

pK_a of about 6.5, meaning that at or below pH 6.5, histidine residues within proteins are often protonated and thus, positively charged, while at pH higher than pH 6.5 they are increasingly unprotonated and neutral in charge. Tumor microenvironments and inflamed tissues are often acidic, and thus, VISTA proteins found in these microenvironments may be at least partially protonated at their histidine residues. The inventors, as discussed herein, have hypothesized that histidine protonation may affect the conformation, surface structure, and/or charge density of VISTA, which, in turn, may create pH-specific or pH-selective epitopes for both receptor-ligand interaction(s) and antibody binding. Targeting VISTA with antibodies that bind at acidic pH but not neutral or physiological pH may prevent target-mediated drug disposition via circulating and lymphoid organ-resident myelomonocytic cells, improving antibody PK, receptor occupancy, and activity in tumor microenvironments. Acidic pH-selective antibodies may also improve the specificity of VISTA antibodies for intratumoral, rather than circulating, target cells in the cases of therapeutic modalities such as antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), and delivery of payloads (antibody-drug conjugates).

Thus, in one aspect, the disclosure provides an isolated antibody that binds specifically to human V-domain Ig Suppressor of T cell Activation (hVISTA), wherein the antibody comprises

(i) VL CDR1, CDR2 and CDR3 of PI-061029, wherein the VL CDR1, CDR2, and CDR3 of PI-061029 comprise sequences of positions 24-35, 51-57, and 90-98 of SEQ ID NO: 68

and VH CDR 1, CDR2 and CDR3 of

PI-068767 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 55),

PI-068761 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 51),

PI-069061 (positions 26-35, 50-66 and 99-112 of SEQ ID NO: 15),

PI-069059 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 11),

PI-068773 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 59),

PI-069073 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 39),

PI-068765 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 63),

PI-068757 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 71),
PI-068771 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 75),
PI-068769 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 83),
PI-068775 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 79),
5 PI-068759 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 87),
PI-068761 E55A (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 193),
PI-068761 H100G (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 201),
PI-068761 E56N (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 197),
PI-068761 E55A E56N (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 213),
10 PI-068761 E30D (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 185),
PI-068761 E30D E55A (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 237),
PI-068761 E56N H100G (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 257),
PI-068761 E30D H100G (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 245),
PI-068761 E30D E56N (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 241),
15 PI-068767 D52N (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 269),
PI-068767 D52N E55A (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 289),
PI-068767 E55A (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 273),
PI-068767 E30D D52N (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 285),
PI-068767 E30D (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 265),
20 PI-068767 E30D E55A (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 301),
PI-068767 E55A E100fF (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 293),
PI-068767 D52N E100fF (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 313),
PI-068767 E100fF (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 277), or
PI-068767 E30D E100fF (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 305),
25 _____ or wherein the antibody comprises
(ii) VL CDR1, CDR2 and CDR3 of PI-061015, wherein the VL CDR1, CDR2, and CDR3 of
PI-061015 comprise sequences of positions 24-35, 51-57, and 90-98 of SEQ ID NO: 96
and VH CDR 1, CDR2 and CDR3 of
PI-068748 (positions 26-35, 50-66, and 99-110 of SEQ ID NO: 99),
30 PI-068744 (positions 26-35, 50-66, and 99-110 of SEQ ID NO: 103),

Pl-068736 (positions 26-35, 50-66, and 99-110 of SEO ID NO: 107), or
Pl-068752 (positions 26-35, 50-66, and 99-110 of SEO ID NO: 111).

In another aspect, the disclosure provides an isolated nucleic acid encoding an antibody of the disclosure.

5 In another aspect, the disclosure provides a composition comprising the isolated nucleic acid of the disclosure.

In another aspect, the disclosure provides a cell comprising the isolated nucleic acid of the disclosure, or the composition of the disclosure.

10 In another aspect, the disclosure provides a method of preparing an antibody, comprising culturing the cell of the disclosure in conditions under which the antibody is expressed.

In another aspect, the disclosure provides a composition comprising an isolated antibody of the disclosure, the isolated nucleic acid of the disclosure, composition of the disclosure or cell of the disclosure and a pharmaceutically acceptable carrier.

15 In another aspect, the disclosure provides a use of the isolated antibody of the disclosure or the composition of the disclosure in the manufacture of a medicament for treating cancer, wherein the medicament stimulates an immune response and/or is a VISTA antagonist antibody.

20 In another aspect, the disclosure provides a use of the isolated antibody of the disclosure or the composition of the disclosure in the manufacture of a medicament for treating infectious disease (e.g., viral disease), wherein the medicament stimulates an immune response and/or is a VISTA antagonist.

BRIEF DESCRIPTION OF THE FIGURES

25 A color version of several of the figures is provided in priority US provisional application No. 62/636,746, filed February 28, 2018. Should that priority application file become available through publication of the present application, it is presumed that copies of the color drawings will be provided by the US Patent and Trademark Office upon request and payment of the necessary fee.

Figs. 1A-C show that VISTA's extracellular domain contains an exceptionally high frequency of histidine residues, that many of these histidine residues are conserved, and that at least some of these histidine residues may participate in receptor-ligand binding. **Fig. 1A** shows a graph of immunoglobulin domain-containing proteins, with the number of

5 extracellular domain amino acid residues for each protein plotted on the x-axis, and the frequency of histidine residues within the extracellular domain for each protein plotted on the y-axis. The size of each data point corresponds to the total number of histidine residues in

CLAIMS

1. An isolated antibody that binds specifically to human V-domain Ig Suppressor of T cell Activation (hVISTA), wherein the antibody comprises

