

CLAIMS

1. An isolated antibody that specifically binds to human VISTA (hVISTA), wherein the antibody has been engineered by replacing from 1-5 glutamic acid (E), aspartic acid
5 (D) or histidine (H) amino acids in VH CDRs of antibody P1-068744 or antibody P1-068748 with the amino acid residue found at the same position in antibody P1-061015, wherein the amino acid sequence of HV CDR 1, VH CDR2, and VH CDR3 of the isolated antibody considered as a whole differs from that of antibody P1-061015.
- 10 2. The isolated antibody of claim 1, wherein the antibody comprises VH CDR1, CDR2 and/or CDR3 of P1-061015 or a variant thereof comprising 1 to 3 or 1 to 2 amino acid differences in VH CDR1, CDR2 and/or CDR3 relative to antibody P1-061015.
3. The isolated antibody of claim 2, wherein no single VH CDR comprises more than 2 amino acid differences relative to the corresponding VH CDR of P1-061015.
- 15 4. The isolated antibody of claim 2 or 3, wherein the amino acid differences comprise substitutions of an amino acid residue in P1-061015 to a glutamic acid residue (E), aspartic acid residue (D) or histidine residue (H).
5. The isolated antibody of any one of claims 2-4, wherein the antibody comprises
- a VH CDR1 comprising GFTFSX₁X₂AMH, wherein X₁ is E, H or S, and wherein X₂ is
20 H or Y;
- a VH CDR2 comprising X₃IWYDGSNX₄X₅X₆ADSVKG, wherein X₃ is H or I, X₄ is D or K; X₅ is D or Y, and X₆ is E or Y; and/or
- a VH CDR3 comprising DSGFYX₇SYFYDX₈, wherein X₇ is D, E or S, and X₈ is E or Y.
- 25 6. The antibody of any one of claims 2-4, wherein the antibody comprises
- a VH CDR1 comprising GFTFSX₁YAMH, wherein X₁ is E or S;
- a VH CDR2 comprising X₂IWYDGSNKYX₃ADSVKG, wherein X₂ is H or I, and X₂ is E or Y; and/or
- a VH CDR3 comprising DSGFYX₄SYFYDX₅, wherein X₄ is E or S, and X₅ is E or Y.
- 30 7. The antibody of any one of claims 2-4, wherein the antibody comprises

- a VH CDR1 comprising GFTFSX₁X₂AMH, wherein X₁ is H or S, and X₂ is H or Y;
- a VH CDR2 comprising IIWYDGSNX₃X₄YADSVKG, wherein X₃ is D or K, and X₄ is D or Y; and/or
- a VH CDR3 comprising DSGFYX₅SYDFDY, wherein X₅ is D or S.

- 5 8. The isolated antibody of any one of claims 1-7, wherein the antibody comprises VH CDR1, CDR2 and/or CDR3 of P1-068744_E31S, P1-68744_H50I, P1-68744_E59Y, P1-068744_E100S, P1-068744_E102Y, P1-068744_E31S_H50I, P1-068744_H50I_E59Y, P1-068744_E59Y_E100S, P1-068744_E100S_E102Y, P1-068744_E31S_E102Y P1-068744_E31S_E59Y, P1-068744_E31S_E100S, P1-068744_H50I_E100S, P1-068744_H50I_E102Y, P1-068744_E59Y_E102Y, P1-068748_H31S, P1-068748_H32Y, P1-068748_D57K, P1-068748_D58Y, P1-068748_D100S, P1-068748_H31S_H32Y, P1-068748_H32Y_D57K, P1-068748_D57K_D58Y, P1-068748_D58Y_D100S, P1-068748_H31S_D57K, P1-068748_H31S_D58Y, P1-068748_H31S_D100S, P1-068748_H32Y_D58Y, P1-068748_H32Y_D100S, or P1-068748_D57K_D100S.
- 10 9. The isolated antibody of any one of claims 1-7, wherein the antibody comprises VH CDR1, CDR2 and CDR3 of P1-068744_E31S, P1-68744_H50I, P1-68744_E59Y, P1-068744_E100S, P1-068744_E102Y, P1-068744_E31S_H50I, P1-068744_H50I_E59Y, P1-068744_E59Y_E100S, P1-068744_E100S_E102Y, P1-068744_E31S_E102Y P1-068744_E31S_E59Y, P1-068744_E31S_E100S, P1-068744_H50I_E100S, P1-068744_H50I_E102Y, P1-068744_E59Y_E102Y, P1-068748_H31S, P1-068748_H32Y, P1-068748_D57K, P1-068748_D58Y, P1-068748_D100S, P1-068748_H31S_H32Y, P1-068748_H32Y_D57K, P1-068748_D57K_D58Y, P1-068748_D58Y_D100S, P1-068748_H31S_D57K, P1-068748_H31S_D58Y, P1-068748_H31S_D100S, P1-068748_H32Y_D58Y, P1-068748_H32Y_D100S, or P1-068748_D57K_D100S.
- 20 10. The isolated antibody of any one of claims 8 or 9, wherein the antibody comprises VL CDR1, CDR2 and CDR3 of the VL of P1-061015.
- 25 11. The isolated antibody of any one of claims 1-10, wherein the antibody comprises a VH comprising an amino acid sequence that is at least 90%, 95%, 97%, 98% or 99%
- 30

