatment of a subject having a cancer.

In an additional example, the disclosure provides a kit for treatment of a cancer or pathogen-mediated disorder, the kit comprising a nucleic acid encoding a CAR

which recognizes an antigen of the cancer or pathogen, and a nucleic acid encoding a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity. Optionally one or both nucleic acids may be comprised in a vector, which may be the same vector (bicistronic) or separate vectors. The nucleic acid encoding the CAR and/or the nucleic acid encoding the scFv may each be operably linked to a promoter which may be the same or different promoters. In particular examples, the kit further contains written instructions for using the cell for the treatment of a subject having a cancer or disorder.In an additional example, the disclosure provides a kit for treatment of a cancer or pathogen-mediated disorder, the kit comprising a nucleic acid encoding a CAR which recognizes an antigen of the cancer or pathogen, and a nucleic acid encoding CD40L. Optionally one or both nucleic acids may be comprised in a vector, which may be the same vector (bicistronic) or separate vectors. The nucleic acid encoding the CAR and/or the nucleic acid encoding CD40L may each be operably linked to a promoter which may be the same or different promoters. In particular examples, the kit further contains written instructions for using the cell for the treatment of a subject having a cancer or disorder.

In various examples of any of the examples delineated herein, the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a human embryonic stem cell, and a pluripotent stem cell from which lymphoid cells may be differentiated. In various examples of any of the examples delineated herein, the immunoresponsive cell is autologous.

In various examples of any of the examples delineated herein, the antigen recognizing receptor is a T cell receptor (TCR) or chimeric antigen receptor (CAR). In various examples of any of the examples delineated herein, the antigen recognizing receptor is exogenous or endogenous. In various examples of any of the examples delineated herein, the antigen recognizing receptor is recombinantly expressed. In various examples of any of the examples delineated herein, the antigen recognizing receptor is expressed from a vector. In various examples of any of the examples delineated herein, the intracellular signaling domain of the antigen recognizing receptor is the CDζ-chain, CD97, CD11a-CD18, CD2, ICOS, CD27, CD154, CDS, OX40, 4-IBB, CD28 signaling domain, a portion thereof,

or combinations thereof. In non-limiting examples, the antigen recognizing receptor is a CAR comprising at least a portion of CD28, 4-IBB, and/or CD3ζ-chain (see, e.g., Zhong et al., 2010, Molecular Ther. 18(2):413-420), together with an antigen binding portion. In non-limiting examples, the antigen recognizing receptor is a CAR described in Kohn et al., 2011, Molecular Ther. 19(3):432-438), optionally where the antigen binding portion is substituted with amino acid sequence that binds to another tumor or pathogen antigen. In various examples, the cell expresses a recombinant or an endogenous antigen receptor that is 1928z or 4H1128z.

In various examples of any of the examples delineated herein, the antigen is a tumor or pathogen antigen. In various examples of any of the examples delineated herein, the tumor antigen is one or more of CD19, MUC16, MUC1, CAlX, CEA, CDS, CD7, CD10, CD20, CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, a cytomegalovirus (CMV) infected cell antigen, EGP-2, EGP-40, EpCAM, erb-B2,3,4, FBP, Fetal acetylcholine receptor, folate receptor-α, GD2, GD3, HER-2, hTERT, IL-13R-a2, κ-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, Mesothelin, NKG2D ligands, NY-ES0-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, or WT-1. In particular examples, the antigen is CD19 or MUC16. Amino acid sequences that specifically bind to said antigens are known in the art or may be prepared using methods known in the art; examples include immunoglobulins, variable regions of immunoglobulins (e.g. variable fragment ("Fv") or bivalent variable fragment ("Fab")), single chain antibodies, etc.

In various examples of any of the examples delineated herein, the soluble scFv is secreted. In various examples of any of the examples delineated herein, the scFv is expressed from a vector. In various examples of any of the examples delineated herein, the immunosuppressive polypeptide is one or more of CD47, PD-1, CTLA-4, and ligands thereof. In various examples of any of the examples delineated herein, the immunostimulatory polypeptide is one or more of CD28, OX-40, 4-1BB, and ligands thereof. In various examples of any of the examples delineated herein, the soluble scFv enhances an immune response of the immunoreponsive cell.

In various examples of any of the examples delineated herein, the immunoresponsive cell secretes a cytokine. In various examples of any of the examples delineated herein, the cytokine is expressed from a vector. In various

examples of any of the examples delineated herein, the pharmaceutical composition containing an immunoresponsive cell of the disclosure contains a cytokine. In various examples of any of the examples delineated herein, an immunoresponsive cell of the disclosure is administered with a cytokine. In various examples of any of the examples delineated herein, the cytokine is one or more of IL-2, IL-3, IL-6, IL-11, IL7, IL12, ILl5, IL21, granulocyte macrophage colony stimulating factor, alpha, beta or gamma interferon and erythropoietin.

