In another aspect, the invention provides the use of an effective amount of an immunoresponsive cell allogeneic to a subject comprising an inhibitory chimeric antigen receptor that binds an antigen, wherein the binding inhibits the allogeneic immunoresponsive cell in the manufacture of a medicament for modulating a graft versus leukemia response or graft versus tumor response in the subject, wherein the medicament is to be administered to the subject, thereby modulating a graft versus leukemia response or graft versus tumor response in the subject.

In another aspect, the invention provides a

T cell comprising:

1) an antigen recognizing receptor that binds to a first antigen that is expressed on a tumor tissue, wherein binding of the antigen recognizing receptor to the first antigen is capable of activating the T cell, and

2) an inhibitory chimeric antigen receptor (iCAR) comprising (a) an antigen-binding domain that binds to a second antigen that is not expressed on said tumor tissue and (b) at least a portion of the signaling domain of an immunoinhibitory receptor selected from the group consisting of CTLA-4, PD-1, LAG-3, 2B4, and BTLA, wherein binding of the iCAR to the second antigen is capable of reducing the cytotoxicity of the T cell against cells that are positive for both the first antigen and the second antigen.

In another aspect, the invention provides a use of an effective amount of T cells in the manufacture of a medicament for reducing tumor burden in a subject, wherein the T cell comprises (a) an antigen recognizing receptor that binds to a first antigen that is expressed on a tumor tissue, wherein binding of the antigen recognizing receptor to the first antigen is capable of activating the T cells, and (b) an inhibitory chimeric antigen receptor (iCAR) comprising an antigen-binding domain that binds to a second antigen that is not expressed on said tumor tissue and (b) at least a portion of the signaling domain of an immunoinhibitory receptor selected from the group consisting of CTLA-4, PD-1, LAG-3, 2B4, and BTLA, wherein binding of the iCAR to the second antigen is capable of reducing the cytotoxicity of the T cell that comprises the iCAR against cells that are positive for both the first antigen and the second antigen.

In another aspect, the invention provides a use of an effective amount of T cells in the manufacture of a medicament for increasing survival of a subject having neoplasia, wherein the T cell comprises (a) an antigen recognizing receptor that binds to a first antigen that is expressed on a tumor tissue, wherein binding of the antigen recognizing receptor to the first antigen is capable of activating the T cells, and (b) an inhibitory chimeric antigen receptor (iCAR) comprising (a) an antigen-binding domain that binds to a second antigen that is not expressed on said tumor tissue and (b) at least a portion of the signaling domain of an immunoinhibitory receptor selected from the group consisting of CTLA-4, PD-1, LAG-3, 2B4, and BTLA, wherein binding of the iCAR to the second antigen is capable of reducing the cytotoxicity of the T cell that comprises the iCAR against cells that are positive for both the first antigen and the second antigen.

In another aspect, the invention provides a method for producing a T cell, the method comprising:

introducing into a T cell a first nucleic acid comprising a first nucleotide sequence that encodes an antigen recognizing receptor that binds to a first antigen that is expressed on a tumor tissue, wherein binding of the antigen recognizing receptor to the first antigen is capable of activating the cell; and

introducing into the T cell a second nucleic acid comprising a second nucleotide sequence that encodes an inhibitory chimeric antigen receptor (iCAR) comprising (a) an antigen-binding domain that binds to a second antigen that is not expressed on said tumor tissue and (b) at least a portion of the signaling domain of an immunoinhibitory receptor selected from the group consisting of CTLA-4, PD-1, LAG-3, 2B4, and BTLA, wherein binding of the iCAR to the second antigen is capable of reducing the cytotoxicity of the T cell against cells that are positive for both the first antigen and the second antigen.

In another aspect, the invention provides a pharmaceutical composition for the treatment of a neoplasia comprising an effective amount of T cells as disclosed herein in a pharmaceutically acceptable excipient.

In another aspect, the invention provides a kit for treatment of a neoplasia, pathogen infection, or an autoimmune disorder, the kit comprising a T cell comprising (a) an antigen recognizing receptor that binds to a first antigen that is expressed on a

tumor tissue and activates the T cell, and (b) an inhibitory chimeric antigen receptor (iCAR) comprising (a) an antigen-binding domain that binds to a second antigen that is not expressed on said tumor tissue and (b) at least a portion of the signaling domain of an immunoinhibitory receptor selected from the group consisting of CTLA-4, PD-1, LAG-3, 2B4, and BTLA, wherein binding of the iCAR to the second antigen is capable of reducing the cytotoxicity of the T cell against cells that are positive for both the first antigen and the second antigen.

