the method further comprises administering a second cancer treatment (e.g., surgery, chemotherapy, radiation therapy, biologic therapy, immunomodulatory therapy, and combinations thereof).

[0021] The invention also provides an antibody or antibody fragment thereof comprising an antigen binding region that binds to a V-domain Ig Suppressor of T cell Activation (VISTA), wherein the antibody binds to a conformational epitope in VISTA (e.g., human VISTA). In some embodiments, the conformational epitope comprises, or is present within, residues 103-111 (NLTLLDSGL (SEQ ID NO62)) and 136-146 (VQTGKDAPSNC (SEQ ID NO:63)) of human VISTA (SEQ ID NO:46). In another embodiment, the conformational epitope comprises, or is present within, residues 24-36 (LLGPVDKGHDVTF (SEQ ID NO:64)), 54-65 (RRPIRDLTFQDL (SEQ ID NO:65) and 100-102 (TMR) of human VISTA (SEQ ID NO:46). In yet another embodiment, the conformational epitope comprises amino acid residues in the FG loop of human VISTA (SEQ ID NO:46).

[0022] In addition, the invention relates to a method of enhancing an immune response in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of an antibody that binds V-domain Ig Suppressor of T cell Activation (VISTA), or an antibody fragment thereof, comprising an antigen binding region that binds to VISTA, thereby enhancing an immune response to the cancer. In a particular embodiment, the immune response is an antitumor immune response.

[0023] In another embodiment, the invention provides a method of eliciting a biological response in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of an antibody that binds V-domain Ig Suppressor of T cell Activation (VISTA), or an antibody fragment thereof, comprising an antigen binding region that binds to VISTA, thereby enhancing an immune response to the cancer. Examples of biological responses include activation of monocytes; induction of T-cell proliferation and cytokine secretion; antibody-dependent cell-mediated cytotoxicity (ADCC) of cells expressing VISTA; and antibody-dependent cellular phagocytosis (ADCP) of cells expressing VISTA.

(SEQ ID NO:75), and $_{54}$RRPIRDLTFQDL$_{65}$ (SEQ ID NO:65) (Figure 28 bottom). There are two other segments showing weak perturbation by VSTB95 and 112, including residues 39-52 and 118-134. However, the levels of the reduction are not as strong as the previous regions (27-36 and 54-65) in the differential map. Although one peptide, $_{100}$TMR$_{102}$ showing strong perturbation by VSTB95 and 112, is located on the other face of VISTA surface, it is distant from the epitope regions, 27-36 and 54-65. This perturbation could be due to allosteric effect. These HDX-MS results provide the peptide level epitopes for anti-VISTA antibodies. There were no overlapping epitope regions for these two epitope groups. These results are in agreement with the previous competition binning data in that they do not compete with each other.

[00287] EXAMPLE 14: STRUCTURE DETERMINATION OF THE HUMAN VISTA ECD:VSTB112 FAB COMPLEX BY PROTEIN CRYSTALLOGRAPHY

[00288] In an effort to determine the VISTA structure and to delineate the epitope and paratope defining the interaction between VISTA extracellular domain (ECD) and the Fab fragment of lead antibody VSTB112, the complex was crystallized and structure determined to 1.85 Å resolution. The structure of the ECD of human VISTA in complex with the Fab fragment of the antibody VSTB112 was determined in an effort both to determine the structure of VISTA ECD itself and to define the epitope/paratope for this interaction. The structure reveals VISTA to adopt an IgV fold with a chain topology similar to the TCR Vα chain. In addition to the canonical disulfide bond bridging B and F strands in the back and front faces of the β-sandwich, the structure reveals the ECD to have two additional disulfide bonds, one tethering the CC' loop to the front sheet and a second between the A' and G' strands. Although crystal contacts between VISTA molecules are present, they are minor and there is no evidence for a dimer of VISTA ECDs based on this structure. The VSTB112 epitope is shown to comprise the portions of the VISTA BC, CC', and FG loops together with residues of the front beta sheet (C'CFG) nearest those loops. The paratope is biased largely toward heavy chain interactions with CDR L3 making minimal contact.

CLAIMS

What is claimed:

1. An isolated antibody or antibody fragment thereof comprising an antigen binding region that binds to a V-domain Ig Suppressor of T cell Activation (VISTA), wherein binding of the antibody or antibody fragment to VISTA modulates or enhances an immune response.

2. The antibody or antibody fragment of claim 1, wherein the antibody fragment is a Fab, $F(ab')_2$, or scFv antibody fragment.

3. The antibody or antibody fragment of claim 1 which comprises an antibody constant region.

4. The antibody or antibody fragment of claim 1, wherein VISTA is expressed on a hematopoietic cell.

5. The antibody or antibody fragment of claim 1, wherein the hematopoietic cell is a myeloid lineage cell and/or a lymphocyte.

6. The antibody or antibody fragment of claim 1, wherein the hematopoietic cell is a monocyte or a neutrophil.

7. The antibody or antibody fragment of claim 1, wherein the hematopoietic cell is a T cell.

8. The antibody or antibody fragment of claim 1, wherein the hematopoietic cell is a B cell, a natural killer (NK) cell or a natural killer T (NKT) cell.

