[0006] It is clear that there continues to be a need for diagnostic, prognostic, and predictive methods to identify patients that are more likely to benefit from anti-tumor treatments that modulate OX40 activity. The invention described herein meets this need and provides other benefits.

[0007] All references cited herein, including patent applications, patent publications, and UniProtKB/Swiss-Prot Accession numbers are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

BRIEF SUMMARY

[0008] The present disclosure describes assays for detecting T cell immune subsets, as well as methods of using these assays to determine prognosis, predict responsiveness, and/or treating or delay progress of cancer.

[0009] The present disclosure provides a method for determining prognosis of a subject having cancer, comprising: (a) measuring the number of CD4+ OX40+ Foxp3+ lymphocytes in a sample comprising cancer cells and lymphocytes obtained from the subject; and (b) determining the prognosis of the subject based on the number of CD4+ OX40+ Foxp3+ lymphocytes in the sample, as compared with a reference, wherein an increased number of CD4+ OX40+ Foxp3+ lymphocytes in the sample indicates that the subject may have an improved prognosis. In some embodiments, the improved prognosis comprises increased overall survival. In some embodiments, the improved prognosis comprises increased progression-free survival.

[0009a] In certain aspects, the present disclosure provides a method for determining prognosis of a subject having cancer, comprising:

(a) measuring the number of CD4+ OX40+ Foxp3+ lymphocytes in a tumor sample comprising cancer cells and lymphocytes obtained from the subject; and

(b) determining the prognosis of the subject based on the number of CD4+ OX40+ Foxp3+ lymphocytes in the tumor sample, as compared with a reference, wherein an increased number of CD4+ OX40+ Foxp3+ lymphocytes in the tumor sample indicates that the subject may have an improved prognosis.

[0010] The present disclosure also provides a method for treating or delaying progression of cancer in a subject, comprising: (a) measuring the number of CD4+ OX40+ Foxp3+ lymphocytes in a sample comprising cancer cells and lymphocytes obtained from the subject; (b) determining the number of CD4+ OX40+ Foxp3+ lymphocytes in the sample, as compared with a reference;

and (c) if the number of CD4+ OX40+ Foxp3+ lymphocytes in the sample is higher than the reference, administering to the subject an effective amount of an OX40 agonist.

[0010a] In certain aspects, the present disclosure provides a use of an OX40 agonist in the manufacture of a medicament for a method for treating or delaying progression of cancer in a subject, wherein said method comprises:

(a) measuring the number of CD4+ OX40+ Foxp3+ lymphocytes in a tumor sample comprising cancer cells and lymphocytes obtained from the subject;

(b) determining the number of CD4+ OX40+ Foxp3+ lymphocytes in the tumor sample, as compared with a reference; and

(c) wherein said OX40 agonist is to be administered to the subject if the number of CD4+ OX40+ Foxp3+ lymphocytes in the tumor sample is higher than the reference.

[0011] The present disclosure also provides a method for treating or delaying progression of cancer in a subject, comprising administering to the subject an effective amount of an OX40 agonist, wherein a sample comprising cancer cells and lymphocytes obtained from the subject has an increased number of CD4+ OX40+ Foxp3+ lymphocytes, as compared with a reference. In some embodiments, the subject has been tested to determine if a sample comprising cancer cells and lymphocytes obtained from the subject has an increased number of CD4+ OX40+ Foxp3+ lymphocytes as compared with a reference before treatment or is selected for

treatment based on a sample comprising cancer cells and lymphocytes obtained from the subject has an increased number of CD4+ OX40+ Foxp3+ lymphocytes as compared with a reference.

[0011a] In certain aspects, the present disclosure provides a use of an OX40 agonist in the manufacture of a medicament for treating or delaying progression of cancer in a subject, wherein the OX40 agonist is to be administered to the subject, and wherein a tumor sample comprising cancer cells and lymphocytes obtained from the subject has been detected to possess an increased number of CD4+ OX40+ Foxp3+ lymphocytes, as compared with a reference.