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 invent ion 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 Cam bridge 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 binding and immunoreceptor tyrosine-based inhibition motifs (ITAMs) a signal transduction cascade is produced. In certain embodiments, when an endogenous TCR

Claims

1. A T cell comprising:

1) an antigen recognizing receptor that binds to a first antigen that is expressed on a tumor tissue, wherein binding of the antigen recognizing receptor to the first antigen is capable of activating the T cell, and

2) an inhibitory chimeric antigen receptor (iCAR) comprising (a) an antigen-binding domain that binds to a second antigen that is not expressed on said tumor tissue and (b) at least a portion of the signaling domain of an immunoinhibitory receptor selected from the group consisting of CTLA-4, PD-1, LAG-3, 2B4, and BTLA, wherein binding of the iCAR to the second antigen is capable of reducing the cytotoxicity of the T cell against cells that are positive for both the first antigen and the second antigen.

2. The T cell of claim 1, wherein the first antigen comprises a tumor antigen.

3. The T cell of claim 1, wherein the second antigen is CD33, CD38, a human leukocyte antigen (HLA), an Epithelial-mesenchymal transition (FMT) antigen, E-cadherin, cytokeratin, Opioid-binding protein/cell adhesion molecule (OPCML), HYLA2, Deleted in Colorectal Carcinoma (DCC), Scaffold/Matrix attachment region-binding protein 1 (SMAR1), or mucin type O-glycan.

4. The T cell of any one of claims 1-3, wherein said inhibitory chimeric antigen receptor (iCAR) is recombinantly expressed.

5. The T cell of any one of claims 1-4, wherein the inhibitory chimeric antigen receptor (iCAR) is expressed from a vector.

6. The T cell of any one of claims 1-5, wherein said antigen recognizing receptor is a T cell receptor (TCR) or a chimeric antigen receptor (CAR).

7. The T cell of any one of claims 1-6, wherein said antigen recognizing receptor is exogenous or endogenous.

8. The T cell of any one of claims l-7, wherein said antigen recognizing receptor is recombinantly expressed.

9. The T cell of any one of claims 1-8, wherein the antigen recognizing receptor is expressed from a vector.

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

11. The T cell of any one of claims 1-9, wherein said T cell is non-autologous.

12. The T cell of any one of claims 1-11, wherein said first antigen is a tumor antigen selected from the group consisting of CD19, CAIX, CEA, CD5, CD7, CD10, CD20, CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD 123, 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-α2, κ-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, Mesothelin, Muc-1, Muc-16, NKG2D ligands, NY-ESO-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, and WT-1.

13. The T cell of any one of claims 1-12, wherein said inhibitory chimeric antigen receptor (iCAR) further comprises a transmembrane domain comprising a CD4 polypeptide, a CD8 polypeptide, a CTLA-4 polypeptide, a PD-l polypeptide, a LAG-3 polypeptide, a 2B4 polypeptide, or a BTLA polypeptide.

14. The T cell of any one of claims 1-13, wherein said antigen-binding domain of said inhibitory chimeric antigen receptor (iCAR) comprises a Fab, a scFv, or a ligand.

15. The T cell of any one of claims 1-14, wherein the antigen recognizing receptor is a chimeric antigen receptor (CAR).

16. The T cell of claim 15, wherein the chimeric antigen receptor (CAR) (a) binds to CD19 and comprises an intracellular signaling domain comprising a co-stimulatory signaling domain that comprises at least a portion of the signaling domain of CD28; (b) binds to PSMA and comprises an intracellular signaling domain comprising a co-stimulatory signaling domain that comprises at

least a portion of the signaling domain of CD28; (c) binds to mesothelin and comprises an intracellular signaling domain comprising a co-stimulatory signaling domain that comprises at least a portion of the signaling domain of CD28, or (d) binds to CD56 and comprises an intracellular signaling domain comprising a co-stimulatory signaling domain that comprises at least a portion of the signaling domain of CD28.