9. The antibody or antibody fragment of claim 1, wherein VISTA is expressed on a tumor cell.

10. The antibody or antibody fragment of claim 1 wherein VISTA is expressed in a tumor microenvironment (TME).

11. The antibody or antibody fragment of any one of claims 1-10, further comprising one or more heavy chain complementary determining regions (CDRs) and one or more light chain CDRs; wherein the one or more heavy chain CDRs comprises an amino acid sequence selected from SEQ ID NOs:1-3, 7-9, 13-15, 19-21, 25-27, 31-33 and the one or more light chain CDRs comprises an amino acid sequence selected from SEQ ID NOs:4-6, 10-12, 16-18, 22-24, 28-30, 34-36).

12. The antibody or antibody fragment of any one of claims 1-10, further comprising at least one heavy chain and at least one light chain.

13. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises SEQ ID NO:1-3.

14. An antibody or antibody fragment according to claim 12, wherein at least one light chain comprises SEQ ID NO:4-6.

15. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises SEQ ID NO:1-3 and wherein at least one light chain comprises SEQ ID NO:4-6.

16. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises the heavy chain variable region sequence as shown in SEQ ID NO:37-40.

17. An antibody or antibody fragment according to claim 12, wherein at least one light chain comprises the light chain variable region sequence as shown in SEQ ID NO:41-45.

18. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises the heavy chain variable region sequence as shown in SEQ ID NO:37-40, and wherein at least one light chain comprises the light chain variable region sequence as shown in SEQ ID NO: 41-45.

19. The antibody or antibody fragment of any one of claims 1-18, wherein the antibody is a monoclonal antibody.

20. The antibody or antibody fragment of any one of claims 1-18, wherein the antibody is a humanized antibody.

21. The antibody or antibody fragment of any one of claims 1-18, wherein the antibody comprises a human constant region.

22. An antibody or antibody fragment according to any one of claims 1-18, wherein the antibody molecule is specific for an epitope within the amino acid sequence SEQ ID NO: 46.

23. The antibody or antibody fragment of any one of claims 1-18, wherein the antibody binds to an epitope of VISTA with an affinity of at least $1x10^{-9}$ liter/mole.

24. The antibody or antibody fragment of any one of claims 1-18, wherein the antibody binds to an epitope of VISTA with an affinity of at least $1x10^{-8}$ liter/mole.

25. The antibody or antibody fragment of any one of claims 1-18, wherein the antibody binds to an epitope of VISTA with an affinity of at least $1x10^{-7}$ liter/mole.

26. The antibody or antibody fragment of any one of claims 1-18, wherein the modulation of the immune response comprises an increase in CD45+ leukocytes, CD4+ T cells, or CD8+ T cells, or a combination thereof, or a decrease in VISTA-expressing immune cells.

27. The antibody or antibody fragment of any one of claims 1-18, wherein the modulation of the immune response comprises enhanced production of cytokines, enhanced T-cell response, and/or modulates Foxp3 expression.

28. A composition comprising the antibody or antibody fragment of any one of claims 1-27 and a pharmaceutically acceptable carrier, diluent, or excipient.

29. A composition comprising:

a VISTA antagonist comprising an antibody or antibody fragment thereof comprising an antigen binding region that binds to a V-domain Ig Suppressor of T cell Activation (VISTA); and

a vaccine.

30. A pharmaceutical composition comprising:

an antibody or antibody fragment thereof comprising an antigen binding region that binds to a V-domain Ig Suppressor of T cell Activation (VISTA); and

a vaccine,

wherein binding of the antibody or antibody fragment to VISTA modulates or enhances an immune response.

31. A method for treating cancer in an individual in need thereof, said method comprising administering to the subject an effective amount of the antibody or antibody fragment of any one of claims 1-27.

32. A method for treating or preventing cancer in an individual in need thereof, said method comprising administering to the individual an effective amount of the composition of any one of claims 28-30.

33. A method of treating a cancer in an individual in need thereof comprising:

administering to the individual a therapeutically effective amount of a V-domain Ig Suppressor of T cell Activation (VISTA) antagonist comprising an antibody or antibody fragment thereof comprising an antigen binding region that binds to VISTA; wherein the antibody or antibody fragment binds to VISTA, thereby modulating or enhancing an immune response to the cancer.

34. The method of any one of Claims 31-32, wherein the individual is a mammal.

35. The method of any one of Claims 31-32, wherein the individual is a human.

36. A method of treating a cancer in an individual in need thereof comprising administering to the individual a therapeutically effective amount of a V-domain Ig Suppressor of T cell Activation (VISTA) antagonist comprising an antibody or antibody fragment thereof comprising an antigen binding region that binds to VISTA; wherein the antibody or antibody fragment binds to VISTA, thereby modulating or enhancing an immune response to the cancer.

37. The method of claim 36, wherein the cancer is a leukemia, lymphoma, myelodisplastic syndrome or myeloma, or a combination thereof.

38. The method of claim 37, wherein the leukemia is a lymphocytic leukemia or a myelogenous leukemia.

39. The method of claim 37, wherein the leukemia is acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid (myelogenous) leukemia (AML), chronic myelogenous leukemia (CML); hairy cell leukemia, T-cell prolymphocytic leukemia, large granular lymphocytic leukemia, or adult T-cell leukemia.

