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 embodiments, the kit further contains written instructions for using the cell for the treatment of a subject having a cancer or disorder.In an additional aspect, the invention 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 embodiments, the kit further contains written instructions for using the cell for the treatment of a subject having a cancer or disorder.

In an additional aspect, the invention provides an immunoresponsive cell comprising:

1) an antigen recognizing receptor that binds a first antigen, wherein the binding is capable of activating the immunoresponsive cell, and

2) a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different from the first antigen.

In an additional aspect, the invention provides a use of an immunoresponsive cell, wherein the immunoresponsive cell comprises (a) an antigen recognizing receptor that binds a first antigen, wherein the binding is capable of activating the immunoresponsive cell, and (b) a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different from the first antigen in the manufacture of a medicament for reducing tumor burden in a subject.

In an additional aspect, the invention provides a use of an immunoresponsive cell, wherein the immunoresponsive cell comprises (a) comprising an antigen recognizing receptor that binds a first antigen, wherein the binding is capable of activating the immunoresponsive cell, and (b) a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity,

wherein the polypeptide is different from the first antigen in the manufacture of a medicament for treating or preventing a neoplasia in the subject.

In an additional aspect, the invention provides a method for producing an immunoresponsive cell as described herein, the method comprising:

(a) introducing into an antigen-specific cell a nucleic acid comprising a sequence that encodes the single-chain variable fragment (scFv) that binds the polypeptide that has immunosuppressive activity or immunostimulatory activity, or

(b) introducing into a cell one or more nucleic acids, which one or more nucleic acids comprise a nucleic acid sequence encoding the antigen recognizing receptor and a nucleic acid sequence encoding the scFv.

In an additional aspect, the invention provides a use of a T cell, wherein the T cell comprises (a) an antigen recognizing receptor that binds CD19, wherein the binding is capable of activating the immunoresponsive cell, and (b) a soluble single-chain variable fragment (scFv) that binds a polypeptide selected from the group consisting of a CD47 polypeptide and ligands thereof, a PD-1 polypeptide and ligands thereof, a CTLA-4 polypeptide and ligands thereof, and combinations thereof in the manufacture of a medicament for treating blood cancer in a subject in need thereof.

In an additional aspect, the invention provides a vector comprising (a) a nucleic acid sequence encoding an antigen recognizing receptor that binds a first antigen, and (b) a nucleic acid sequence encoding a soluble single chain variable fragment (scFv) that binds a polypeptide having immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different from the first antigen.

In an additional aspect, the invention provides a pharmaceutical composition for the treatment of a neoplasia comprising an effective amount of a tumor antigen-specific T cell as described herein in a pharmaceutically acceptable excipient.

In an additional aspect, the invention provides a kit for treatment of a neoplasia, pathogen infection, an autoimmune disorder, or an allogeneic transplant, the kit comprising an immunoresponsive cell comprising (a) an antigen recognizing receptor that binds a first antigen and the binding is capable of activating the immunoresponsive cell, and (b) a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different from the first antigen.

In an additional aspect, the invention provides a kit for treatment of a neoplasia, pathogen infection, an autoimmune disorder, or an allogeneic transplant comprising (a) a nucleic acid encoding a CAR which recognizes an antigen of the neoplasia, pathogen, autoimmune disorder, or transplant to be treated, and (b) a nucleic acid encoding a soluble

single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different form the antigen.

In an additional aspect, the invention provides a kit for treatment of a cancer, the kit comprising (a) a nucleic acid encoding a CAR which recognizes an antigen of the cancer, and (b) a nucleic acid encoding a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different form the antigen.

In an additional aspect, the invention provides a kit for treatment of a cancer or pathogen-mediated disorder, the kit comprising (a) a nucleic acid encoding a CAR which recognizes an antigen of the cancer or pathogen, and (b) a nucleic acid encoding a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different form the antigen.

In an additional aspect, the invention provides a composition comprising (a) a nucleic acid comprising a sequence encoding an antigen recognizing receptor that binds a first antigen and (b) a nucleic acid comprising a sequence encoding a soluble single-chain variable fragment (scFv) that binds a polypeptide having immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different from the first antigen, wherein the nucleic acid of (a) and/or the nucleic acid of (b) is optionally a vector.

In various embodiments of any of the aspects 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 embodiments of any of the aspects delineated herein, the immunoresponsive cell is autologous.