(i) VL CDR1, CDR2 and CDR3 of PI-061029, wherein the VL CDR1, CDR2, and CDR3 of PI-061029 comprise sequences of positions 24-35, 51-57, and 90-98 of SEQ ID NO: 68

and VH CDR 1, CDR2 and CDR3 of

PI-068767 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 55),

PI-068761 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 51),

PI-069061 (positions 26-35, 50-66 and 99-112 of SEQ ID NO: 15),

PI-069059 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 11),

PI-068773 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 59),

PI-069073 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 39),

PI-068765 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 63),

PI-068757 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 71),

PI-068771 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 75),

PI-068769 (positions 26-35, 50-66, and 99-112 of SEO ID NO: 83),

PI-068775 (positions 26-35, 50-66, and 99-112 of SEO ID NO: 79),

PI-068759 (positions 26-35, 50-66, and 99-112 of SEO ID NO: 87),

PI-068761 E55A (positions 26-35, 50-66, and 99-112 of SEO ID NO: 193),

PI-068761 H100G (positions 26-35, 50-66, and 99-112 of SEO ID NO: 201),

PI-068761 E56N (positions 26-35, 50-66, and 99-112 of SEO ID NO: 197),

PI-068761 E55A E56N (positions 26-35, 50-66, and 99-112 of SEO ID NO: 213),

PI-068761 E30D (positions 26-35, 50-66, and 99-112 of SEO ID NO: 185),

PI-068761 E30D E55A (positions 26-35, 50-66, and 99-112 of SEO ID NO: 237),

PI-068761 E56N H100G (positions 26-35, 50-66, and 99-112 of SEO ID NO: 257),

PI-068761 E30D H100G (positions 26-35, 50-66, and 99-112 of SEO ID NO: 245),

PI-068761 E30D E56N (positions 26-35, 50-66, and 99-112 of SEO ID NO: 241),

PI-068767 D52N (positions 26-35, 50-66, and 99-112 of SEO ID NO: 269),

PI-068767 D52N E55A (positions 26-35, 50-66, and 99-112 of SEO ID NO: 289),

PI-068767 E55A (positions 26-35, 50-66, and 99-112 of SEO ID NO: 273),

PI-068767 E30D D52N (positions 26-35, 50-66, and 99-112 of SEO ID NO: 285),

PI-068767 E30D (positions 26-35, 50-66, and 99-112 of SEO ID NO: 265),

PI-068767 E30D E55A (positions 26-35, 50-66, and 99-112 of SEO ID NO: 301),

PI-068767 E55A E100fF (positions 26-35, 50-66, and 99-112 of SEO ID NO: 293),

PI-068767 D52N E100fF (positions 26-35, 50-66, and 99-112 of SEO ID NO: 313),

PI-068767 E100fF (positions 26-35, 50-66, and 99-112 of SEO ID NO: 277), or

PI-068767 E30D E100fF (positions 26-35, 50-66, and 99-112 of SEO ID NO: 305),

or wherein the antibody comprises

(ii) VL CDR1, CDR2 and CDR3 of PI-061015, wherein the VL CDR1, CDR2, and CDR3 of PI-061015 comprise sequences of positions 24-35, 51-57, and 90-98 of SEQ ID NO: 96

and VH CDR 1, CDR2 and CDR3 of

PI-068748 (positions 26-35, 50-66, and 99-110 of SEO ID NO: 99),

PI-068744 (positions 26-35, 50-66, and 99-110 of SEO ID NO: 103),
PI-068736 (positions 26-35, 50-66, and 99-110 of SEO ID NO: 107), or
PI-068752 (positions 26-35, 50-66, and 99-110 of SEO ID NO: 111).

2. The isolated antibody of claim 1, wherein the antibody binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-5} M or more, wherein neutral conditions are conditions having a pH of 7.0, and wherein physiological conditions are conditions having a pH of 7.4.

3. The isolated antibody of claim 1 or 2, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-7} M or less, wherein acidic conditions are conditions having a pH of 6.0 to 6.5.

4. The isolated antibody of any one of claims 1-3 wherein the isolated antibody has one or more of the following properties:

- a. binds to hVISTA in acidic conditions with a K_D that is at least 10 fold lower than its K_D in neutral or physiological conditions, wherein acidic conditions are conditions having a pH of 6.0 to 6.5, wherein neutral conditions are conditions having a pH of 7.0, and wherein physiological conditions are conditions having a pH of 7.4;
- b. binds to hVISTA in acidic conditions with a k_{off} that is at least 5 fold lower than its k_{off} in neutral or physiological conditions, wherein acidic conditions are conditions having a pH of 6.0 to 6.5, wherein neutral conditions are conditions having a pH of 7.0, and wherein physiological conditions are conditions having a pH of 7.4;
- c. inhibits the binding of hVISTA to human T cells in conditions having a pH of less than pH 7.0;
- d. inhibits the binding of hVISTA to huPSGL-1 in conditions having a pH of less than pH 7.0;
- e. inhibits the binding of hVISTA to heparan sulfate proteoglycans;
- f. inhibits the binding of hVISTA to heparan sulfate proteoglycans in conditions having a pH of less than pH 7.0;
- g. stimulates T cell activation in conditions having a pH of less than pH 7.0;
- h. reduces VISTA mediated cell-cell adhesion;
- i. has a mean residence time of at least 500 days in Cynomolgus macaques;
- j. does not significantly bind to VISTA positive cells in the peripheral blood of a subject to whom it is to be administered; and
- k. does not significantly deplete VISTA positive cells in peripheral blood of a subject to whom it is to be administered

~~1. An isolated antibody that binds specifically to hVISTA in acidic conditions.~~

- ~~2. The isolated antibody of claim 1, which binds specifically to hVISTA in acidic conditions, but not significantly in neutral or physiological conditions.~~
- ~~3. The isolated antibody of claim 1 or 2, wherein the antibody binds to hVISTA in acidic conditions with a K_D that is at least 10 fold lower than its K_D in neutral or physiological conditions.~~
- ~~4. The isolated antibody of any one of claims 1 to 3, wherein the antibody binds to hVISTA in acidic conditions with a K_D that is at least 100 fold lower than its K_D in neutral or physiological conditions.~~
- ~~5. The isolated antibody of any one of claims 1-4, wherein the antibody binds to hVISTA in acidic conditions with a K_D that is at least 1000 fold lower than its K_D in neutral or physiological conditions.~~
- ~~6. The isolated antibody of any one of claims 1-5, wherein the antibody binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-5} M or more.~~
- ~~7. The isolated antibody of any one of claims 1-6, wherein the antibody binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-4} M or more.~~
- ~~8. The isolated antibody of any one of claims 1-7, wherein the antibody binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-3} M or more.~~
- ~~9. The isolated antibody of any one of claims 1-8, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-7} M or less.~~
- ~~10. The isolated antibody of any one of claims 1-9, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-8} M or less.~~
- ~~11. The isolated antibody of any one of claims 1-10, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-9} M or less.~~
- ~~12. The isolated antibody of any one of claims 1-11, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-7} or less and binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-4} or more.~~
- ~~13. The isolated antibody of any one of claims 1-12, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-7} or less and binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-5} or more.~~