[0012] The present disclosure also provides a method for predicting responsiveness of a subject having cancer to an OX40 agonist treatment, comprising: (a) measuring the number of CD4+ OX40+ Foxp3+ lymphocytes in a sample comprising cancer cells and lymphocytes obtained from the subject; and (b) classifying the subject as a responsive or non-responsive subject based on the number of CD4+ OX40+ Foxp3+ lymphocytes in the sample, as compared with a reference, wherein an increased number of CD4+ OX40+ Foxp3+ lymphocytes in the sample indicates the subject may be responsive to the OX40 agonist treatment.

[0013] In some embodiments, the number of CD4+ OX40+ Foxp3+ lymphocytes in one or more regions of interest in the sample of a subject is measured. In some embodiments, the region of interest includes tumor cells and stroma. In some embodiments, the number of CD4+ OX40+ Foxp3+ lymphocytes is a median, mean or average number of CD4+ OX40+ Foxp3+ lymphocytes in different regions of interest of the sample from the subject. In some embodiments, the number of CD4+ OX40+ Foxp3+ lymphocytes is normalized to total cells in the region of interest of the sample. In some embodiments, the reference is based on the number of CD4+ OX40+ Foxp3+ lymphocytes in a sample comprising cancer cells and lymphocytes obtained from a cancer having the same type and/or stage as the cancer of the subject. In some embodiments, the reference is a median, mean, or average number of CD4+ OX40+ Foxp3+ lymphocytes in samples obtained from cancers having the same type and/or stage as the cancer of the subject. In some embodiments, the OX40 agonist is an agonist anti-human OX40 antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a humanized or human antibody. In some embodiments, the antibody comprises an IgG1 Fc region. In some embodiments, the antibody comprises an IgG4 Fc region. In some embodiments, the antibody comprises an Fc region comprising a mutation that decreases binding to an Fc receptor. In some embodiments, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:2, 8 or 9; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:3, 10, 11, 12, 13, or 14; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:4, 15 or 19; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising an amino

comprising an amino acid sequence selected from SEQ ID NO:7. In some embodiments, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:3; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:4; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:26. In some embodiments, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:3; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:4; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:27. In some embodiments, the antibody is MEDI6469 or MEDI0562. In some embodiments, the OX40 agonist comprises one or more extracellular domains of OX40L. In some embodiments, the OX40 agonist is MEDI6383. In some embodiments, measuring the number of CD4+ OX40+ Foxp3+ lymphocytes in the sample comprises: (a) labeling lymphocytes that show CD4 expression in the sample; (b) labeling lymphocytes that show OX40 expression in the sample after step (a); (c) labeling lymphocytes that show Foxp3 expression in the sample after step (b); and (d) measuring the number of CD4+ OX40+ Foxp3+ lymphocytes in the sample after step (c).

[0014] The present disclosure also provides a method for measuring the number of CD4+ OX40+ Foxp3+ lymphocytes in a sample comprising cancer cells and lymphocytes obtained from a subject, comprising the steps of: (a) labeling lymphocytes that show CD4 expression in the sample; (b) labeling lymphocytes that show OX40 expression in the sample after step (a); (c) labeling lymphocytes that show Foxp3 expression in the sample after step (b); and (d) measuring the number of CD4+ OX40+ Foxp3+ lymphocytes in the sample after step (c). In some embodiments, the sample is treated with a solution comprising EDTA or citrate buffer between steps (a) and (b) and/or between steps (b) and (c). In some embodiments, lymphocytes showing CD4 expression, OX40 expression, and Foxp3 expression are labeled by immunofluorescence staining. In some embodiments, lymphocytes showing OX40 expression are labeled using an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:3; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:4; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:7, 26 or 27. In some embodiments, quantifying the number of CD4+ OX40+ Foxp3+ lymphocytes in the sample comprises imaging said immunofluorescence staining.