In various embodiments of any of the aspects delineated herein, the antigen recognizing receptor is a T cell receptor (TCR) or chimeric antigen receptor (CAR). In various embodiments of any of the aspects delineated herein, the antigen recognizing receptor is exogenous or endogenous. In various embodiments of any of the aspects delineated herein, the antigen recognizing receptor is recombinantly expressed. In various embodiments of any of the aspects delineated herein, the antigen recognizing receptor is expressed from a vector. In various embodiments of any of the aspects 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,

Claim

1. An immunoresponsive cell comprising:

1) an antigen recognizing receptor that binds a first antigen, wherein the binding is capable of activating the immunoresponsive cell, and

2) a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different from the first antigen.

2. The immunoresponsive cell of claim 1, wherein the antigen comprises ~~is~~ a tumor or pathogen antigen.

3. The immunoresponsive cell of claim 1, wherein the soluble scFv is secreted by the cell.

4. The immunoresponsive cell of any one of claims 1-3, wherein said antigen recognizing receptor is a T cell receptor (TCR) or a chimeric antigen receptor (CAR).

5. The immunoresponsive cell of any one of claims 1-4, wherein said antigen recognizing receptor is exogenous or endogenous.

6. The immunoresponsive cell of any one of claims 1-5, wherein said antigen recognizing receptor is recombinantly expressed.

7. The immunoresponsive cell of any one of claims 1-6, wherein the antigen recognizing receptor is expressed from a vector.

8. The immunoresponsive cell of any one of claims 1-7, wherein the scFv is expressed from a vector.

9. The immunoresponsive cell of any one of claims 1-8, wherein the cell is of a type selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell.

10. The immunoresponsive cell of any one of claims 1-9, wherein said immunoresponsive cell is autologous.

11. The immunoresponsive cell of any one of claims 1-10, wherein said antigen is a tumor antigen selected from the group consisting of CD19, MUC16, MUCl, CAIX, 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-a, GD2, GD3, HER-2, hTERT, IL-13R-a2, K-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, and WT-1.

12. The immunoresponsive cell of any one of claims 1-11, wherein said polypeptide is selected from the group consisting of a CD47 polypeptide and ligands thereof, a PD-1 polypeptide and ligands thereof, a CTLA-4 polypeptide and ligands thereof, and combinations thereof.

13. The immunoresponsive cell of any one of claims 1-11, wherein said polypeptide has immunostimulatory activity and is selected from the group consisting of a CD28 polypeptide and ligands thereof, an OX-40 polypeptide and ligands thereof, a 4-1BB polypeptide and ligands thereof, and combinations thereof.

14. The immunoresponsive cell of any one of claims 1-13, wherein said antigen is CD19 or MUC16.

15. The immunoresponsive cell of any one of claims 1-14, wherein the intracellular signaling domain of said antigen recognizing receptor comprises an intracellular signaling domain of a CD3ζ-chain, CD97, CD11a-CD18, CD2, ICOS, CD27, CD154, CDS, OX40, 4-1BB, CD28, or combinations thereof.

16. The immunoresponsive cell of any one of claims 1-15, wherein the antigen recognizing receptor that is 1928z or 4H1128z.

17. The immunoresponsive cell of any one of claims 1-16, wherein the soluble scFv enhances an immune response of the immunoresponsive cell.

18. The immunoresponsive cell of claim 16, wherein the 1928z comprises (a) an amino acid sequence having at least 90 %, at least 99 % or at least 95 % identity to SEQ ID NO: 7 (b) the amino acid sequence set forth in SEQ ID NO: 7, optionally without the CDS leader sequence at amino acids 1-18, or (c) an amino acid sequence encoded by SEQ ID NO: 8, with or without the leader sequence encoded by SEQ ID NO: 8.

19. The immunoresponsive cell of claim 16 wherein the 4H1128z comprises (a) an amino acid sequence having at least 90 %, at least 99 % or at least 95 % identity to SEQ ID NO: 9, (b) the amino acid sequence set forth in SEQ ID NO: 9, optionally without the CDS leader sequence at amino acids 1-18, or (c) an amino acid sequence encoded by SEQ ID NO: 10, with or without the leader sequence encoded by SEQ ID NO: 10.

20. The immunoresponsive cell of any one of claims 1-19, wherein the immunoresponsive cell is a T cell.

21. Use of an immunoresponsive cell, wherein the immunoresponsive cell comprises (a) an antigen recognizing receptor that binds a first antigen, wherein the binding is capable of activating the immunoresponsive cell, and (b) a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different from the first antigen in the manufacture of a medicament for reducing tumor burden in a subject.

