

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),

- Pl-068757 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 71),
 Pl-068771 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 75),
 Pl-068769 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 83),
 Pl-068775 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 79),
 5 Pl-068759 (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 87),
 Pl-068761 E55A (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 193),
 Pl-068761 H100G (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 201),
 Pl-068761 E56N (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 197),
 Pl-068761 E55A E56N (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 213),
 10 Pl-068761 E30D (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 185),
 Pl-068761 E30D E55A (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 237),
 Pl-068761 E56N H100G (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 257),
 Pl-068761 E30D H100G (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 245),
 Pl-068761 E30D E56N (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 241),
 15 Pl-068767 D52N (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 269),
 Pl-068767 D52N E55A (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 289),
 Pl-068767 E55A (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 273),
 Pl-068767 E30D D52N (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 285),
 Pl-068767 E30D (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 265),
 20 Pl-068767 E30D E55A (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 301),
 Pl-068767 E55A E100fF (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 293),
 Pl-068767 D52N E100fF (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 313),
 Pl-068767 E100fF (positions 26-35, 50-66, and 99-112 of SEQ ID NO: 277), or
 Pl-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 Pl-061015, wherein the VL CDR1, CDR2, and CDR3 of
 Pl-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
 Pl-068748 (positions 26-35, 50-66, and 99-110 of SEQ ID NO: 99),
 30 Pl-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

5 (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

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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),
15 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),
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25 PI-068761 E30D E55A (positions 26-35, 50-66, and 99-112 of SEO ID NO: 237),
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30 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),
35 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

40 (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),

Pl-068744 (positions 26-35, 50-66, and 99-110 of SEO 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).

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

6. The isolated antibody of any one of claims 1-5, 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.
- 5 7. The isolated antibody of any one of claims 1-6, wherein the antibody has an isoelectric point (pI) between 6.5 and 6.8, as measured by icIEF.
8. The isolated antibody of any one of claims 1-7, which is an IgG1, IgG2 or IgG4 antibody.
9. The isolated antibody of claim 8, wherein the IgG4 antibody has an S228P substitution.
- 10 10. The isolated antibody of any one of claims 1-9, wherein the antibody is an effectorless antibody.
11. The isolated antibody of claim 10, wherein the effectorless antibody comprises a heavy chain constant region comprising 1-5 mutations that reduce effector function of the antibody.
- 15 12. The isolated antibody of any one of claims 1-9, wherein the heavy chain constant region of the antibody is an is IgG1.3, an IgG1.1 or an IgG1 with a P238K substitution.
13. The isolated antibody of any one of claims 1-9, wherein the antibody has effector function.
- 20 14. The isolated antibody of claim 13, wherein the antibody is afucosylated.
15. The isolated antibody of claim 13 or 14, wherein the antibody comprises a heavy chain constant region comprising 1-5 mutations that enhance effector function of the antibody.
- 25 16. The isolated antibody of any one of claims 1- 15, which is a full length antibody.
17. The isolated antibody of any one of claims 1-7, which is an antigen binding fragment of the antibody.
18. The isolated antibody of any one of claims 1-17, which is a multimeric antibody.
19. The isolated antibody of any one of claims 1-18, which is linked to another molecule.
- 30 20. The isolated antibody of claim 19, wherein said another molecule is a label

or is a peptide.

21. The isolated antibody of any one of claims 1-20, which is an antibody drug conjugate (ADC).

22. An isolated nucleic acid encoding an antibody of any one of claims 1-21.

5 23. A composition comprising the isolated nucleic acid of claim 22.

24. A cell comprising the isolated nucleic acid of claim 22, or the composition of claim 23.

25. A method of preparing an antibody, comprising culturing the cell of claim 24 in conditions under which the antibody is expressed.

10 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 and a pharmaceutically acceptable carrier.

27. 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 cancer, wherein the
15 medicament stimulates an immune response and/or is a VISTA antagonist antibody.

28. 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.

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