40. The method of claim 36, wherein the cancer is an acute myeloid (myelogenous) leukemia (AML).

41. The method of claim 36, wherein the cancer is a chronic myelogenous leukemia (CML).

42. The method of claim 36, wherein the cancer is a solid tumor.

43. The method of claim 36, wherein the solid tumor is surrounded by a tumor stroma comprising myeloid cells, T-cells, or a combination of myeloid cells and T-cells.

44. The method of claim 42 or 43, wherein the solid tumor is infiltrated with myeloid cells, T cells or a combination of myeloid cells and T-cells.

45. The method of claim 35, further comprising administering a vaccine.

46. A method for suppressing tumor growth in an individual in need thereof, said method comprising administering an effective antibody or antibody fragment of claims 1-27 to a subject in need thereof.

47. The method of any one of claims 31-46, wherein the composition, antibody or fragment is administered parenterally or nonparenterally, for example, intravenously, subcutaneously or orally.

48. The method of any one of claims 31-46, wherein the dosage of the administered composition, antibody or fragment is 0.1-15 mg/kg per administration.

49. The method of any one of claims 31-46, wherein the composition, antibody or fragment is administered weekly, once every two weeks, once every three weeks, once every month, once every 2 months, or once every 3 months.

50. An antibody or fragment thereof that binds VISTA and which comprises an antibody VH domain comprising a VH CDR1 having an amino acid sequence that is substantially similar to SEQ ID NO:1, a VH CDR2 having an amino acid sequence that is substantially similar to SEQ ID NO:2 and a VH CDR3 having an amino acid sequence that is substantially similar to SEQ ID NO:3, and which further comprises an antibody VL domain comprising a VL CDR1 having an amino acid sequence that is substantially similar to SEQ ID NO:4, a VL CDR2 having an amino acid sequence that is substantially similar to SEQ ID NO:5 and a VL CDR3 having an amino acid sequence that is identical to SEQ ID NO:6.

51. An antibody or fragment thereof of claim 12, wherein the variable light chain VL domain comprises a human framework region.

52. An antibody or fragment thereof of claim 1, which comprises a whole antibody.

53. An isolated nucleic acid which comprises a nucleotide sequence encoding the antibody of claim 1.

54. An expression vector comprising the nucleic acid of claim 51 operably linked to a promoter.

55. A host cell transformed with the expression vector of claim 52.

56. A method of producing antibody or fragment thereof of claim 1, the method comprising culturing host cells according to claim 53 under conditions for production of said antibody or fragment.

57. The method according to claim 54 further comprising isolating said antibody.

58. An article of manufacture comprising the composition of claim 30 and a container, and further comprising a package insert or label indicating that the composition can be used to treat cancer.

59. An antibody or antibody fragment thereof, wherein the antibody is selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

60. The antibody or antibody fragment of any one of claims 1-10, further comprising one or more heavy chain complementary determining regions (CDRs) and one or more light chain CDRs of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

61. The antibody or antibody fragment of any one of claims 1-10, further comprising at least one heavy chain and at least one light chain of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

62. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises all three heavy chain CDRs of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

63. An antibody or antibody fragment according to claim 12, wherein at least one light chain comprises all three light chain CDRs of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

64. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises all three heavy chain CDRs of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47), and wherein at least one light chain comprises all three light chain CDRs of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

65. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises the heavy chain variable region sequence of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

66. An antibody or antibody fragment according to claim 12, wherein at least one light chain comprises the light chain variable region sequence of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

67. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises the heavy chain variable region sequence of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47), and wherein at least one light chain comprises the light chain variable region sequence of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

68. A method of treating a cancer in an individual in need thereof comprising:

administering to the individual a therapeutically effective amount of a V-domain Ig Suppressor of T cell Activation (VISTA) antagonist comprising an antibody or antibody fragment thereof comprising an antigen binding region that binds to VISTA; wherein the antibody or antibody fragment binds to VISTA, thereby modulating or enhancing an immune response to the cancer; and

administering a second cancer treatment.

69. The method of claim 68, wherein the second cancer treatment is surgery, chemotherapy, radiation therapy, biologic therapy, targeted therapy, or immunomodulatory therapy, or a combination thereof.

70. The composition of claim 29, pharmaceutical composition of claim 30, or method of claim 45, wherein the vaccine is a viral vector vaccine, bacterial vaccine, DNA vaccine, RNA vaccine, peptide vaccine, or protein vaccine.

71. The method of claim 36, wherein the cancer is a lung cancer.

72. The method of claim 69, wherein the lung cancer is a non-small cell lung carcinoma (NSCLC).

73. An isolated antibody or antibody fragment thereof comprising an antigen binding region that binds to a V-domain Ig Suppressor of T cell Activation (VISTA), wherein the antibody comprises an antibody VH domain comprising a VH CDR1 having the amino acid sequence of SEQ ID NO:25, a VH CDR2 having the amino acid sequence of SEQ ID NO:26 and a VH CDR3 having the amino acid sequence of SEQ ID NO:27, and which further comprises an antibody VL domain comprising a VL CDR1 having the amino acid sequence of SEQ ID NO:28, a VL CDR2 having the amino acid sequence of SEQ ID NO:29 and a VL CDR3 having the amino acid sequence of SEQ ID NO:30.