22. The use of claim 21, wherein the use reduces the number of tumor cells.

23. The use of claim 21, wherein the use reduces tumor size.

24. The use of claim 21, wherein the use eradicates the tumor in the subject.

25. Use of an immunoresponsive cell, wherein the immunoresponsive cell comprises (a) an antigen recognizing receptor that binds a first antigen, wherein the binding is capable of activating the immunoresponsive cell, and (b) a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different from the first antigen in the manufacture of a medicament for treating or preventing a neoplasia in the subject.

26. The use of claim 25, wherein the neoplasia is selected from the group consisting of blood cancer, B cell leukemia, multiple myeloma, lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, non-Hodgkin's lymphoma, and ovarian cancer.

27. The use of claim 26, wherein the neoplasia is B cell leukemia, the antigen is CD19, and the polypeptide has immunosuppressive activity is selected from the group consisting of a CD47 polypeptide and ligands thereof, a PD-1 polypeptide and ligands thereof, a CTLA-4 polypeptide and ligands thereof, and combinations thereof.

28. The use of claim 26, wherein the neoplasia is multiple myeloma, the antigen is CD19, and the polypeptide has immunosuppressive activity is of CD47, PD-1, CTLA-4, and ligands thereof.

29. The use of claim 26, wherein the neoplasia is acute lymphoblastic leukemia (ALL), the antigen is CD19, and the polypeptide has immunosuppressive activity is selected from the group consisting of a CD47 polypeptide and ligands thereof, a PD-1 polypeptide and ligands thereof, a CTLA-4 polypeptide and ligands thereof, and combinations thereof.

30. The use of claim 26, wherein the neoplasia is chronic lymphocytic leukemia, the antigen is CD19, and the polypeptide has immunosuppressive activity is selected from the group consisting of a CD47 polypeptide and ligands thereof, a PD-1 polypeptide and ligands thereof, a CTLA-4 polypeptide and ligands thereof, and combinations thereof.

31. The use of claim 26, wherein the neoplasia is non-Hodgkin's lymphoma, the antigen is CD19, and the polypeptide has immunosuppressive activity is selected from the group consisting of a CD47 polypeptide and ligands thereof, a PD-1 polypeptide and ligands thereof, a CTLA-4 polypeptide and ligands thereof, and combinations thereof.

32. The use of claim 26, wherein the neoplasia is ovarian, the antigen is MUC16, and the polypeptide that has immunosuppressive activity is selected from the group consisting of a CD47 polypeptide and ligands thereof, a PD-1 polypeptide and ligands thereof, a CTLA-4 polypeptide and ligands thereof, and combinations thereof.

33. The use of any one of claims 25-32, wherein the soluble scFv is secreted.

34. The use of any one of claims 25-33, wherein the scFv is expressed from a vector.

35. The use of any one of claims 25-34, wherein the soluble scFv enhances an immune response of the immunoresponsive cell.

36. The use of any one of claims 25-35, wherein said antigen recognizing receptor is a T cell receptor (TCR) or chimeric antigen receptor (CAR).

37. The use of any one of claims 25-36, wherein said antigen recognizing receptor is exogenous or endogenous.

38. The use of any one of claims 25-37, wherein said antigen recognizing receptor is recombinantly expressed.

39. The use of any one of claims 25-38, wherein the antigen recognizing receptor is expressed from a vector.

40. The use of any one of claims 25-39, wherein the cell is of a type selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell.

41. The use of any one of claims 25-40, wherein the use reduces or eradicates the tumor burden in the subject.

42. A method for producing an immunoresponsive cell of any one of claims 1-20, the method comprising:

(a) introducing into an antigen-specific cell a nucleic acid comprising a sequence that encodes the single-chain variable fragment (scFv) that binds the polypeptide that has immunosuppressive activity or immunostimulatory activity, or

(b) introducing into a cell one or more nucleic acids, which one or more nucleic acids comprise a nucleic acid sequence encoding the antigen recognizing receptor and a nucleic acid sequence encoding the scFv.

43. The method of claim 42, wherein the soluble scFv is secreted.

44. The method of claim 42 or 43, wherein the scFv is expressed from a vector.

45. The method of any one of claims 42-44, wherein the soluble scFv enhances an immune response of the immunoresponsive cell.

46. The method of any one of claims 42-45, wherein said immunosuppressive polypeptide is selected from the group consisting of a CD47 polypeptide and ligands thereof, a PD-1 polypeptide and ligands thereof, a CTLA-4 polypeptide and ligands thereof, and combinations thereof.