14. The isolated antibody of any one of claims 1-13, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} that is at least 5 fold lower than its k_{off} in neutral or physiological conditions.
15. The isolated antibody of any one of claims 1-14, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} that is at least 10 fold lower than its k_{off} in neutral or physiological conditions.
16. The isolated antibody of any one of claims 1-15, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} that is at least 50 fold lower than its k_{off} in neutral or physiological conditions.
17. The isolated antibody of any one of claims 1-16, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} that is at least 100 fold lower than its k_{off} in neutral or physiological conditions.
18. The isolated antibody of any one of claims 1-17, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of $7 \times 10^{-3} \text{ s}^{-1}$ or less.
19. The isolated antibody of any one of claims 1-18, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of $5 \times 10^{-3} \text{ s}^{-1}$ or less.
20. The isolated antibody of any one of claims 1-19 wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of $3 \times 10^{-3} \text{ s}^{-1}$ or less.
21. The isolated antibody of any one of claims 1-20, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 10^{-3} s^{-1} or less.
22. The isolated antibody of any one of claims 1-21, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of $7 \times 10^{-4} \text{ s}^{-1}$ or less.
23. The isolated antibody of any one of claims 1-22, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of $5 \times 10^{-4} \text{ s}^{-1}$ or less.
24. The isolated antibody of any one of claims 1-23, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of $3 \times 10^{-4} \text{ s}^{-1}$ or less.
25. The isolated antibody of any one of claims 1-24, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 10^{-4} s^{-1} or less.
26. The isolated antibody of any one of claims 1-25, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of $7 \times 10^{-5} \text{ s}^{-1}$ or less.

27. The isolated antibody of any one of claims 1-26, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of $5 \times 10^{-5} \text{ s}^{-1}$ or less.
28. The isolated antibody of any one of claims 1-21, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of $3 \times 10^{-5} \text{ s}^{-1}$ or less.
- 5 29. The isolated antibody of any one of claims 1-28, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 10^{-5} s^{-1} or less.
30. The isolated antibody of any one of claims 1-29, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 10^{-3} s^{-1} or more.
31. The isolated antibody of any one of claims 1-30, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of $3 \times 10^{-3} \text{ s}^{-1}$ or more.
- 10 32. The isolated antibody of any one of claims 1-31, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of $5 \times 10^{-3} \text{ s}^{-1}$ or more.
33. The isolated antibody of any one of claims 1-32, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of $7 \times 10^{-3} \text{ s}^{-1}$ or more.
- 15 34. The isolated antibody of any one of claims 1-33, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 10^{-2} s^{-1} or more.
35. The isolated antibody of any one of claims 1-34, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of $3 \times 10^{-2} \text{ s}^{-1}$ or more.
36. The isolated antibody of any one of claims 1-35, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of $5 \times 10^{-2} \text{ s}^{-1}$ or more.
- 20 37. The isolated antibody of any one of claims 1-36, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of $7 \times 10^{-2} \text{ s}^{-1}$ or more.
38. The isolated antibody of any one of claims 1-37, wherein binding of the antibody to hVISTA in neutral or physiological conditions is not detectable, e.g., via SPR.
- 25 39. The isolated antibody of any one of claims 1-38, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of $5 \times 10^{-3} \text{ s}^{-1}$ or less and in neutral or physiological conditions with a k_{off} of $7 \times 10^{-3} \text{ s}^{-1}$ or more.
40. The isolated antibody of any one of claims 1-39, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 10^{-4} s^{-1} or less and in neutral or physiological conditions with a k_{off} of 10^{-2} s^{-1} or more.
- 30

41. The isolated antibody of any one of claims 1-40, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-8} M or less and a k_{off} of $5 \times 10^{-3} s^{-1}$ or less and in neutral or physiological conditions with a K_D of 10^{-6} M or more and a k_{off} of $7 \times 10^{-3} s^{-1}$ or more.

42. The isolated antibody of any one of claims 1-41, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-8} M or less and a k_{off} of $3 \times 10^{-3} s^{-1}$ or less and in neutral or physiological conditions with a K_D of 10^{-6} M or more and a k_{off} of $10^{-2} s^{-1}$ or more.

43. The isolated antibody of any one of claims 1-42, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-12} to 10^{-8} M and a k_{off} of 10^{-4} to $5 \times 10^{-3} s^{-1}$ and in neutral or physiological conditions with a K_D of 10^{-7} to 10^{-4} M and a k_{off} of 3×10^{-3} to $10^{-2} s^{-1}$ or more.

44. The isolated antibody of any one of claims 1-43, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-12} to 10^{-8} M and a k_{off} of 10^{-4} to $5 \times 10^{-3} s^{-1}$ and in neutral or physiological conditions with a K_D of 10^{-7} to 10^{-4} M and a k_{off} of 3×10^{-3} to $10^{-2} s^{-1}$ or more; and wherein the antibody binds to cyno VISTA with a K_D of 10^{-7} or less.

45. The isolated antibody of any one of claims 1-44, wherein the antibody binds to hVISTA with a K_D that is at least 10 fold lower at pH 6.9 than at pH 7.4 and/or a K_D that is at least 100 fold lower at pH 6.5 than at pH 7.4 and at least 1000 fold lower at pH 6.0 than at pH 7.4.

46. The isolated antibody of any one of claims 1-45, wherein the antibody binds to cyno VISTA.

47. The isolated antibody of claim 46, wherein the antibody binds to cyno VISTA with higher affinity in acidic conditions relative to physiologic conditions.

48. The isolated antibody of any one of claims 45-47, wherein the antibody binds to cyno VISTA in acidic conditions with a K_D of 10^{-8} or less and/or a k_{off} of 10^{-2} or less and in physiologic conditions with a K_D of 10^{-6} or more and/or a k_{off} of 10^{-2} or more.

49. The isolated antibody of any one of claims 1-48, wherein acidic conditions are conditions having a pH of 6.5 or less.

50. The isolated antibody of any one of claims 1-49, wherein acidic conditions are conditions having a pH of 6.0 to 6.5.

51. The isolated antibody of any one of claim 1-50, wherein neutral conditions are conditions having a pH of 7.0.

5 52. The isolated antibody of any one of claims 1-51, wherein physiological conditions are conditions having a pH of 7.35 to 7.45.