identical to that of P1-068744_E31S, P1-68744_H50I, P1-68744_E59Y, P1-068744_E100S, P1-068744_E102Y, P1-068744_E31S_H50I, P1-068744_H50I_E59Y, P1-068744_E59Y_E100S, P1-068744_E100S_E102Y, P1-068744_E31S_E102Y P1-068744_E31S_E59Y, P1-068744_E31S_E100S, P1-068744_H50I_E100S, P1-068744_H50I_E102Y, P1-068744_E59Y_E102Y, P1-068748_H31S, P1-068748_H32Y, P1-068748_D57K, P1-068748_D58Y, P1-068748_D100S, P1-068748_H31S_H32Y, P1-068748_H32Y_D57K, P1-068748_D57K_D58Y, P1-068748_D58Y_D100S, P1-068748_H31S_D57K, P1-068748_H31S_D58Y, P1-068748_H31S_D100S, P1-068748_H32Y_D58Y, P1-068748_H32Y_D100S, or P1-068748_D57K_D100S;

or

comprises the VH of P1-068744_E31S, P1-68744_H50I, P1-68744_E59Y, P1-068744_E100S, P1-068744_E102Y, P1-068744_E31S_H50I, P1-068744_H50I_E59Y, P1-068744_E59Y_E100S, P1-068744_E100S_E102Y, P1-068744_E31S_E102Y P1-068744_E31S_E59Y, P1-068744_E31S_E100S, P1-068744_H50I_E100S, P1-068744_H50I_E102Y, P1-068744_E59Y_E102Y, P1-068748_H31S, P1-068748_H32Y, P1-068748_D57K, P1-068748_D58Y, P1-068748_D100S, P1-068748_H31S_H32Y, P1-068748_H32Y_D57K, P1-068748_D57K_D58Y, P1-068748_D58Y_D100S, P1-068748_H31S_D57K, P1-068748_H31S_D58Y, P1-068748_H31S_D100S, P1-068748_H32Y_D58Y, P1-068748_H32Y_D100S, or P1-068748_D57K_D100S, modified by 1, 2, 3, 4, or 5 amino acid substitutions.

12. The isolated antibody of any one of claims 1-10, wherein the antibody comprises a VH comprising the amino acid sequence of the VH of P1-068744_E31S, P1-68744_H50I, P1-68744_E59Y, P1-068744_E100S, P1-068744_E102Y, P1-068744_E31S_H50I, P1-068744_H50I_E59Y, P1-068744_E59Y_E100S, P1-068744_E100S_E102Y, P1-068744_E31S_E102Y P1-068744_E31S_E59Y, P1-068744_E31S_E100S, P1-068744_H50I_E100S, P1-068744_H50I_E102Y, P1-068744_E59Y_E102Y, P1-068748_H31S, P1-068748_H32Y, P1-068748_D57K, P1-068748_D58Y, P1-068748_D100S, P1-068748_H31S_H32Y, P1-