47. The method of any one of claims 42-45, wherein said immunostimulatory polypeptide is selected from the group consisting a CD28 polypeptide and ligands thereof, an OX-40 polypeptide and ligands thereof, a 4-1BB polypeptide and ligands thereof, and combinations thereof.

48. The method of any one of claims 42-47, wherein said antigen recognizing receptor is a T cell receptor (TCR) or chimeric antigen receptor (CAR).

49. The method of any one of claims 42-48, wherein said antigen recognizing receptor is exogenous or endogenous.

50. The method of any one of claims 42-49, wherein said antigen recognizing receptor is recombinantly expressed.

51. The method of any one of claims 42-50, wherein the antigen recognizing receptor is expressed from a vector.

52. The method of any one of claims 42-51, wherein the cell is of a type selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell.

53. The method of any one of claims 42-52, wherein the intracellular signaling domain of said antigen recognizing receptor ~~is~~ comprises an intracellular signaling domain of a CD3ζ-chain, CD97, CD11a-CD18, CD2, ICOS, CD27, CD154, CDS, OX40, 4-lBB, CD28, or combinations thereof.

54. Use of a T cell, wherein the T cell comprises (a) an antigen recognizing receptor that binds CD19, wherein the binding is capable of activating the immunoresponsive cell, and (b) a soluble single-chain variable fragment (scFv) that binds a polypeptide selected from the group consisting of a CD47 polypeptide and ligands thereof, a PD-1 polypeptide and ligands thereof, a CTLA-4 polypeptide and ligands thereof, and combinations thereof in the manufacture of a medicament for treating blood cancer in a subject in need thereof.

55. The use of claim 54, wherein the blood cancer is selected from the group consisting of B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, and non-Hodgkin's lymphoma.

56. A vector comprising (a) a nucleic acid sequence encoding an antigen recognizing receptor that binds a first antigen, and (b) a nucleic acid sequence encoding a soluble single chain variable fragment (scFv) that binds a polypeptide having immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different from the first antigen.

57. A pharmaceutical composition comprising an effective amount of an immunoresponsive cell of any one of claims 1-20 in a pharmaceutically acceptable excipient.

58. A pharmaceutical composition for the treatment of a neoplasia comprising an effective amount of a tumor antigen-specific T cell of any one of claims 1-20 in a pharmaceutically acceptable excipient.

59. A kit for treatment of a neoplasia, pathogen infection, an autoimmune disorder, or an allogeneic transplant, the kit comprising an immunoresponsive cell comprising (a) an antigen recognizing receptor that binds a first antigen and the binding is capable of activating the immunoresponsive cell, and (b) a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different from the first antigen.

60. The kit of claim 59, wherein the kit further comprises written instructions for using said cell for the treatment of a subject having a neoplasia, a pathogen infection, an autoimmune disorder, or an allogeneic transplant.

61. A kit for treatment of a neoplasia, pathogen infection, an autoimmune disorder, or an allogeneic transplant comprising (a) a nucleic acid encoding a CAR which recognizes an antigen of

the neoplasia, pathogen, autoimmune disorder, or transplant to be treated, and (b) a nucleic acid encoding a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different form the antigen.

62. A kit for treatment of a cancer, the kit comprising (a) a nucleic acid encoding a CAR which recognizes an antigen of the cancer, and (b) a nucleic acid encoding a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different form the antigen.

63. A kit for treatment of a cancer or pathogen-mediated disorder, the kit comprising (a) a nucleic acid encoding a CAR which recognizes an antigen of the cancer or pathogen, and (b) a nucleic acid encoding a soluble single-chain variable fragment (scFv) that binds a polypeptide that has immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different form the antigen.

64. A composition comprising (a) a nucleic acid comprising a sequence encoding an antigen recognizing receptor that binds a first antigen and (b) a nucleic acid comprising a sequence encoding a soluble single-chain variable fragment (scFv) that binds a polypeptide having immunosuppressive activity or immunostimulatory activity, wherein the polypeptide is different from the first antigen, wherein the nucleic acid of (a) and/or the nucleic acid of (b) is optionally a vector.

65. The vector of claim 56 or the composition of claim 64, wherein:

introduction of the vector into a cell is capable of producing the immunoresponsive cell of any one of claims 1-20; or

introduction of the composition into a cell is capable of producing the immunoresponsive cell of any one of claims 1-20.