In various examples of any of the examples delineated herein, the method reduces the number of tumor cells, reduces tumor size, eradicates the tumor in the subject, reduces the tumor burden in the subject, and/or eradicates the tumor burden in the subject.

Illustrative neoplasms for which the disclosure can be used include, but are not limited to leukemias (e.g., acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemi a, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), polycythemia vera, lymphoma (Hodgkin's disease, non-Hodgkin's disease), 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, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, nile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodenroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma).

In various non-limiting examples of any of the examples delineated herein, the neoplasia is one or more of blood cancer, B cell leukemia, multiple myeloma,

lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, non-Hodgkin's lymphoma, and ovarian cancer. In certain examples, the blood cancer is one or more of B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, and non-Hodgkin's lymphoma. In particular examples, the neoplasia is B cell leukemia, the antigen is CD19, and the polypeptide that has immunosuppressive activity is one or more of CD47, PD-1, CTLA-4, and ligands thereof. In particular examples, the neoplasia is multiple myeloma, the antigen is CD19, and the polypeptide that has immunosuppressive activity is one or more of CD47, PD-1, CTLA-4, and ligands thereof. In particular examples, the neoplasia is acute lymphoblastic leukemia (ALL), the antigen is CD19, and the polypeptide that has immunosuppressive activity is one or more of CD47, PD-1, CTLA-4, and ligands thereof. In particular examples, the neoplasia is chronic lymphocytic leukemia, the antigen is CD19, and the polypeptide that has immunosuppressive activity is one or more of CD47, PD-1, CTLA-4, and ligands thereof. In particular examples, the neoplasia is non- Hodgkin's lymphoma, the antigen is CD19, and the polypeptide that has immunosuppressive activity is one or more of CD47, PD-1, CTLA-4, and ligands thereof. In particular examples, the neoplasia is ovarian cancer, the antigen is MUC16, and the polypeptide that has immunosuppressive activity is one or more of CD47, PD-1, CTLA-4, and ligands thereof.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: 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 following terms have the meanings ascribed to them below, unless specified otherwise.

By "activates an immunoresponsive cell" is meant induction of signal transduction or changes in protein expression in the cell resulting in initiation of an immune response. For example, when CD3 Chains cluster in response to ligand

What is claimed is:

1. An immunoresponsive cell comprising:

1) a chimeric antigen receptor (CAR) that binds to ROR1 and comprises an intracellular signaling domain of a CD3ζ polypeptide and an intracellular signaling domain of a co-stimulatory receptor; and

2) a nucleic acid encoding a recombinant CD40L.

2. The immunoresponsive cell of claim 1, wherein the nucleic acid is comprised in a vector.

3. The immunoresponsive cell of claim 2, wherein the vector is a viral vector.

4. The immunoresponsive cell of claim 3, wherein the viral vector is a retroviral vector.

5. The immunoresponsive cell of claim 1, wherein the immunoresponsive cell is selected from the group consisting of T cells, Natural Killer cells, cytotoxic T cells, regulatory T cells, and combinations thereof.

6. The immunoresponsive cell of claim 5, wherein the immunoresponsive cell is a T cell.

7. The immunoresponsive cell of claim 1, wherein the recombinant CD40L enhances an immune response of the immunoresponsive cell.

8. The immunoresponsive cell of claim 1, wherein the co-stimulatory receptor is CD28, 4-1BB, or OX-40.

9. The immunoresponsive cell of claim 1, wherein the co-stimulatory receptor is CD28.

10. A pharmaceutical composition comprising an effective amount of an immunoresponsive cell of claim 1 and a pharmaceutically acceptable excipient.

11. The pharmaceutical composition of claim 10, wherein said composition is for treating a neoplasm and the immunoresponsive cell is a T cell.

12. A kit for treating a neoplasm, the kit comprising an immunoresponsive cell comprising: a) a chimeric antigen receptor (CAR) that binds to ROR1 and comprises an intracellular signaling domain of a CD3ζ polypeptide and an intracellular signaling domain of a co-stimulatory receptor; and b) a nucleic acid encoding a recombinant CD40L.

13. The kit of claim 12, wherein the kit further comprises written instructions for using said cell for the treatment of a subject having a neoplasm.

14. The kit of claim 12, wherein the co-stimulatory receptor is CD28, 4-1BB, or OX40.

15. The kit of claim 12, wherein the co-stimulatory receptor is CD28.