53. The isolated antibody of any one of claims 1-52, wherein physiological conditions are conditions having a pH of 7.4.

10 54. The isolated antibody of any one of claims 1-53, wherein the antibody inhibits the binding of hVISTA to human T cells, such as human CD4+ T cells (an antagonist antibody).

55. The isolated antibody of claim 54, wherein the antibody inhibits the binding of hVISTA to human T cells in conditions having a pH of less than pH 7.0.

15 56. The isolated antibody of any one of claims 1-55, wherein the antibody inhibits the binding of hVISTA to huPSGL-1.

57. The isolated antibody of claim 56, wherein the antibody inhibits the binding of hVISTA to huPSGL-1 in conditions having a pH of less than pH 7.0.

58. The isolated antibody of anyone of claims 1-57, wherein the antibody inhibits the binding of hVISTA to heparan sulfate proteoglycans.

20 59. The isolated antibody of claim 58, wherein the antibody inhibits the binding of hVISTA to heparan sulfate proteoglycans in conditions having a pH of less than pH 7.0.

25 60. The isolated antibody of anyone of claims 55, 57 or 59, wherein a condition having a pH of less than pH 7.0 is a tumor or any diseased area having a pH of less than pH 7.0 in a subject and in which an immune stimulation is desired.

61. The isolated antibody of any one of claims 1-60, wherein the antibody stimulates T cell activation.

62. The isolated antibody of claim 61, wherein the antibody stimulates T cell activation in conditions having a pH of less than pH 7.0.

~~63. The isolated antibody of any one of claims 1-62, wherein the antibody reduces VISTA-mediated cell-cell adhesion.~~

~~64. The isolated antibody of any one of claims 1-53, wherein the antibody stimulates the activity of VISTA (an agonist antibody).~~

5 ~~65. The isolated antibody of any one of claims 1-53 and 64, wherein the antibody stimulates the binding of hVISTA to human T cells, such as human CD4⁺ T cells.~~

~~66. The isolated antibody of claim 65, wherein the antibody stimulates the binding of hVISTA to human T cells in conditions having a pH of less than pH 7.0.~~

10 ~~67. The isolated antibody of any one of claims 1-53 and 64-66, wherein the antibody stimulates the binding of hVISTA to huPSGL-1.~~

~~68. The isolated antibody of claim 67, wherein the antibody stimulates the binding of hVISTA to huPSGL-1 in conditions having a pH of less than pH 7.0.~~

~~69. The isolated antibody of anyone of claims 1-53 and 64-68, wherein the antibody stimulates the binding of hVISTA to heparan sulfate proteoglycans.~~

15 ~~70. The isolated antibody of claim 69, wherein the antibody stimulates the binding of hVISTA to heparan sulfate proteoglycans in conditions having a pH of less than pH 7.0.~~

~~71. The isolated antibody of anyone of claims 66, 68 and 70, wherein a condition having a pH of less than pH 7.0 is an autoimmune (e.g., rheumatoid arthritis and lupus)~~

20 ~~milieu, an inflammation site, or any diseased area having a pH of less than pH 7.0 in a subject and in which an immune inhibition is desired.~~

~~72. The isolated antibody of any one of claims 1-71, wherein the antibody has a mean residence time of at least 500 days in Cynomolgus macaques.~~

25 ~~73. The isolated antibody of any one of claims 1-72, wherein the antibody is not significantly binding to VISTA positive cells, e.g., neutrophils, in the peripheral blood of a subject to whom it is administered.~~

~~74. The isolated antibody of any one of claims 1-73, wherein the antibody is not significantly depleting VISTA positive cells, e.g., neutrophils, in peripheral blood of a subject to whom it is administered.~~

75. The antibody of any one of claims 1-74, wherein the antibody has been engineered to bind to hVISTA at acidic pH, but not at neutral or physiological pH.
76. The antibody of claim 75, wherein the antibody has been engineered by replacing from 1-8 amino acids in VH CDR1, CDR2 and/or CDR3 with a glutamic acid, aspartic acid or histidine residue.
77. The antibody of any one of claims 1-63 and 72-76, wherein the antibody comprises VH CDR1, CDR2 and/or CDR3 of P1-061029 or a variant thereof comprising 1 to 6 or 1 to 8 amino acid differences in CDR1, CDR2 and/or CDR3 relative to P1-061029, wherein at most 1, 2 or 3 amino acid variations are present in any one CDR.
78. The antibody of claim 77, wherein the antibody comprises VH CDR1, CDR2 and CDR3 of P1-061029 or a variant thereof comprising 1 to 6 or 1 to 8 amino acid differences in CDR1, CDR2 and/or CDR3 relative to P1-061029.
79. The antibody of claim 77 or 78, wherein an amino acid variation is a substitution to a glutamic acid residue (E), aspartic acid residue (D) or a histidine residue (H).
80. The antibody of any one of claims 77-79, wherein the antibody comprises
-a CDR1 comprising GFTX₁X₂DX₃AMH, wherein X₁ is D or L, X₂ is E or D and X₃ is E or Y (SEQ ID NO: 463);
-a CDR2 comprising GIX₄WX₅SX₆X₇IGYADSVKG, wherein X₄ is D or N, X₅ is D or N, X₆ is D, E, H or A and X₇ is D, E, H or N (SEQ ID NO: 464); and/or
-a CDR3 comprising VPGYSX₈GWIDAX₉DX₁₀, wherein X₈ is E, H or G, X₉ is E, D or F and X₁₀ is D, E or V (SEQ ID NO: 465).
81. The antibody of claim 80, wherein the antibody comprises
-a CDR1 comprising GFTX₁X₂DX₃AMH, wherein X₁ is D or L, X₂ is E or D and X₃ is E or Y (SEQ ID NO: 463);
-a CDR2 comprising GIX₄WX₅SX₆X₇IGYADSVKG, wherein X₄ is D or N, X₅ is D or N, X₆ is D, E, H or A and X₇ is D, E, H or N (SEQ ID NO: 464); and
-a CDR3 comprising VPGYSX₈GWIDAX₉DX₁₀, wherein X₈ is E, H or G, X₉ is E, D or F and X₁₀ is D, E or V (SEQ ID NO: 465).
82. The antibody of claim 80 or 81, wherein X₃ is E and X₉ is E.
83. The antibody of any one of claims 80-82, wherein X₂ is E, X₇ is E, and/or X₈ is H.