74. The antibody or antibody fragment of claim 73, comprising one or more humanized or human framework regions.

75. The antibody or antibody fragment of claim 74, wherein the antibody VH domain comprises SEQ ID NO:37.

76. The antibody of claim 74 or 75, wherein the antibody VL domain comprises SEQ ID NO:44.

77. The antibody or antibody fragment of any one of claims 73-76, wherein the antibody comprises a heavy chain constant region.

78. The antibody or antibody fragment of claim 77, wherein the heavy chain constant region is a human heavy chain constant region.

79. The antibody or antibody fragment of claim 77 or 78, wherein the heavy chain constant region is an IgG1 heavy chain constant region.

80. The antibody or antibody fragment of claim 79, wherein the IgG1 heavy chain constant region comprises the amino acid sequence of the IgG1 heavy chain constant region present in SEQ ID NO:61.

81. The antibody or antibody fragment of claim 79, wherein the IgG1 heavy chain constant region has been modified to enhance protease resistance of the antibody.

82. The antibody or antibody fragment of claim 81, wherein the IgG1 heavy chain constant region that has been modified to enhance protease resistance of the antibody comprises the amino acid sequence of the IgG1 heavy chain constant region present in SEQ ID NO:60.

83. The antibody or antibody fragment of any one of claims 77-82, wherein the antibody comprises a light chain constant region.

84. The antibody or antibody fragment of claim 83, wherein the light chain constant region is a human light chain constant region.

85. The antibody or antibody fragment of claim 84, wherein the human light chain constant region comprises the amino acid sequence of the light chain constant region present in SEQ ID NO:56.

86. The antibody or antibody fragment of any one of claims 73-85, wherein the antibody comprises a heavy chain comprising SEQ ID NO:60 and a light chain comprising SEQ ID NO:56.

87. The antibody or antibody fragment of any one of claims 73-85, wherein the antibody comprises a heavy chain comprising SEQ ID NO:61 and a light chain comprising SEQ ID NO:56.

88. The antibody or antibody fragment of any one of claims 73-87, wherein the antibody or antibody fragment is expressed in a cell that is deficient in fucosylation enzymes.

89. The antibody or antibody fragment of claim 88, wherein the cell is a Chinese hamster ovary (CHO) cell.

90. A composition comprising the antibody or antibody fragment of any one of claims 73-89 and a pharmaceutically acceptable carrier, diluent, or excipient.

91. A method for treating cancer in an individual in need thereof, said method comprising administering to the subject an effective amount of the antibody or antibody fragment of any one of claims 73-89, or the composition of claim 90.

92. The method of claim 91, wherein the cancer is a lung cancer.

93. The method of claim 91 or 92, further comprising administering a second cancer treatment.

94. The method of claim 93, wherein the second cancer treatment is surgery, chemotherapy, radiation therapy, biologic therapy, targeted therapy, or immunomodulatory therapy, or a combination thereof.

95. A method for treating a non-small cell lung carcinoma (NSCLC) in an individual in need thereof, said method comprising administering to the subject an effective amount of the antibody or antibody fragment of any one of claims 73-89, or the composition of claim 90.

96. The antibody or antibody fragment of claim 22, wherein the epitope is a conformational epitope.

97. The antibody or antibody fragment of claim 96, wherein the conformational epitope comprises or is present within residues 103-111 (NLTLLDSGL (SEQ ID NO:62)) and 136-146 (VQTGKDAPSNC (SEQ ID NO:63)) of human VISTA (SEQ ID NO:46).

98. The antibody or antibody fragment of claim 96, wherein the conformational epitope comprises or is present within residues 24-36 (LLGPVDKGHDVTF (SEQ ID NO:64)), and 54-65 (RRPIRDLTFQDL (SEQ ID NO:65) of human VISTA (SEQ ID NO:46).

99. The antibody or antibody fragment of claim 96, wherein the conformational epitope comprises amino acid residues in the FG loop of human VISTA (SEQ ID NO:46).

100. A method of enhancing an immune response in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of an antibody that binds V-domain Ig Suppressor of T cell Activation (VISTA), or an antibody fragment thereof, comprising an antigen binding region that binds to VISTA, thereby enhancing an immune response to the cancer.

101. The method of Claim 100, wherein the immune response is an antitumor immune response.

102. A method of eliciting a biological response in an individual in need thereof, comprising administering to the individual a therapeutically effective amount

of an antibody that binds V-domain Ig Suppressor of T cell Activation (VISTA), or an antibody fragment thereof, comprising an antigen binding region that binds to VISTA, thereby enhancing an immune response to the cancer, wherein the biological response is selected from the group consisting of:

a. activation of monocytes;
b. induction of T-cell proliferation and cytokine secretion;
c. increased survival of monocytes;
d. induction of antibody-dependent cell-mediated cytotoxicity (ADCC) in cells-expressing VISTA; and
e. induction of antibody-dependent cellular phagocytosis (ADCP) in cells-expressing VISTA.

103. An isolated antibody or antibody fragment thereof that competitively inhibits binding of the antibody or antibody fragment of Claim 1 to human VISTA (SEQ ID NO:46).

the method further comprises administering a second cancer treatment (e.g., surgery, chemotherapy, radiation therapy, biologic therapy, immunomodulatory therapy, and combinations thereof).