068748_H32Y_D57K, P1-068748_D57K_D58Y, P1-068748_D58Y_D100S, P1-068748_H31S_D57K, P1-068748_H31S_D58Y, P1-068748_H31S_D100S, P1-068748_H32Y_D58Y, P1-068748_H32Y_D100S, or P1-068748_D57K_D100S;
or

comprises the VH of P1-068744_E31S, P1-68744_H50I, P1-68744_E59Y, P1-068744_E100S, P1-068744_E102Y, P1-068744_E31S_H50I, P1-068744_H50I_E59Y, P1-068744_E59Y_E100S, P1-068744_E100S_E102Y, P1-068744_E31S_E102Y P1-068744_E31S_E59Y, P1-068744_E31S_E100S, P1-068744_H50I_E100S, P1-068744_H50I_E102Y, P1-068744_E59Y_E102Y, P1-068748_H31S, P1-068748_H32Y, P1-068748_D57K, P1-068748_D58Y, P1-068748_D100S, P1-068748_H31S_H32Y, P1-068748_H32Y_D57K, P1-068748_D57K_D58Y, P1-068748_D58Y_D100S, P1-068748_H31S_D57K, P1-068748_H31S_D58Y, P1-068748_H31S_D100S, P1-068748_H32Y_D58Y, P1-068748_H32Y_D100S, or P1-068748_D57K_D100S, modified by 1, 2, 3, 4, or 5 amino acid substitutions in the framework regions.

13. The isolated antibody of any one of claims 1-12, 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.

14. The isolated antibody of claim 13, wherein the antibody comprises the VL of P1-061015 or the VL of P1-061015 modified by a T85V substitution.

~~15. The isolated antibody of claim 13, wherein the antibody comprises a VL comprising the amino acid sequence of the VL of P1-061015 modified by a T85V substitution.~~

~~16.~~15. The isolated antibody of any one of claims 1-~~15~~14, wherein the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 182, 183, or 184.

~~17.~~16. The isolated antibody of any one of claims 1-~~15~~14, wherein the antibody comprises the LC of P1-061015, or the LC of P1-061015 modified by a T85V substitution.

18.17. ~~_____~~ The isolated ~~isolated~~ antibody of any one of claims 1-~~17~~16, wherein the antibody binds at or near the histidine rich region of hVISTA, such as the histidine-rich β -sheet extension, optionally wherein the antibody binds in conditions having a pH of 6.0-6.5.

5 19. ~~The isolated isolated antibody of claim 18, wherein the antibody binds at or near the histidine rich region of hVISTA, such as the histidine rich β sheet extension, in conditions having a pH of 6.0-6.5.~~

20.18. ~~_____~~ The isolated ~~isolated~~ antibody of any one of claims 1-~~19~~17, wherein the antibody competes or cross-competes for binding to hVISTA with one or more antibodies ~~described herein, e.g.,~~ comprising the VH and VL of P1-061015, P1-068744, P1-068748, P1-068744_E31S, P1-68744_H50I, P1-68744_E59Y, P1-068744_E100S, P1-068744_E102Y, P1-068744_E31S_H50I, P1-068744_H50I_E59Y, P1-068744_E59Y_E100S, P1-068744_E100S_E102Y, P1-068744_E31S_E102Y P1-068744_E31S_E59Y, P1-068744_E31S_E100S, P1-068744_H50I_E100S, P1-068744_H50I_E102Y, P1-068744_E59Y_E102Y, P1-068748_H31S, P1-068748_H32Y, P1-068748_D57K, P1-068748_D58Y, P1-068748_D100S, P1-068748_H31S_H32Y, P1-068748_H32Y_D57K, P1-068748_D57K_D58Y, P1-068748_D58Y_D100S, P1-068748_H31S_D57K, P1-068748_H31S_D58Y, P1-068748_H31S_D100S, P1-068748_H32Y_D58Y, P1-068748_H32Y_D100S, or P1-068748_D57K_D100S), as determined, ~~e.g.,~~ by the a competitive bio-layer interferometry (BLI) epitope binning assay ~~described in the Examples~~ or as determined using competitive surface plasmon resonance (SPR) epitope binning ~~as described in the Examples.~~

21.19. ~~_____~~ The isolated antibody of any one of claims 1-~~20~~18, wherein the antibody bins to epitope Group A, as determined, ~~e.g.,~~ by the a competitive bio-layer interferometry (BLI) epitope binning assay ~~described in the Examples.~~

22.20. ~~_____~~ The isolated antibody of any one of claims 1-~~21~~19, wherein the antibody does not bind significantly to a hVISTA modified in that 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, ~~e.g., as determined by the yeast mutational analysis described in the Examples.~~

~~23, 21.~~ 21. An isolated antibody (Ab) according to any one of claims 1-~~22~~ 20 that specifically binds to hVISTA under acidic conditions, ~~e.g., at a pH of 6.5 (as measured, e.g., by one of the assays described in the Examples),~~ wherein the Ab:

- o inhibits the interaction between hVISTA and (a) T cells and/or (b) PSGL-1 (~~e.g., inhibits the interaction between H153 and H154 of hVISTA having SEQ ID NO: 1 and PSGL-1 tyrosines Y46 and Y48~~) and/or (c) VSIG-3;
- o enhances T cell activation ~~by, e.g., enhancing~~ enhances T cell proliferation; ~~enhancing~~ enhances IFN- γ production from T cells; and/or stimulates ~~ing~~ ing T cell receptor mediated NF-kB signaling;
- o contacts hVISTA through one or more (~~e.g., at least 1-3, 1-5, 1-10, 5-10, 5-15 or all~~) energetically important contact residues Y37, T39, R54, F62, H66, V117, I119 or S124, as determined, ~~e.g., using the~~ by yeast surface display and NGS assay ~~described in the Examples;~~ and wherein numbering is that of mature hVISTA (SEQ ID NO: 2);
- o binds to the histidine-rich β -sheet extension of hVISTA, ~~as determined, e.g., by crystallography, as described, e.g., in the Examples;~~
- o contacts H121, H122 and/or H123 of mature hVISTA (~~distance of 4.0 Ångströms (Å) or less~~), ~~such as through hydrogen bonds, as determined, e.g., by crystallography, as described, e.g., in the Examples;~~
- o binds to Region 1: ⁵⁷LGPVDKGHDVTF₆₈ (SEQ ID NO: 566); Region 2: ⁸⁶RRPIRNLTfQDL₉₇ (SEQ ID NO: 567); and Region 3: ¹⁴⁸VVEIRHHHSEHRVHGAME₁₆₅ (SEQ ID NO: 568) of hVISTA having SEQ ID NO: 1, ~~and optionally wherein the binding is strongest to Region 2, as determined by MS-HDX as described in the Examples;~~
- o competes for binding to hVISTA (~~two-way competition~~) ~~with one or more antibodies described herein, e.g., with~~ with P1-061015, P1-068744, P1-068748, P1-061029, P1-068761, P1-068767 ~~and or~~ or VISTA.4;

- o contacts hVISTA through at least one or more glutamic acid, aspartic acid or histidine residue that is located in VH CDR1, CDR2 or CDR3;
 - o has ~~low target mediated drug disposition, leading to a~~ mean residence time (MRT) of at least 100, 200, 300, 400, 500, 600 or 700 hours, ~~as measured, e.g., as described in the Examples;~~
 - o accumulates preferably in tumor tissues in comparison to lung, liver, and spleen in hVISTA knock-in mice harboring MC38 tumors; and/or
 - o accumulates preferably in tumor tissues in comparison to lung, liver, and spleen in a subject to which the antibody was administered.
- 24.22. ~~_____~~ An isolated antibody (Ab) according to any one of claims 1-23 ~~21~~ that specifically binds to hVISTA under acidic conditions, e.g., at a pH of 6.5 with a K_D (and/or k_{off}) that is at least 10 fold, 100 fold or 1000 fold lower than its K_D or k_{off} of binding to hVISTA under neutral or physiological pH ~~(as measured, e.g., by one of the assays described in the Examples)~~, wherein the Ab:
- o specifically binds to hVISTA, ~~e.g., at a~~ histidine rich region of the ECD or a polypeptide comprising amino acid residues 35-127 of SEQ ID NO: 2, at acidic pH, ~~e.g., pH 6.0 or pH 6.5;~~
 - o lacks significant binding to hVISTA, ~~e.g., at a~~ histidine rich region of the ECD or a polypeptide comprising amino acid residues 35-127 of SEQ ID NO: 2, at physiological pH or neutral pH, ~~e.g., pH 7.4 or pH 7.0;~~
 - o specifically binds to cyno VISTA, ~~e.g., at a~~ histidine rich region of the ECD, at acidic pH, ~~e.g., pH 6.0 or pH 6.5;~~
 - o lacks significant binding to cyno VISTA ~~at a, e.g.,~~ histidine rich region of the ECD, at physiological pH or neutral pH, ~~e.g., pH 7.4 or pH 7.0;~~
 - o has reduced binding to hVISTA-ECD having a substitution at one or more of the following amino acids: T35, Y37, K38, T39, Y41, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127 relative to hVISTA ECD having SEQ ID NO: 2;
 - o cross-competes for binding to hVISTA with P1-061015, P1-068744, and/or P1-068748;