84. ~~The antibody of claim 83, wherein two or more of X₂ is E, X₇ is E, and X₈ is H are met.~~
85. ~~The antibody of claim 84, wherein X₂ is E, X₇ is E and X₈ is H.~~
86. ~~The antibody of any one of claims 80-85, wherein X₆ is E.~~
- 5 87. ~~The antibody of any one of claims 80-85, wherein X₆ is A.~~
88. ~~The antibody of any one of claims 80-86, wherein X₁ is L, X₄ is N, X₅ is N and/or X₁₀ is V.~~
89. ~~The antibody of claim 88, wherein two or more of X₁ is L, X₄ is N, X₅ is N and/or X₁₀ is V are met.~~
- 10 90. ~~The antibody of claim 89, wherein three or more of X₁ is L, X₄ is N, X₅ is N and/or X₁₀ is V are met.~~
91. ~~The antibody of claim 90, wherein X₁ is L, X₄ is N, X₅ is N and/or X₁₀ is V.~~
92. ~~The antibody of any one of claims 80-91, wherein X₁₀ is D.~~
93. ~~The antibody of any one of claims 80-92, wherein X₉ is E.~~
- 15 94. ~~The antibody of any one of claims 80-93, wherein X₂ is E, X₄ is D and/or X₆ is E.~~
95. ~~The antibody of claim 94, wherein two or more of X₂ is E, X₄ is D and/or X₆ is E are met.~~
96. ~~The antibody of claim 95, wherein X₂ is E, X₄ is D and X₆ is E.~~
97. ~~The antibody of any one of claims 80-96, wherein X₁ is L, X₃ is Y, X₅ is N, X₇ is N and/or X₈ is G.~~
- 20 98. ~~The antibody of claim 97, wherein two or more of X₁ is L, X₃ is Y, X₅ is N, X₇ is N and/or X₈ is G are met.~~
99. ~~The antibody of claim 98, wherein three or more of X₁ is L, X₃ is Y, X₅ is N, X₇ is N and/or X₈ is G are met.~~
- 25 100. ~~—The antibody of claim 99, wherein four or more of X₁ is L, X₃ is Y, X₅ is N, X₇ is N and/or X₈ is G are met.~~
101. ~~—The antibody of claim 100, wherein X₁ is L, X₃ is Y, X₅ is N, X₇ is N and X₈ is G.~~
102. ~~—The antibody of any one of claims 77-101, wherein the antibody comprises~~
- 30 ~~VH CDR1, CDR2 and/or CDR3 of P1-061029, P1-068757, P1-068759, P1-068761,~~

~~P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, or P1-068761_E30D_E56N, P1-068761_E100FF, P1-068761_E55A_E100FF, P1-068761_H100G_E100FF, P1-068761_E30D_E100FF, P1-068761_E56N_E100FF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, or P1-068761_E32Y_E100FF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100FF_D102V, P1-068767_E55A_E100FF, P1-068767_D52N_E100FF, P1-068767_E100FF or P1-068767_E30D_E100FF.~~

103. ~~———— The antibody of claim 102, wherein the antibody comprises VH CDR1, CDR2 and CDR3 of P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-069077, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, or P1-068761_E30D_E56N, P1-068761_E100FF, P1-068761_E55A_E100FF, P1-068761_H100G_E100FF, P1-068761_E30D_E100FF, P1-068761_E56N_E100FF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100FF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100FF_D102V, P1-068767_E55A_E100FF, P1-068767_D52N_E100FF, P1-068767_E100FF or P1-068767_E30D_E100FF.~~

104. — The antibody of any one of claims 77-103, wherein the antibody comprises VL CDR1, CDR2 and CDR3 of the VL of P1-061029.

105. — The antibody of any one of claims 1-63 and 72-104, wherein the antibody comprises a VH comprising an amino acid sequence that is at least 90%, 95%, 97%, 98% or 99% identical to that of P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-069077, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, or P1-068761_E30D_E56N, P1-068761_E100F, P1-068761_E55A_E100F, P1-068761_H100G_E100F, P1-068761_E30D_E100F, P1-068761_E56N_E100F, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100F, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100F_D102V, P1-068767_E55A_E100F, P1-068767_D52N_E100F, P1-068767_E100F or P1-068767_E30D_E100F.

106. — The antibody of any one of claims 1-63 and 72-104, wherein the antibody comprises a VH comprising the amino acid sequence of the VH of P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-069077, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, or P1-068761_E30D_E56N, P1-068761_E100F, P1-068761_E55A_E100F, P1-068761_H100G_E100F, P1-068761_E30D_E100F, P1-068761_E56N_E100F, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-

068761_E32Y_H100G, or P1-068761_E32Y_E100F, P1-068767_D52N_D102V,
P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-
068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-
068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-
068767_E100F_D102V, P1-068767_E55A_E100F, P1-068767_D52N_E100F, P1-
068767_E100F or P1-068767_E30D_E100F.

107. — The antibody of any one of claims 1-106, wherein the antibody comprises a
VL comprising an amino acid sequence that is at least 90%, 95%, 97%, 98% or 99%
identical to that of P1-061029.

108. — The antibody of claim 107, wherein the antibody comprises the VL of P1-
061029.

109. — The antibody of any one of claims 1-63 and 72-76, wherein the antibody
comprises VH CDR1, CDR2 and/or CDR3 of P1-061015 or a variant thereof
comprising 1-6 or 1-8 amino acid differences in CDR1, CDR2 and/or CDR3 relative
to P1-061015, wherein at most 1, 2 or 3 amino acid variations are present in any one
CDR.

110. — The antibody of claim 109, wherein the antibody comprises VH CDR1, CDR2
and CDR3 of P1-061015 or a variant thereof comprising 1-6 amino acid differences
in CDR1, CDR2 and/or CDR3 relative to the P1-061015, wherein at most 1, 2 or 3
amino acid variations are present in any one CDR.

111. — The antibody of claims 108 or 109, wherein an amino acid variation is a
substitution to a glutamic acid residue (E), aspartic acid residue (D) or a histidine
residue (H).