[0021] The invention also provides an antibody or antibody fragment thereof comprising an antigen binding region that binds to a V-domain Ig Suppressor of T cell Activation (VISTA), wherein the antibody binds to a conformational epitope in VISTA (e.g., human VISTA). In some embodiments, the conformational epitope comprises, or is present within, residues 103-111 (NLTLLDSGL (SEQ ID N062)) and 136-146 (VQTGKDAPSNC (SEQ ID NO:63)) of human VISTA (SEQ ID NO:46). In another embodiment, the conformational epitope comprises, or is present within, residues 24-36 (LLGPVDKGHDVTF (SEQ ID NO:64)), 54-65 (RRPIRNLTFQDL (SEQ ID NO:65) and 100-102 (TMR) of human VISTA (SEQ ID NO:46). In yet another embodiment, the conformational epitope comprises amino acid residues in the FG loop of human VISTA (SEQ ID NO:46).

[0022] In addition, the invention relates to a method of enhancing an immune response in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of an antibody that binds V-domain Ig Suppressor of T cell Activation (VISTA), or an antibody fragment thereof, comprising an antigen binding region that binds to VISTA, thereby enhancing an immune response to the cancer. In a particular embodiment, the immune response is an antitumor immune response.

[0023] In another embodiment, the invention provides a method of eliciting a biological response in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of an antibody that binds V-domain Ig Suppressor of T cell Activation (VISTA), or an antibody fragment thereof, comprising an antigen binding region that binds to VISTA, thereby enhancing an immune response to the cancer. Examples of biological responses include activation of monocytes; induction of T-cell proliferation and cytokine secretion; antibody-dependent cell-mediated cytotoxicity (ADCC) of cells expressing VISTA; and antibody-dependent cellular phagocytosis (ADCP) of cells expressing VISTA.

(SEQ ID NO:75), and $_{54}$RRPIRNLTFQDL$_{65}$ (SEQ ID NO:65) (Figure 28 bottom). There are two other segments showing weak perturbation by VSTB95 and 112, including residues 39-52 and 118-134. However, the levels of the reduction are not as strong as the previous regions (27-36 and 54-65) in the differential map. Although one peptide, $_{100}$TMR$_{102}$ showing strong perturbation by VSTB95 and 112, is located on the other face of VISTA surface, it is distant from the epitope regions, 27-36 and 54-65. This perturbation could be due to allosteric effect. These HDX-MS results provide the peptide level epitopes for anti-VISTA antibodies. There were no overlapping epitope regions for these two epitope groups. These results are in agreement with the previous competition binning data in that they do not compete with each other.

[00287] EXAMPLE 14: STRUCTURE DETERMINATION OF THE HUMAN VISTA ECD:VSTB112 FAB COMPLEX BY PROTEIN CRYSTALLOGRAPHY

[00288] In an effort to determine the VISTA structure and to delineate the epitope and paratope defining the interaction between VISTA extracellular domain (ECD) and the Fab fragment of lead antibody VSTB112, the complex was crystallized and structure determined to 1.85 Å resolution. The structure of the ECD of human VISTA in complex with the Fab fragment of the antibody VSTB112 was determined in an effort both to determine the structure of VISTA ECD itself and to define the epitope/paratope for this interaction. The structure reveals VISTA to adopt an IgV fold with a chain topology similar to the TCR Vα chain. In addition to the canonical disulfide bond bridging B and F strands in the back and front faces of the β-sandwich, the structure reveals the ECD to have two additional disulfide bonds, one tethering the CC' loop to the front sheet and a second between the A' and G' strands. Although crystal contacts between VISTA molecules are present, they are minor and there is no evidence for a dimer of VISTA ECDs based on this structure. The VSTB112 epitope is shown to comprise the portions of the VISTA BC, CC', and FG loops together with residues of the front beta sheet (C'CFG) nearest those loops. The paratope is biased largely toward heavy chain interactions with CDR L3 making minimal contact.

CLAIMS

What is claimed:

1. An isolated antibody or antibody fragment thereof comprising an antigen binding region that binds to a V-domain Ig Suppressor of T cell Activation (VISTA), wherein binding of the antibody or antibody fragment to VISTA modulates or enhances an immune response.

2. The antibody or antibody fragment of claim 1, wherein the antibody fragment is a Fab, $F(ab')_2$, or scFv antibody fragment.

3. The antibody or antibody fragment of claim 1 which comprises an antibody constant region.

4. The antibody or antibody fragment of claim 1, wherein VISTA is expressed on a hematopoietic cell.

5. The antibody or antibody fragment of claim 1, wherein the hematopoietic cell is a myeloid lineage cell and/or a lymphocyte.

6. The antibody or antibody fragment of claim 1, wherein the hematopoietic cell is a monocyte or a neutrophil.

7. The antibody or antibody fragment of claim 1, wherein the hematopoietic cell is a T cell.

8. The antibody or antibody fragment of claim 1, wherein the hematopoietic cell is a B cell, a natural killer (NK) cell or a natural killer T (NKT) cell.