17. Use of an effective amount of T cells in the manufacture of a medicament for reducing tumor burden in a subject, wherein the T cell comprises (a) an antigen recognizing receptor that binds to a first antigen that is expressed on a tumor tissue, wherein binding of the antigen recognizing receptor to the first antigen is capable of activating the T cells, and (b) an inhibitory chimeric antigen receptor (iCAR) comprising an antigen-binding domain that binds to a second antigen that is not expressed on said tumor tissue and (b) at least a portion of the signaling domain of an immunoinhibitory receptor selected from the group consisting of CTLA-4, PD-1, LAG-3, 2B4, and BTLA, wherein binding of the iCAR to the second antigen is capable of reducing the cytotoxicity of the T cell that comprises the iCAR against cells that are positive for both the first antigen and the second antigen.

18. The use of claim 17, wherein the medicament selectively targets tumor cells compared to non-tumor cells.

19. The use of claim 17, wherein the medicament reduces the number of tumor cells.

20. The use of claim 17, wherein the medicament reduces tumor size.

21. The use of claim 17, wherein the medicament eradicates the tumor in the subject.

22. Use of an effective amount of T cells in the manufacture of a medicament for increasing survival of a subject having neoplasia, wherein the T cell comprises (a) an antigen recognizing receptor that binds to a first antigen that is expressed on a tumor tissue, wherein binding of the antigen recognizing receptor to the first antigen is capable of activating the T cells, and (b) an inhibitory chimeric antigen receptor (iCAR) comprising (a) an antigen-binding domain that binds to a second antigen that is not expressed on said tumor tissue and (b) at least a portion of the

signaling domain of an immunoinhibitory receptor selected from the group consisting of CTLA-4, PD-1, LAG-3, 2B4, and BTLA, wherein binding of the iCAR to the second antigen is capable of reducing the cytotoxicity of the T cell that comprises the iCAR against cells that are positive for both the first antigen and the second antigen.

23. The use of claim 22, wherein the neoplasia is selected from the group consisting of blood cancer, B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, non-Hodgkin's lymphoma, ovarian cancer, prostate cancer, pancreatic cancer, lung cancer, breast cancer, and sarcoma, and acute myeloid leukemia (AML).

24. The use of any one of claims 17-23, where in the first antigen is a tumor antigen.

25. The use of any one of claims 17-24, wherein the second antigen is CD33, CD38, a human leukocyte antigen (HLA), an Epithelial-mesenchymal transition (EMT) antigen, E-cadherin, cytokeratin, Opioid-binding protein/cell adhesion molecule (OPCML), HYLA2, Deleted in Colorectal Carcinoma (DCC), Scaffold/Matrix attachment region-binding protein 1 (SMAR1), or mucin type O-glycan.

26. The use of any one of claims 17-24, wherein said inhibitory chimeric antigen receptor (iCAR) is recombinantly expressed.

27. The use of any one of claims 17-26, wherein the inhibitory chimeric antigen receptor (iCAR) is expressed from a vector.

28. The use of any one of claims 17-27, wherein said antigen recognizing receptor is a T cell receptor (TCR) or chimeric antigen receptor (CAR).

29. The use of any one of claims 17-28, wherein said antigen recognizing receptor is exogenous or endogenous.

30. The use of any one of claims 17-29, wherein said antigen recognizing receptor is recombinantly expressed.

31. The use of any one of claims 17-30, wherein the antigen recognizing receptor is expressed from a vector.

32. The use of any one of claims 17-31, wherein said T cell is autologous.

33. The use of any one of claims 17-31, wherein said T cell is non-autologous.

34. The use of any one of claims 17-33, wherein said first antigen is a tumor antigen selected from the group consisting of CD19, CAIX, CEA, CD5, CD7, CD10, CD20, CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD123, 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-α2, κ-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, Mesothelin, Muc-1, Muc-16, NKG2D ligands, NY-ESO-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, and WT-1.

35. The use of any one of claims 17-34, wherein said inhibitory chimeric antigen receptor (iCAR) further comprises a transmembrane domain comprising a CD4 polypeptide, a CD8 polypeptide, a CTLA-4 polypeptide, a PD-1 polypeptide, a LAG-3 polypeptide, a 2B4 polypeptide, or a BTLA polypeptide.

36. The use of any one of claims 17-35, wherein said antigen-binding domain of said inhibitory chimeric antigen receptor (iCAR) comprises a Fab, a scFv, or a ligand.