112. — The antibody of any one of claims 108-111, wherein the antibody comprises
— a CDR1 comprising GFTFX₁X₂X₃MH, wherein X₁ is E, D, H or S; X₂ is H or Y; and X₃
is D or A (SEQ ID NO: 466);
— a CDR2 comprising X₄X₅WX₆DGSX₇X₈X₉X₁₀ADSVKG, wherein X₄ is E, H or I; X₅ is
D or I; X₆ is D or Y; X₇ is D or N; X₈ is D, H or K; X₉ is D, H or Y; and X₁₀ is E or Y
(SEQ ID NO: 467); and/or

~~—a CDR3 comprising DX₁₁X₁₂FYX₁₃X₁₄YYDFX₁₅, wherein X₁₁ is E or S; X₁₂ is E or G; X₁₃ is D, E or S; X₁₄ is D or S; and X₁₅ is D, E or Y (SEQ ID NO: 468).~~

113. ~~—The antibody of any one of claims 108–112, wherein the antibody comprises~~
~~—a CDR1 comprising GFTFX₁X₂X₃MH, wherein X₁ is E, D, H or S; X₂ is H or Y; and X₃~~
5 ~~is D or A (SEQ ID NO: 466);~~

~~—a CDR2 comprising X₄X₅WX₆DGSX₇X₈X₉X₁₀ADSVKG, wherein X₄ is E, H or I; X₅ is~~
~~D or I; X₆ is D or Y; X₇ is D or N; X₈ is D, H or K; X₉ is D, H or Y; and X₁₀ is E or Y~~
~~(SEQ ID NO: 467); and~~

~~—a CDR3 comprising DX₁₁X₁₂FYX₁₃X₁₄YYDFX₁₅, wherein X₁₁ is E or S; X₁₂ is E or G;~~
10 ~~X₁₃ is D, E or S; X₁₄ is D or S; and X₁₅ is D, E or Y (SEQ ID NO: 468).~~

114. ~~—The antibody of anyone of claims 98–102, wherein the antibody comprises VH~~
~~CDR1, CDR2 and/or CDR3 of P1-061015, P1-068736, P1-068738, P1-068740, P1-~~
~~068742, P1-068744, P1-068748, P1-068750, P1-068752 or P1-068754.~~

115. ~~—The antibody of claim 114, wherein the antibody comprises VH CDR1, CDR2~~
15 ~~and CDR3 of P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-~~
~~068744, P1-068748, P1-068750, P1-068752 or P1-068754.~~

116. ~~—The antibody of any one of claims 98–115, wherein the antibody comprises~~
~~VL CDR1, CDR2 and CDR3 of the VL of P1-061015.~~

117. ~~—The antibody of any one of claims 98–116, wherein the antibody comprises a~~
20 ~~VH comprising an amino acid sequence that is at least 90%, 95%, 97%, 98% or 99%~~
~~identical to that of P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-~~
~~068744, P1-068748, P1-068750, P1-068752 or P1-068754.~~

118. ~~—The antibody of any one of claims 98–117, wherein the antibody comprises a~~
~~VH comprising the amino acid sequence of the VH of P1-061015, P1-068736, P1-~~
25 ~~068738, P1-068740, P1-068742, P1-068744, P1-068748, P1-068750, P1-068752 or~~
~~P1-068754.~~

119. ~~—The antibody of any one of claims 98–118, wherein the antibody comprises a~~
~~VL comprising an amino acid sequence that is at least 90%, 95%, 97%, 98% or 99%~~
~~identical to that of P1-061015.~~

120. ~~_____The antibody of claim 119, wherein the antibody comprises a VL comprising the amino acid sequence of P1-061015.~~

121. ~~_____The isolated antibody of any one of claims 1-120, wherein the antibody binds at or near the histidine rich region of hVISTA.~~

5 ~~122.5. _____~~ The isolated antibody of any one of claims 1-4~~of claim 121~~, wherein the antibody binds at or near the histidine rich region of hVISTA in conditions having a pH of 6.0-6.5.

10 ~~123. _____The isolated antibody of any one of claims 1-122, wherein the antibody competes or cross-competes for binding to hVISTA with one or more antibodies comprising the VH and VL of P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-069077, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, or P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068748, P1-068750, P1-068752 or P1-068754.~~

25 ~~124.6. _____~~ The isolated antibody of any one of claims 1-~~123~~5, wherein the antibody does not bind significantly to hVISTA, in which one or more of the following amino acid residues have been mutated: T35, Y37, K38, T39, Y41, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127.

125. ~~_____ An antibody that binds to hVISTA consisting of SEQ ID NO: 1 or 2, but does not bind significantly to hVISTA, in which one or more of the following amino acid residues have been mutated: T35, Y37, K38, T39, Y41, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127.~~

126. ~~_____ The antibody of any one of claims 1-125, wherein the antibody does not bind significantly to hVISTA, in which 2, 3, 4, 5 or more of the following amino acid residues have been mutated: T35, Y37, K38, T39, Y41, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127.~~

127. ~~_____ The antibody of any one of claims 124-126, wherein the antibody does not bind to a hVISTA in which one or more of the following residues have been mutated to one of the corresponding residue shown in Table 15.~~

128. ~~7. _____ The isolated antibody of any one of claims 1-127, wherein the antibody has an isoelectric point (pI) between 6.5 and 6.8, as measured, e.g., by icIEF.~~

129. ~~_____ The isolated antibody of any one of claims 1-128, wherein the antibody exhibits low aggregation, e.g., an aggregation that is similar or lower than that of '029, '761 or '767, e.g., as determined in the Examples.~~

130. ~~_____ The isolated antibody of any one of claims 1-129, wherein the antibody exhibits a viscosity that is similar or lower than that of '029, '761 or '767, e.g., as determined in the Examples.~~

131. ~~_____ The isolated antibody of any one of claims 1-130, wherein the antibody exhibits a hydrodynamic radius that is similar or lower than that of '029, '761 or '767, e.g., as determined in the Examples.~~

132. ~~_____ The isolated antibody of any one of claims 1-131, wherein the antibody exhibits a Tm1 that is similar or higher than that of '029, '761 or '767, e.g., as determined in the Examples.~~

133. ~~_____ The isolated antibody of any one of claims 1-132, wherein the antibody exhibits an amount of high molecular weight species that is similar to or lower than that of '029, '761 or '767, e.g., as determined in the Examples.~~

134. ~~8. _____ The isolated antibody of any one of claims 1-133, which is an IgG antibody.~~

~~135. The isolated antibody of claim 134, which is an IgG1, IgG2 or IgG4 antibody (IgG4 optionally with S228P).~~

9. The isolated antibody of claim 8, wherein the IgG4 antibody has an S228P substitution.

5 ~~136.10.~~ The isolated antibody of any one of claims 1-~~1359~~, wherein the antibody is an effectorless antibody.