9. The antibody or antibody fragment of claim 1, wherein VISTA is expressed on a tumor cell.

10. The antibody or antibody fragment of claim 1 wherein VISTA is expressed in a tumor microenvironment (TME).

11. The antibody or antibody fragment of any one of claims 1-10, further comprising one or more heavy chain complementary determining regions (CDRs) and one or more light chain CDRs; wherein the one or more heavy chain CDRs comprises an amino acid sequence selected from SEQ ID NOs:1-3, 7-9, 13-15, 19-21, 25-27, 31-33 and the one or more light chain CDRs comprises an amino acid sequence selected from SEQ ID NOs:4-6, 10-12, 16-18, 22-24, 28-30, 34-36).

12. The antibody or antibody fragment of any one of claims 1-10, further comprising at least one heavy chain and at least one light chain.

13. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises SEQ ID NO:1-3.

14. An antibody or antibody fragment according to claim 12, wherein at least one light chain comprises SEQ ID NO:4-6.

15. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises SEQ ID NO:1-3 and wherein at least one light chain comprises SEQ ID NO:4-6.

16. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises the heavy chain variable region sequence as shown in SEQ ID NO:37-40.

17. An antibody or antibody fragment according to claim 12, wherein at least one light chain comprises the light chain variable region sequence as shown in SEQ ID NO:41-45.

18. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises the heavy chain variable region sequence as shown in SEQ ID NO:37-40, and wherein at least one light chain comprises the light chain variable region sequence as shown in SEQ ID NO: 41-45.

19. The antibody or antibody fragment of any one of claims 1-18, wherein the antibody is a monoclonal antibody.

20. The antibody or antibody fragment of any one of claims 1-18, wherein the antibody is a humanized antibody.

21. The antibody or antibody fragment of any one of claims 1-18, wherein the antibody comprises a human constant region.

22. An antibody or antibody fragment according to any one of claims 1-18, wherein the antibody molecule is specific for an epitope within the amino acid sequence SEQ ID NO: 46.

23. The antibody or antibody fragment of any one of claims 1-18, wherein the antibody binds to an epitope of VISTA with an affinity of at least $1x10^{-9}$ liter/mole.

24. The antibody or antibody fragment of any one of claims 1-18, wherein the antibody binds to an epitope of VISTA with an affinity of at least $1x10^{-8}$ liter/mole.

25. The antibody or antibody fragment of any one of claims 1-18, wherein the antibody binds to an epitope of VISTA with an affinity of at least $1x10^{-7}$ liter/mole.

26. The antibody or antibody fragment of any one of claims 1-18, wherein the modulation of the immune response comprises an increase in CD45+ leukocytes, CD4+ T cells, or CD8+ T cells, or a combination thereof, or a decrease in VISTA-expressing immune cells.

27. The antibody or antibody fragment of any one of claims 1-18, wherein the modulation of the immune response comprises enhanced production of cytokines, enhanced T-cell response, and/or modulates Foxp3 expression.

28. A composition comprising the antibody or antibody fragment of any one of claims 1-27 and a pharmaceutically acceptable carrier, diluent, or excipient.

29. A composition comprising:

a VISTA antagonist comprising an antibody or antibody fragment thereof comprising an antigen binding region that binds to a V-domain Ig Suppressor of T cell Activation (VISTA); and

a vaccine.

30. A pharmaceutical composition comprising:

an antibody or antibody fragment thereof comprising an antigen binding region that binds to a V-domain Ig Suppressor of T cell Activation (VISTA); and

a vaccine,

wherein binding of the antibody or antibody fragment to VISTA modulates or enhances an immune response.

31. A method for treating cancer in an individual in need thereof, said method comprising administering to the subject an effective amount of the antibody or antibody fragment of any one of claims 1-27.

32. A method for treating or preventing cancer in an individual in need thereof, said method comprising administering to the individual an effective amount of the composition of any one of claims 28-30.

33. A method of treating a cancer in an individual in need thereof comprising:

administering to the individual a therapeutically effective amount of a V-domain Ig Suppressor of T cell Activation (VISTA) antagonist comprising an antibody or antibody fragment thereof comprising an antigen binding region that binds to VISTA; wherein the antibody or antibody fragment binds to VISTA, thereby modulating or enhancing an immune response to the cancer.

34. The method of any one of Claims 31-32, wherein the individual is a mammal.

35. The method of any one of Claims 31-32, wherein the individual is a human.

36. A method of treating a cancer in an individual in need thereof comprising administering to the individual a therapeutically effective amount of a V-domain Ig Suppressor of T cell Activation (VISTA) antagonist comprising an antibody or antibody fragment thereof comprising an antigen binding region that binds to VISTA; wherein the antibody or antibody fragment binds to VISTA, thereby modulating or enhancing an immune response to the cancer.

37. The method of claim 36, wherein the cancer is a leukemia, lymphoma, myelodisplastic syndrome or myeloma, or a combination thereof.

38. The method of claim 37, wherein the leukemia is a lymphocytic leukemia or a myelogenous leukemia.

39. The method of claim 37, wherein the leukemia is acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid (myelogenous) leukemia (AML), chronic myelogenous leukemia (CML); hairy cell leukemia, T-cell prolymphocytic leukemia, large granular lymphocytic leukemia, or adult T-cell leukemia.

40. The method of claim 36, wherein the cancer is an acute myeloid (myelogenous) leukemia (AML).

41. The method of claim 36, wherein the cancer is a chronic myelogenous leukemia (CML).

42. The method of claim 36, wherein the cancer is a solid tumor.

43. The method of claim 36, wherein the solid tumor is surrounded by a tumor stroma comprising myeloid cells, T-cells, or a combination of myeloid cells and T-cells.