37. The use of any one of claims 17-36, wherein the medicament reduces or eradicates the tumor burden in the subject.

38. A method for producing a T cell, the method comprising:

introducing into a T cell a first nucleic acid comprising a first nucleotide sequence that encodes an antigen recognizing receptor that binds to a first antigen that is expressed on a tumor tissue, wherein binding of the antigen recognizing receptor to the first antigen is capable of activating the cell; and

introducing into the T cell a second nucleic acid comprising a second nucleotide sequence that encodes an inhibitory chimeric antigen receptor (iCAR) comprising (a) an antigen-

binding domain that binds to a second antigen that is not expressed on said tumor tissue and (b) at least a portion of the signaling domain of an immunoinhibitory receptor selected from the group consisting of CTLA-4, PD-1, LAG-3, 2B4, and BTLA, wherein binding of the iCAR to the second antigen is capable of reducing the cytotoxicity of the T cell against cells that are positive for both the first antigen and the second antigen.

39. The method of claim 38, wherein the first antigen comprises a tumor antigen.

40. The method of claim 38 or 39, wherein the second antigen is CD33, CD38, a human leukocyte antigen (HLA), an Epithelial-mesenchymal transition (EMT) antigen, E-cadherin, cytokeratin, Opioid -binding protein/cell adhesion molecule (OPCML), HYLA2, Deleted in Colorectal Carcinoma (DCC), Scaffold/Matrix attachment region-binding protein 1 (SMAR1), or mucin type O-glycan.

41. The method of any one of claims 38-40, wherein said inhibitory chimeric antigen receptor (iCAR) is recombinantly expressed.

42. The method of any one of claims 38-41, wherein the inhibitory chimeric antigen receptor (iCAR) is expressed from a vector.

43. The method of any one of claims 38-42, wherein said antigen recognizing receptor is a T cell receptor (TCR) or chimeric antigen receptor (CAR).

44. The method of any one of claims 38-43, wherein said antigen recognizing receptor is exogenous or endogenous.

45. The method of any one of claims 38-44, wherein said antigen recognizing receptor is recombinantly expressed.

46. The method of any one of claims 38-45, wherein the antigen recognizing receptor is expressed from a vector.

47. The method of any one of claims 38-46, wherein said T cell is autologous.

48. The method of any one of claims 38-47, wherein said T cell is non-autologous.

49. The method of any one of claims 38-48, wherein said first antigen is a tumor antigen selected from the group consisting of CD19, CAIX, CEA, CD4, CD7, CD10, CD20, CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD123, 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-α2, κ-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-Al, Mesothelin, Muc-1, Muc-16, NKG2D ligands, NY-ESO-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, and WT-1.

50. The method of any one of claims 38-49, wherein said inhibitory chimeric antigen receptor (iCAR) further comprises a transmembrane domain comprising a CTLA-4 polypeptide, a PD-1 polypeptide, a LAG-3 polypeptide, a 2B4 polypeptide, and a BTLA polypeptide.

51. The method of any one of claims 38-50, wherein said antigen-binding domain of said inhibitory chimeric antigen receptor (iCAR) comprises a Fab, a scFv, or a ligand.

52. A pharmaceutical composition comprising an effective amount of T cells of any one of claims 1-16 in a pharmaceutically acceptable excipient.

53. A pharmaceutical composition for the treatment of a neoplasia comprising an effective amount of T cells of any one of claims 1-16 in a pharmaceutically acceptable excipient.

54. A kit for treatment of a neoplasia, pathogen infection, or an autoimmune disorder, , the kit comprising a T cell comprising (a) an antigen recognizing receptor that binds to a first antigen that is expressed on a tumor tissue and activates the T cell, and (b) an inhibitory chimeric antigen receptor (iCAR) comprising (a) an antigen-binding domain that binds to a second antigen that is not expressed on said tumor tissue and (b) at least a portion of the signaling domain of an immunoinhibitory receptor selected from the group consisting of CTLA-4, PD-1, LAG-3, 2B4, and BTLA, wherein binding of the iCAR to the second antigen is capable of reducing the

cytotoxicity of the T cell against cells that are positive for both the first antigen and the second antigen.

55. The kit of claim 54, wherein the kit further comprises written instructions for using said cell for the treatment of a subject having a neoplasia.