~~137.11.~~ The isolated antibody of claim ~~13610~~, wherein the effectorless antibody comprises a heavy chain~~wherein the~~ constant region ~~comprises comprising~~ 1-5 mutations ~~in a wild-type heavy chain constant region~~ that reduce ~~the~~ effector function of the antibody.

~~138.12.~~ The isolated antibody of any one of claims 1-~~1379~~, wherein the heavy chain constant region of the antibody is an~~wherein the constant region of the antibody is~~ IgG1.3, an IgG1.1 or ~~is~~ an IgG1 with a P238K substitution.

15 ~~139.13.~~ The isolated antibody of any one of claims 1-~~1359~~, wherein the antibody has effector function.

~~140.14.~~ The isolated antibody of claim ~~13913~~, wherein the antibody is afucosylated (e.g., ~~an afucosylated IgG1 antibody~~).

20 ~~141.15.~~ The isolated antibody of claim ~~139 or 14013 or 14~~, wherein the antibody comprises a heavy chain constant region comprising~~wherein the constant region comprises~~ 1-5 mutations that enhance ~~the~~ effector function of the antibody.

~~142.16.~~ The isolated antibody of any one of claims 1- ~~14115~~, which is a full length antibody.

~~143.17.~~ The isolated antibody of any one of claims 1-~~1417~~, which is an antigen binding fragment of the antibody.

25 ~~144.18.~~ The isolated antibody of any one of claims 1-~~14317~~, which is a multimeric (e.g., ~~dimeric or trimeric~~) antibody.

~~145.19.~~ The isolated antibody of any one of claims 1-~~14418~~, which is linked (e.g., ~~covalently~~) to another molecule.

30 ~~146.20.~~ The isolated antibody of claim ~~14519~~, wherein said another molecule~~the other molecule~~ is a label.

147. ~~The isolated antibody of claim 145 or 146, wherein the other molecule or~~ is a peptide.
- 148.21. ~~The isolated antibody of any one of claims 1-147~~20, which is an antibody drug conjugate (ADC).
- 5 149.22. ~~An isolated nucleic acid encoding an antibody of any one of claims 1-148~~21.
150. ~~An isolated nucleic acid encoding the heavy chain and/or the light chain of an antibody of anyone of claims 1-149.~~
- 151.23. ~~A composition comprising the isolated nucleic acid of claim 22a nucleic acid encoding the heavy chain of an antibody of anyone of claims 1-150 and a nucleic acid encoding the light chain of the antibody.~~
- 10 152.24. ~~A cell comprising the isolated nucleic acid of claim 22, or the composition of claim 23~~of anyone of claims 149-151.
- 153.25. ~~A method of preparing an antibody, comprising culturing the cell of claim 152~~24 in conditions under which the antibody is expressed.
- 15 154.26. ~~A composition comprising an isolated antibody of any one of claims 1-21, the isolated nucleic acid of claim 22, composition of claim 23 or cell of claim 24~~comprising an isolated antibody, nucleic acid, composition or cell of anyone of claims 1-152 and a pharmaceutically acceptable carrier.
155. ~~The composition of claim 154, comprising a second therapeutic agent.~~
- 20 156. ~~The composition of claim 155, wherein the second therapeutic agent is an immunostimulatory agent.~~
157. ~~The composition of claim 156, wherein the immunostimulating agent is an antagonist of an immunosuppressive molecule, e.g., the PD-1/PD-L1, a CTLA-4 and LAG-3, or an agonist of an immunostimulating molecule, e.g., GITR and OX40.~~
- 25 158.27. ~~A method of treating a subject having cancer, comprising administering to the subject a therapeutically effective amount of a composition or~~Use of the isolated antibody of any one of claims 1-152-21 or the composition of claim 26 in the manufacture of a medicament for treating cancer, wherein the medicament~~and 154-157 that~~ stimulates an immune response and/or is a VISTA antagonist antibody.

159. ——— The method of claim 158, wherein the subject has ~~VISTA positive cells in a tumor of the cancer.~~

160. ——— The method of claim 159, wherein cells are ~~VISTA positive infiltrating lymphocytic (e.g., T cells) or myelomonocytic cells.~~

161. ——— The method of anyone of claims 158-160, wherein the subject is first tested for the presence of ~~VISTA positive cells in a tumor.~~

162. ——— The method of anyone of claims 158-161, wherein the method further comprises administering a second therapy.

163. ——— The method of claim 162, wherein the second therapy is radiotherapy, surgery or administration of a second agent.

164. ——— The method of claim 163, wherein the second therapy is a second agent and the second agent is an immunostimulatory agent.

165. ——— The method of claim 164, wherein the immunostimulatory agent is an antagonist of an immunosuppressive molecule, e.g., the PD-1/PD-L1, an CTLA-4 and LAG-3, or an agonist of an immunostimulating molecule, e.g., GITR and OX40.

166.28. A method of treating an infectious disease (e.g., viral disease) in a subject, comprising administering to the subject a therapeutically effective amount of a composition or isolated antibody of any one of claims 1-152 and 154-157 that Use of the isolated antibody of any one of claims 1-21 or the composition of claim 26 in the manufacture of a medicament for treating infectious disease (e.g., viral disease), wherein the medicament stimulates an immune response and/or is a VISTA antagonist.

167. ——— A method of treating an inflammation, an inflammatory condition, and autoimmune disease, graft versus host disease or a disease that benefits from reducing an immune response, comprising administering to the subject a therapeutically effective amount of a composition or isolated antibody of any one of claims 1-152 and 154-157 that inhibits an immune response, e.g., T cell activation or is a VISTA agonist.