44. The method of claim 42 or 43, wherein the solid tumor is infiltrated with myeloid cells, T cells or a combination of myeloid cells and T-cells.

45. The method of claim 35, further comprising administering a vaccine.

46. A method for suppressing tumor growth in an individual in need thereof, said method comprising administering an effective antibody or antibody fragment of claims 1-27 to a subject in need thereof.

47. The method of any one of claims 31-46, wherein the composition, antibody or fragment is administered parenterally or nonparenterally, for example, intravenously, subcutaneously or orally.

48. The method of any one of claims 31-46, wherein the dosage of the administered composition, antibody or fragment is 0.1-15 mg/kg per administration.

49. The method of any one of claims 31-46, wherein the composition, antibody or fragment is administered weekly, once every two weeks, once every three weeks, once every month, once every 2 months, or once every 3 months.

50. An antibody or fragment thereof that binds VISTA and which comprises an antibody VH domain comprising a VH CDR1 having an amino acid sequence that is substantially similar to SEQ ID NO:1, a VH CDR2 having an amino acid sequence that is substantially similar to SEQ ID NO:2 and a VH CDR3 having an amino acid sequence that is substantially similar to SEQ ID NO:3, and which further comprises an antibody VL domain comprising a VL CDR1 having an amino acid sequence that is substantially similar to SEQ ID NO:4, a VL CDR2 having an amino acid sequence that is substantially similar to SEQ ID NO:5 and a VL CDR3 having an amino acid sequence that is identical to SEQ ID NO:6.

51. An antibody or fragment thereof of claim 12, wherein the variable light chain VL domain comprises a human framework region.

52. An antibody or fragment thereof of claim 1, which comprises a whole antibody.

53. An isolated nucleic acid which comprises a nucleotide sequence encoding the antibody of claim 1.

54. An expression vector comprising the nucleic acid of claim 51 operably linked to a promoter.

55. A host cell transformed with the expression vector of claim 52.

56. A method of producing antibody or fragment thereof of claim 1, the method comprising culturing host cells according to claim 53 under conditions for production of said antibody or fragment.

57. The method according to claim 54 further comprising isolating said antibody.

58. An article of manufacture comprising the composition of claim 30 and a container, and further comprising a package insert or label indicating that the composition can be used to treat cancer.

59. An antibody or antibody fragment thereof, wherein the antibody is selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

60. The antibody or antibody fragment of any one of claims 1-10, further comprising one or more heavy chain complementary determining regions (CDRs) and one or more light chain CDRs of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

61. The antibody or antibody fragment of any one of claims 1-10, further comprising at least one heavy chain and at least one light chain of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

62. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises all three heavy chain CDRs of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

63. An antibody or antibody fragment according to claim 12, wherein at least one light chain comprises all three light chain CDRs of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

64. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises all three heavy chain CDRs of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47), and wherein at least one light chain comprises all three light chain CDRs of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

65. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises the heavy chain variable region sequence of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

66. An antibody or antibody fragment according to claim 12, wherein at least one light chain comprises the light chain variable region sequence of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

67. An antibody or antibody fragment according to claim 12, wherein at least one heavy chain comprises the heavy chain variable region sequence of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47), and wherein at least one light chain comprises the light chain variable region sequence of an antibody selected from the group consisting of: VSTB112 (S2), VSTB116 (S5), VSTB95 (S16), VSTB50 (S41), VSTB53 (S43) and VSTB60 (S47).

68. A method of treating a cancer in an individual in need thereof comprising:

administering to the individual a therapeutically effective amount of a V-domain Ig Suppressor of T cell Activation (VISTA) antagonist comprising an antibody or antibody fragment thereof comprising an antigen binding region that binds to VISTA; wherein the antibody or antibody fragment binds to VISTA, thereby modulating or enhancing an immune response to the cancer; and

administering a second cancer treatment.

69. The method of claim 68, wherein the second cancer treatment is surgery, chemotherapy, radiation therapy, biologic therapy, targeted therapy, or immunomodulatory therapy, or a combination thereof.

70. The composition of claim 29, pharmaceutical composition of claim 30, or method of claim 45, wherein the vaccine is a viral vector vaccine, bacterial vaccine, DNA vaccine, RNA vaccine, peptide vaccine, or protein vaccine.

71. The method of claim 36, wherein the cancer is a lung cancer.

72. The method of claim 69, wherein the lung cancer is a non-small cell lung carcinoma (NSCLC).

73. An isolated antibody or antibody fragment thereof comprising an antigen binding region that binds to a V-domain Ig Suppressor of T cell Activation (VISTA), wherein the antibody comprises an antibody VH domain comprising a VH CDR1 having the amino acid sequence of SEQ ID NO:25, a VH CDR2 having the amino acid sequence of SEQ ID NO:26 and a VH CDR3 having the amino acid sequence of SEQ ID NO:27, and which further comprises an antibody VL domain comprising a VL CDR1 having the amino acid sequence of SEQ ID NO:28, a VL CDR2 having the amino acid sequence of SEQ ID NO:29 and a VL CDR3 having the amino acid sequence of SEQ ID NO:30.