[0015] In some embodiments, the cancer is colorectal cancer. In some embodiments, the cancer is selected from the group consisting of non-small cell lung cancer, renal cell carcinoma, bladder cancer, ovarian cancer, glioblastoma, neuroblastoma, melanoma, breast carcinoma, gastric cancer, and hepatocellular carcinoma. In some embodiments, the breast carcinoma is triple-negative breast carcinoma. In some embodiments, the sample comprising cancer cells and lymphocytes, wherein said cancer cells are from a primary tumor. In some embodiments, the sample comprising cancer cells and lymphocytes, wherein said cancer cells are from a metastasis. In some embodiments, the CD4+ OX40+ Foxp3+ lymphocytes are tumor-infiltrating lymphocytes.

[0016] The present disclosure also provides a method for determining prognosis of a subject having cancer, comprising: (a) measuring the number of OX40+, CD4+ OX40+ Foxp3+, or CD4+ OX40+ Foxp3- lymphocytes in a sample comprising metastatic cancer cells and lymphocytes obtained from the subject; and (b) determining the prognosis of the subject based on the number of OX40+, CD4+ OX40+ Foxp3+, or CD4+ OX40+ Foxp3- lymphocytes in the sample, as compared with a reference, wherein an increased number of OX40+, CD4+ OX40+ Foxp3+, or CD4+ OX40+ Foxp3- lymphocytes in the sample indicates that the subject may have an improved prognosis. In some embodiments, the improved prognosis comprises increased overall survival. In some embodiments, the improved prognosis comprises increased progression-free survival.

[0017] The present disclosure also provides a method for treating or delaying progression of cancer in a subject, comprising: (a) measuring the number of OX40+, CD4+ OX40+ Foxp3+, or CD4+ OX40+ Foxp3- lymphocytes in a sample comprising metastatic cancer cells and lymphocytes obtained from the subject; (b) determining the number of OX40+, CD4+ OX40+ Foxp3+, or CD4+ OX40+ Foxp3- lymphocytes in the sample, as compared with a reference; and (c) if the number of OX40+, CD4+ OX40+ Foxp3+, or CD4+ OX40+ Foxp3- lymphocytes in the sample is higher than the reference, administering to the subject an effective amount of an OX40 agonist.

[0018] The present disclosure also provides a method for treating or delaying progression of cancer in a subject, comprising administering to the subject an effective amount of an OX40 agonist, wherein a sample comprising metastatic cancer cells and lymphocytes obtained from the subject has an increased number of OX40+, CD4+ OX40+ Foxp3+, or CD4+ OX40+ Foxp3- lymphocytes, as compared with a reference.

[0019] The present disclosure also provides a method for predicting responsiveness of a subject having cancer to an OX40 agonist treatment, comprising: (a) measuring the number of OX40+, CD4+ OX40+ Foxp3+, or CD4+ OX40+ Foxp3- lymphocytes

CLAIMS

What is claimed is:

1. A method for determining prognosis of a subject having cancer, comprising:

(a) measuring the number of CD4+ OX40+ Foxp3+ lymphocytes in a tumor sample comprising cancer cells and lymphocytes obtained from the subject; and

(b) determining the prognosis of the subject based on the number of CD4+ OX40+ Foxp3+ lymphocytes in the tumor sample, as compared with a reference, wherein an increased number of CD4+ OX40+ Foxp3+ lymphocytes in the tumor sample indicates that the subject may have an improved prognosis.

2. The method of claim 1, wherein the improved prognosis comprises increased overall survival.

3. The method of claim 1 or claim 2, wherein the improved prognosis comprises increased progression-free survival.

4. Use of an OX40 agonist in the manufacture of a medicament for a method for treating or delaying progression of cancer in a subject, wherein said method comprises:

(a) measuring the number of CD4+ OX40+ Foxp3+ lymphocytes in a tumor sample comprising cancer cells and lymphocytes obtained from the subject;

(b) determining the number of CD4+ OX40+ Foxp3+ lymphocytes in the tumor sample, as compared with a reference; and

(c) wherein said OX40 agonist is to be administered to the subject if the number of CD4+ OX40+ Foxp3+ lymphocytes in the tumor sample is higher than the reference.