- o inhibits binding of hVISTA to human T cells expressing VISTA (e.g., naïve or activated T cells) at acidic pH ~~e.g., pH 6.0 or pH 6.5~~;
- o inhibits binding of hVISTA to PSGL-1 at acidic pH e.g., pH 6.0 or pH 6.5 (e.g., inhibiting the interaction between H153 and H154 of hVISTA having SEQ ID NO: 1 and PSGL-1 tyrosines Y46 and Y48), wherein PSGL-1 is with or without siayl lewis X, and wherein the tyrosines are preferably sulfotyrosines;
- o has a mean residence time (MRT) of at least 100, 200, 300, 350, 400, 450, 500, 600 or 700 hours (e.g., at least 350 hours) in cynomolgus monkeys, ~~measured, e.g., as described in the Examples~~;
- o stimulates T cell activation by, e.g., enhancing T cell proliferation; enhancing IFN- γ production from T cells; and/or stimulating T cell receptor mediated NF-kB signaling;
- o inhibits VISTA mediated cell:cell adhesion;
- o specifically binds to hVISTA in samples of human tumor cells or samples of inflamed human tissue that express VISTA;
- o contacts hVISTA through one or more ~~(e.g., at least 1-3, 1-5, 1-10, 5-10, 5-15 or all)~~ energetically important contact residues Y37, T39, R54, F62, H66, V117, I119 or S124, as determined, ~~e.g., using the a~~ yeast surface display and NGS assay ~~described in the Examples~~; and wherein numbering is that of mature hVISTA (SEQ ID NO: 2);
- o binds to Region 1 of hVISTA: ₅₇LGPVDKGHDVTF₆₈ (SEQ ID NO: 566); Region 2: ₈₆RRPIRNLTfQDL₉₇ (SEQ ID NO: 567); and Region 3: ₁₄₈VVEIRHHHSEHRVHGAME₁₆₅ (SEQ ID NO: 568) having SEQ ID NO: 1, and optionally wherein the binding is strongest to Region 2, as determined by MS-HDX ~~as described in the Examples~~;
- o binds to the histidine-rich β -sheet extension of hVISTA, as determined, ~~e.g., by~~ crystallography, ~~as described, e.g., in the Examples~~;
- o contacts H121, H122 and/or H123 of mature hVISTA (distance of 4.0 Ångströms (Å) or less), such as through hydrogen bonds, as determined, ~~e.g., by~~ crystallography, ~~as described, e.g., in the Examples~~; and/or

- o contacts hVISTA through at least one or more glutamic acid, aspartic acid or histidine residue that is located in VH CDR1, CDR2 or CDR3.

~~25.23.~~_____The isolated antibody of any one of claims 1-~~24~~22, wherein the antibody has an isoelectric point (pI) between 6.5 and 6.8, as measured, ~~e.g.,~~ by icIEF.

~~26.24.~~_____The isolated antibody of any one of claims 1-~~25~~23, which is an IgG antibody.

~~27.25.~~_____The isolated antibody of claim ~~26~~23, which is an IgG1, IgG2 or IgG4 antibody (IgG4 optionally with S228P).

~~28.~~ The isolated antibody of any one of claims 1-27, wherein the antibody is an effectorless antibody, e.g., lacks ADCC and/or CDC, and/or an antibody that does not significantly bind to one or more FcγRs, e.g., FcγRIII.

~~29.~~ The isolated antibody of claim 28, wherein the constant region comprises 1-5 mutations in a wild-type heavy chain constant region that reduce the effector function of the antibody and/or the ability to bind to one or more FcγRs, e.g., FcγRIII, relative to that of the corresponding wildtype heavy chain constant region.

~~30.~~ The isolated antibody of any one of claims 1-29, wherein the constant region of the antibody is IgG1.3, IgG1.1 or is an IgG1 with a P238K substitution (e.g., IgG1.P238K).

~~31.~~ The isolated antibody of any one of claims 1-30, wherein the antibody has effector function and/or binds to one or more FcγRs, e.g., FcγRIII.

~~32.~~ The isolated antibody of claim 31, wherein the antibody is afucosylated (e.g., an afucosylated IgG1 antibody).