168. ——— A method for identifying an Ab that binds to hVISTA ECD at pH 6.5 or less with a K_D of 10^{-7} M or less, comprising contacting a test Ab or a plurality of test Abs

with a polypeptide comprising hVISTA-ECD or a fragment thereof comprising the IgV domain of hVISTA-ECD or comprising amino acids 20-95, 20-70, 35-70-127, or 35-127 of SEQ ID NO:2 at pH 6.5 or less, and selecting the test Ab or Abs that bind to the polypeptide with a K_D of 10^{-7} M or less.

169. — A method for identifying an Ab that binds to hVISTA-ECD at pH 6.5 or less with a k_{off} of 5×10^{-3} sec⁻¹ or less, comprising contacting a test Ab or a plurality of test Abs with a polypeptide comprising hVISTA-ECD or a fragment thereof comprising the IgV domain of hVISTA-ECD or comprising amino acids 20-95, 20-70, 35-70, or 35-127 of SEQ ID NO:2 at pH 6.5 or less, and selecting the test Ab or Abs that bind to the polypeptide with a k_{off} of 5×10^{-3} sec⁻¹ or less.

170. — A method for identifying an Ab that specifically binds to hVISTA-ECD at pH 6.5 with similar affinity at pH 7.0 comprising:

- a. — contacting a test Ab or plurality of test Abs at pH 6.5 with a polypeptide comprising hVISTA-ECD or a fragment thereof comprising the IgV domain of hVISTA-ECD or comprising amino acids 20-95, 20-70, or 35-70, or 35-127 of SEQ ID NO:2;
- b. — contacting the test Ab or plurality of test Abs at pH 7.0 with the polypeptide of (a); and
- c. — selecting a test Ab if it binds to the polypeptide with a K_D of 10^{-7} M or less at pH 6.5 and at pH 7.0.

171. — A method for identifying an Ab that binds with higher affinity to hVISTA-ECD at pH 6.5 than at pH 7.0 comprising:

- a. — contacting a test Ab or plurality of test Abs at pH 6.5 with a polypeptide comprising hVISTA-ECD or a fragment thereof comprising the IgV domain of hVISTA-ECD or comprising amino acids 20-95, 20-70, 35-70, or 35-127 of SEQ ID NO:2;
- b. — contacting the test Ab or plurality of test Abs at pH 7.0 with the polypeptide of (a); and
- c. — selecting a test Ab if it binds to the polypeptide with a K_D or k_{off} at least 2 fold lower at pH 6.5 than at pH 7.0.

172. ~~—A method for identifying an Ab that specifically binds to hVISTA-ECD for use in treating cancer, comprising~~
- 5 ~~a. identifying Abs that specifically bind to hVISTA-ECD at pH 6.5 or less, such as according to the methods of claims 168-171; and~~
- ~~b. selecting the Abs of (a) that trigger or enhance an immune response in a tumor model or that inhibit tumor growth at pH 6.5 or less.~~
173. ~~—The method of claim 172, wherein step (b) comprises measuring T cell activity.~~
174. ~~—The method of claim 172 or 173, further comprising measuring the anti-tumor effect of the Ab.~~
- 10 ~~175. —A method for improving the anti-tumor efficacy of an Ab that binds to hVISTA-ECD, comprising~~
- ~~a. Providing an Ab that binds to hVISTA-ECD at pH 6.5 or less with an affinity that is less than a desired value;~~
- 15 ~~b. substituting 1 to 5 amino acid residues in the heavy or light chain of the Ab with a different amino acid residue, wherein the 1 to 5 amino acid residues are contact residues with hVISTA-ECD;~~
- ~~c. Determining if the Ab obtained in (b) has higher affinity for hVISTA-ECD at pH 6.5 or less relative to the Ab of (a); and~~
- 20 ~~d. Repeating steps (a) —(c), for a number of rounds sufficient to obtain an Ab that binds to hVISTA-ECD at pH 6.5 or less with a K_D of 10^{-7} M or less.~~
176. ~~—A method for improving the anti-tumor efficacy of an Ab that binds to hVISTA-ECD, comprising:~~
- 25 ~~a. Providing an Ab that binds to hVISTA-ECD at pH 6.5 or less with an affinity that is less than a desired value;~~
- ~~b. Preparing a library of variants of the Ab of (a), wherein each variant comprises a substitution of 1 to 5 amino acid residues in the heavy or light chain of the Ab with a different amino acid residue, wherein the 1 to 5 amino acid residues are contact residues with hVISTA-ECD;~~

- c. ~~Selecting Abs of the library of variants of (b) that bind to hVISTA-ECD at pH 6.5 or less with a K_D of 10^{-7} M or less; and~~
- d. ~~Testing the anti-tumor efficacy of the Abs of (c) in a tumor model.~~

5

177. ~~— A method for improving the pharmacokinetics of an antibody that binds to human VISTA-ECD, comprising enhancing the ability of the antibody to bind to human VISTA in acidic conditions, e.g., equal to or lower than pH 6.5.~~

10

178. ~~— A method for selecting an antibody that binds to human VISTA and having an extended half-life (good pharmacokinetic properties), wherein the method comprises selecting an antibody that binds to human VISTA in acidic conditions, e.g., equal to or lower than pH 6.5.~~

15

179. ~~— A method for improving the efficacy of antibodies that bind to hVISTA, comprising increasing the number of aspartic acid, glutamic acid and/or histidine residues in one or more VH-CDRs of the antibody, to enhance binding of the antibody to hVISTA at acidic pH.~~

20

180. ~~— A method for isolating antibodies that bind to hVISTA and have a long half-life in human blood and/or stimulate T cells in a tumor environment, comprising screening a library of antibodies binding to hVISTA for those that bind at acidic pH, but not at neutral pH.~~

25

181. ~~— The method of anyone of claims 168–180, comprising counter-selecting for those antibodies that bind at physiological pH.~~

182. ~~— The method of any one of claims 168–181, further comprising selecting antibodies that are either a VISTA antagonist or a VISTA agonist.~~

183. ~~— The method of any one of claims 168–182, comprising selecting for those antibodies that inhibit the interaction between VISTA and a VISTA co-receptor and/or the interaction between VISTA and T cells or myelomonocytic cells.~~

184. ~~— The method of any one of claims 168–183, further comprising selecting antibodies that have one or more of the properties of P1-068761 or P1-068767.~~

185. ~~A method of detecting VISTA in a sample, comprising contacting the sample with a VISTA antibody of any one of claims 1-148.~~