5. Use of an OX40 agonist in the manufacture of a medicament for treating or delaying progression of cancer in a subject, wherein the OX40 agonist is to be administered to the subject, and wherein a tumor sample comprising cancer cells and lymphocytes obtained from the subject has been detected to possess an increased number of CD4+ OX40+ Foxp3+ lymphocytes, as compared with a reference.

6. The method or use of any one of claims 1-5, wherein the number of CD4+ OX40+ Foxp3+ lymphocytes is a median, mean or average number of CD4+ OX40+ Foxp3+ lymphocytes in different regions of interest of the tumor sample from the subject.

7. The method or use of claim 6, wherein the number of CD4+ OX40+ Foxp3+ lymphocytes is normalized to total cells in the region of interest of the tumor sample.

8. The method or use of any one of claims 1-7, wherein the reference is based on the number of CD4+ OX40+ Foxp3+ lymphocytes in a tumor sample comprising cancer cells and lymphocytes obtained from a cancer having the same type and/or stage as the cancer of the subject.

9. The method or use of claim 8, wherein the reference is a median, mean, or average number of CD4+ OX40+ Foxp3+ lymphocytes in tumor samples obtained from cancers having the same type and/or stage as the cancer of the subject.

10. The method or use of claim 4 or claim 5, wherein the OX40 agonist is an agonist anti-human OX40 antibody.

11. The method or use of claim 10, wherein the antibody is a monoclonal antibody.

12. The method or use of claim 10, wherein the antibody is a humanized or human antibody.

13. The method or use of claim 10, wherein the antibody comprises an IgG1 Fc region.

14. The method or use of claim 10, wherein the antibody comprises an IgG4 Fc region.

15. The method or use of claim 10, wherein the antibody comprises an Fc region comprising a mutation that decreases binding to an Fc receptor.

16. The method or use of claim 10, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:2, 8 or 9; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:3, 10, 11, 12, 13, or 14; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:4, 15 or 19; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:7, 22, 23, 24, 25, 26, 27 or 28.

17. The method or use of claim 10, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:3; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:4; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:7.

18. The method or use of claim 10, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:3; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:4; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:26.

19. The method or use of claim 10, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:3; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:4; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:27.

20. The method or use of claim 10, wherein the antibody is MEDI6469 or MEDI0562.

21. The method or use of claim 4 or claim 5, wherein the OX40 agonist comprises one or more extracellular domains of OX40L.

22. The method or use of claim 4 or claim 5, wherein the OX40 agonist is MEDI6383.

23. The method or use of any one of claims 1-22, wherein measuring the number of CD4+ OX40+ Foxp3+ lymphocytes in the tumor sample comprises:

(a) labeling lymphocytes that show CD4 expression in the tumor sample;

(b) labeling lymphocytes that show OX40 expression in the tumor sample after step (a);

(c) labeling lymphocytes that show Foxp3 expression in the tumor sample after step (b); and

(d) measuring the number of CD4+ OX40+ Foxp3+ lymphocytes in the tumor sample after step (c).

24. The method or use of any one of claims 1-23, wherein the cancer is colorectal cancer.

25. The method or use of any one of claims 1-23, wherein the cancer is selected from the group consisting of non-small cell lung cancer, renal cell carcinoma, bladder cancer, ovarian cancer, glioblastoma, neuroblastoma, melanoma, breast carcinoma, gastric cancer, and hepatocellular carcinoma.

26. The method or use of claim 25, wherein the breast carcinoma is triple-negative breast carcinoma.

27. The method or use of any one of claims 1-26, wherein the tumor sample comprising cancer cells and lymphocytes is from a primary tumor.

28. The method or use of any one of claims 1-26, wherein the tumor sample comprising cancer cells and lymphocytes is from a metastasis.

29. The method or use of any one of claims 1-28, wherein the CD4+ OX40+ Foxp3+ lymphocytes are tumor-infiltrating lymphocytes.