~~33.~~ The isolated antibody of claim 31 or 32, wherein the constant region comprises 1-5 mutations that enhance the effector function of the antibody and/or the ability to bind to one or more FcγRs, e.g., FcγRIII, relative to the corresponding wildtype constant region.

~~34.~~ The isolated antibody of any one of claims 1-33, which is a full length antibody or an antibody comprising a full length heavy chain (with or without a C-terminal lysine) and a full length light chain.

~~35.~~ The isolated antibody of any one of claims 1-33, which is an antigen binding fragment of the antibody.

36. ~~The isolated antibody of any one of claims 1-35, which is a multimeric (e.g., dimeric or trimeric) antibody.~~

37. ~~The isolated antibody of any one of claims 1-36, which is linked (e.g., covalently) to another molecule.~~

38. ~~The isolated antibody of claim 37, wherein the other molecule is a label.~~

39. ~~The isolated antibody of claim 37 or 38, wherein the other molecule is a peptide.~~

40. ~~26. _____ The isolated antibody of any one of claims 1-39~~25, which is an antibody drug conjugate (ADC) or an activatable antibody.

41. ~~27. _____ An isolated nucleic acid encoding an antibody of any one of claims 1-40~~26.

42. ~~28. _____ An isolated nucleic acid encoding the heavy chain and/or the light chain of an antibody of any one of claims 1-40~~26.

43. ~~29. _____ A composition comprising an isolated nucleic acid encoding the heavy chain of an antibody of any one of claims 1-40~~26 and a nucleic acid encoding the light chain of the antibody.

44. ~~30. _____ A cell comprising the isolated nucleic acid of any one of claims 41-43~~27-4329.

45. ~~31. _____ A method of preparing an antibody, comprising culturing the cell of claim 44~~30 in conditions under which the antibody is expressed.

46. ~~32. _____ A composition comprising an isolated antibody, nucleic acid, composition or cell of any one of claims 1-40~~26 and a pharmaceutically acceptable carrier.

47. ~~The composition of claim 46, comprising a second therapeutic agent.~~

48. ~~The composition of claim 47, wherein the second therapeutic agent is an immunostimulatory agent or a chemotherapeutic agent.~~

49. ~~The composition of claim 48, wherein the second therapeutic agent is an immunostimulating agent that 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.~~

50. ~~33. _____ Use of the isolated antibody of any one of claims 1-26 in the manufacture of a medicament for treating cancer~~
A method of treating cancer in a subject, wherein a therapeutically effective amount of the isolated antibody is to be administered
~~comprising administering to the subject, wherein the~~ a therapeutically

effective amount of a composition or isolated antibody of any one of claims 1-40 that stimulates an immune response and/or is a VISTA antagonist antibody.

51. ~~The method of claim 50, wherein the subject has VISTA positive cells, e.g., in a tumor of the cancer.~~

52. ~~The method of claim 51, wherein the VISTA positive cells are VISTA positive infiltrating lymphocytic (e.g., T cells) or myelomonocytic cells.~~

53. ~~The method of any one of claims 50-52, wherein the subject is first tested for the presence of VISTA positive cells in a tumor.~~

54. ~~The method of any one of claims 50-53, wherein the method further comprises administering a second therapy.~~

55. ~~The method of claim 54, wherein the second therapy is chemotherapy, radiotherapy, surgery or administration of a second agent.~~

56. ~~The method of claim 55, wherein the second therapy is a second agent and the second agent is an immunostimulatory agent or a chemotherapeutic agent.~~

57. ~~The method of claim 55, wherein the second therapeutic agent is an immunostimulatory agent that 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.~~

34. Use of the isolated antibody of any one of claims 1-26 in the manufacture of a medicament for treating an infectious disease~~A method of treating an infectious disease (e.g., viral disease) in a subject, wherein a therapeutically effective amount of the isolated antibody is to be administered comprising administering to the subject a therapeutically effective amount of an isolated antibody of any one of claims 1-40, wherein the antibody stimulates an immune response and/or is a VISTA antagonist.~~

35. Use of the isolated antibody of any one of claims 1-26 in the manufacture of a medicament for treating~~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, wherein a therapeutically effective amount of the isolated antibody is to be administered comprising administering to the subject a therapeutically effective amount of an isolated antibody of any one of claims 1-40,~~

wherein the antibody inhibits an immune response, e.g., T cell activation, or is a VISTA agonist.