74. The antibody or antibody fragment of claim 73, comprising one or more humanized or human framework regions.

75. The antibody or antibody fragment of claim 74, wherein the antibody VH domain comprises SEQ ID NO:37.

76. The antibody of claim 74 or 75, wherein the antibody VL domain comprises SEQ ID NO:44.

77. The antibody or antibody fragment of any one of claims 73-76, wherein the antibody comprises a heavy chain constant region.

78. The antibody or antibody fragment of claim 77, wherein the heavy chain constant region is a human heavy chain constant region.

79. The antibody or antibody fragment of claim 77 or 78, wherein the heavy chain constant region is an IgG1 heavy chain constant region.

80. The antibody or antibody fragment of claim 79, wherein the IgG1 heavy chain constant region comprises the amino acid sequence of the IgG1 heavy chain constant region present in SEQ ID NO:61.

81. The antibody or antibody fragment of claim 79, wherein the IgG1 heavy chain constant region has been modified to enhance protease resistance of the antibody.

82. The antibody or antibody fragment of claim 81, wherein the IgG1 heavy chain constant region that has been modified to enhance protease resistance of the antibody comprises the amino acid sequence of the IgG1 heavy chain constant region present in SEQ ID NO:60.

83. The antibody or antibody fragment of any one of claims 77-82, wherein the antibody comprises a light chain constant region.

84. The antibody or antibody fragment of claim 83, wherein the light chain constant region is a human light chain constant region.

85. The antibody or antibody fragment of claim 84, wherein the human light chain constant region comprises the amino acid sequence of the light chain constant region present in SEQ ID NO:56.

86. The antibody or antibody fragment of any one of claims 73-85, wherein the antibody comprises a heavy chain comprising SEQ ID NO:60 and a light chain comprising SEQ ID NO:56.

87. The antibody or antibody fragment of any one of claims 73-85, wherein the antibody comprises a heavy chain comprising SEQ ID NO:61 and a light chain comprising SEQ ID NO:56.

88. The antibody or antibody fragment of any one of claims 73-87, wherein the antibody or antibody fragment is expressed in a cell that is deficient in fucosylation enzymes.

89. The antibody or antibody fragment of claim 88, wherein the cell is a Chinese hamster ovary (CHO) cell.

90. A composition comprising the antibody or antibody fragment of any one of claims 73-89 and a pharmaceutically acceptable carrier, diluent, or excipient.

91. A method for treating cancer in an individual in need thereof, said method comprising administering to the subject an effective amount of the antibody or antibody fragment of any one of claims 73-89, or the composition of claim 90.

92. The method of claim 91, wherein the cancer is a lung cancer.

93. The method of claim 91 or 92, further comprising administering a second cancer treatment.

94. The method of claim 93, wherein the second cancer treatment is surgery, chemotherapy, radiation therapy, biologic therapy, targeted therapy, or immunomodulatory therapy, or a combination thereof.

95. A method for treating a non-small cell lung carcinoma (NSCLC) in an individual in need thereof, said method comprising administering to the subject an effective amount of the antibody or antibody fragment of any one of claims 73-89, or the composition of claim 90.

96. The antibody or antibody fragment of claim 22, wherein the epitope is a conformational epitope.

97. The antibody or antibody fragment of claim 96, wherein the conformational epitope comprises or is present within residues 103-111 (NLTLLDSGL (SEQ ID NO:62)) and 136-146 (VQTGKDAPSNC (SEQ ID NO:63)) of human VISTA (SEQ ID NO:46).

98. The antibody or antibody fragment of claim 96, wherein the conformational epitope comprises or is present within residues 24-36 (LLGPVDKGHDVTF (SEQ ID NO:64)), and 54-65 (RRPIRNLTFQDL (SEQ ID:65) of human VISTA (SEQ ID NO:46).

99. The antibody or antibody fragment of claim 96, wherein the conformational epitope comprises amino acid residues in the FG loop of human VISTA (SEQ ID NO:46).

100. A method of enhancing an immune response in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of an antibody that binds V-domain Ig Suppressor of T cell Activation (VISTA), or an antibody fragment thereof, comprising an antigen binding region that binds to VISTA, thereby enhancing an immune response to the cancer.

101. The method of Claim 100, wherein the immune response is an antitumor immune response.

102. A method of eliciting a biological response in an individual in need thereof, comprising administering to the individual a therapeutically effective amount

of an antibody that binds V-domain Ig Suppressor of T cell Activation (VISTA), or an antibody fragment thereof, comprising an antigen binding region that binds to VISTA, thereby enhancing an immune response to the cancer, wherein the biological response is selected from the group consisting of:

a. activation of monocytes;
b. induction of T-cell proliferation and cytokine secretion;
c. increased survival of monocytes;
d. induction of antibody-dependent cell-mediated cytotoxicity (ADCC) in cells-expressing VISTA; and
e. induction of antibody-dependent cellular phagocytosis (ADCP) in cells-expressing VISTA.

103. An isolated antibody or antibody fragment thereof that competitively inhibits binding of the antibody or antibody fragment of Claim 1 to human VISTA (SEQ ID NO:46).

104. The method of claim 91, wherein the cancer is a bladder cancer.

105. The method of claim 91, wherein the cancer is a breast cancer.