~~59.36. The isolated antibody of any one of claims 1-26 for use in therapy.~~

~~60. The method of any one of claims 50-59, wherein the antibody that binds to hVISTA is an antibody of any one of claims 1-40.~~

~~61. A method for improving the anti-tumor efficacy of an antibody (Ab) that specifically binds to human VISTA extracellular domain (hVISTA-ECD), comprising~~

~~a. Providing an Ab P1-061015, P1-068744, or P1-068748;~~

~~b. substituting 1 to 5 amino acid residues in the heavy or light chain of the Ab with a different amino acid residue (e.g., with a glutamic acid, aspartic acid or histidine residue, or in the case of P1-06844 and P1-06848, with the corresponding residue of P1-061015), 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~~

~~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.~~

~~62. A method for improving the anti-tumor efficacy of an antibody (Ab) that specifically binds to human VISTA extracellular domain (hVISTA-ECD), comprising:~~

~~a. Providing an Ab P1-061015, P1-068744, or P1-068748;~~

~~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 (e.g., with a glutamic acid, aspartic acid or histidine residue, or in the case of P1-06844 and P1-06848, with the corresponding residue of P1-061015), 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. Optionally testing the anti-tumor efficacy of the Abs of (c) in a tumor model.~~

63. ~~A method for improving the efficacy of antibodies that bind to human VISTA (hVISTA), comprising increasing the number of aspartic acid, glutamic acid and/or histidine residues in one or more VH CDRs of Ab P1-061015, P1-068744, or P1-068748, to enhance binding of the antibody to hVISTA at acidic pH.~~
- 5 64. ~~The method of any one of claims 61-63, further comprising counter-selecting for those antibodies that bind VISTA at physiological pH.~~
65. ~~The method of any one of claims 61-64, further comprising selecting antibodies that are either a VISTA antagonist or a VISTA agonist.~~
- 10 66. ~~The method of any one of claims 61-65, further comprising selecting for those antibodies that inhibit the interaction between VISTA and a VISTA co-receptor (e.g., PSGL-1 and/or VSIG-3) and/or the interaction between VISTA and T cells or myelomonocytic cells.~~
67. ~~The method of any one of claims 61-66, further comprising selecting antibodies that have one or more of the properties of antibodies P1-068744 or P1-068748.~~
- 15 68. ~~A method for improving the anti-tumor efficacy of an antibody (Ab) that specifically binds to human VISTA extracellular domain (hVISTA-ECD), comprising~~
 - ~~a. Providing Ab P1-061015, P1-068744, or P1-068748;~~
 - ~~b. substituting 1 to 5 amino acid residues in the heavy or light chain, e.g., CDRs, of the Ab with a different amino acid residue, wherein, e.g., the 1 to 5 amino acid residues are contact residues with hVISTA-ECD, e.g., one or more of~~

20 ~~amino acid residues 31, 32, 50, 57, 58, 59, 100, or 102;~~

 - ~~c. determining if the Ab obtained in (b) has higher affinity for hVISTA-ECD at pH 6.5 than at pH 7.0 and/or determining whether the Ab obtained in (b) has similar or higher affinity at pH 6.5 relative to the antibody of (a) and/or lower~~

25 ~~affinity at pH 7.0 relative to the antibody of (a); and~~

 - ~~d. repeating steps (a)-(c) for a number of rounds sufficient to obtain an Ab that binds to hVISTA-ECD at pH 6.5 with a K_D of 10^{-7} M or less and binds to hVISTA-ECD at pH 7.0 with a K_D of 10^{-6} M or more.~~
- 30 69. ~~A method of detecting VISTA in a sample, comprising contacting the sample with the VISTA antibody of any one of claims 1-40.~~

70. The antibody, composition or method of any one of claims 1-69, wherein the antibody inhibits the interaction between hVISTA extracellular domain (ECD) and PSGL-1.
71. The antibody, composition or method of any one of claims 1-70, wherein the antibody competes for binding to hVISTA ECD with an antibody described herein (e.g., using BLI described herein).
72. The antibody, composition or method of any one of claims 1-71, wherein the antibody interacts with the histidine triad (H121, 122 and 123 of mature hVISTA), as determined by X-ray crystallography, e.g., as determined herein.
73. A method for selectively targeting an anti-VISTA Ab to a tumor in a subject, comprising administering to a human having at least one tumor, an Ab that binds specifically to human VISTA in acidic conditions.
74. The method of claim 73, wherein the anti-VISTA Ab accumulates preferably in tumor cells in comparison to myeloid cells, in comparison to blood, and/or in comparison to liver, lung and spleen cells.
75. The method of claims 73 or 74, wherein the anti-VISTA Ab accumulates to levels at least twice as high in the tumor relative to the blood 24 to 51 hours after administration of the Ab to the subject.
76. The method of any one of claims 73-75, wherein the anti-VISTA Ab does not accumulate significantly more in the liver or lungs relative to the blood of the subject.
77. The method of any one of claims 73-76, wherein the anti-VISTA Ab has been shown in a VISTA knock-in mouse to accumulate preferably to tumors over the blood.
78. The method of any one of claims 73-77, wherein accumulation of the anti-VISTA Ab in the tumor is sustained over time, e.g., up to at least 51 hours post administration of the VISTA Ab to the subject.
79. The method of any one of claims 73-78, wherein accumulation of the anti-VISTA Ab in the subject is determined by administering to the subject the Ab labeled with a PET tracer.
80. The method of any one of claims 73-79, wherein the anti-VISTA Ab specifically binds to VISTA at pH 6.5, but not significantly at pH 7.0.

81. The method of any one of claims 73-80, wherein the anti-VISTA Ab preferably accumulates in tumor tissue of hVISTA knock-in mice harboring MC-38 tumors in comparison to blood and/or in comparison to lung, liver, and spleen.

82. The method of any one of claims 73-81, wherein the anti-VISTA Ab is any one of VISTA.4, P1-061015, 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, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752, P1-068754, 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, 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, P1-061029_F100ff_V102D, P1-061029_F100ff, P1-061029_V102D, P1-061029_Y32E, P1-061029_Y32E_F100ff, P1-068744_E31S, P1-068744_H50I, P1-068744_E59Y, P1-068744_E100S, P1-068744_E102Y, P1-068744_E31S_H50I, P1-068744_H50I_E59Y, P1-068744_E59Y_E100S, P1-068744_E100S_E102Y, P1-068744_E31S_E102Y, P1-068744_E31S_E59Y, P1-068744_E31S_E100S, P1-068744_H50I_E100S, P1-068744_H50I_E102Y, P1-068744_E59Y_E102Y, P1-068748_H31S, P1-068748_H32Y, P1-068748_D57K, P1-068748_D58Y, P1-068748_D100S, P1-068748_H31S_H32Y, P1-068748_H32Y_D57K, P1-068748_D57K_D58Y, P1-068748_D58Y_D100S, P1-068748_H31S_D57K, P1-068748_H31S_D58Y, P1-068748_H31S_D100S, P1-

068748_H32Y_D58Y, P1-068748_H32Y_D100S, or P1-068748_D57K_D100S,
optionally, in the case of P1-061029 or its progeny wherein the VH comprises one or
both of K16R and T84A substitutions, and optionally, in the case of P1-061015 or its
progeny wherein the VL comprises a T85V substitution.

5 83. An isolated antibody that specifically binds to human VISTA (hVISTA) under acidic
conditions, e.g., at a pH of 6.5 with a K_D (and/or k_{off}) that is at least 10 fold, 100 fold
or 1000 fold lower than its K_D or k_{off} of binding to hVISTA under neutral or
physiological pH as determined by surface plasmon resonance (SPR) as described in
10 Example 30 herein, optionally wherein the anti-VISTA Ab is (a) an antibody
according to any one of claims 1-40, or (b) wherein the antibody comprises the heavy
and light chain CDRs of any one of antibodies: VISTA.4, P1-061015, 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,
15 P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766,
P1-068748, P1-068750, P1-068752 P1-068754, 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, P1-
068761_E30D_E56N, P1-068761_E100FF, P1-068761_E55A_E100FF, P1-
20 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-
25 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-
061029_F100FE_V102D, P1-061029_F100FE, P1-061029_V102D, P1-
061029_Y32E, P1-061029_Y32E_F100FE, P1-068744_E31S, P1-68744_H50I, P1-
30 68744_E59Y, P1-068744_E100S, P1-068744_E102Y, P1-068744_E31S_H50I, P1-

068744_H50I_E59Y, P1-068744_E59Y_E100S, P1-068744_E100S_E102Y, P1-
068744_E31S_E102Y P1-068744_E31S_E59Y, P1-068744_E31S_E100S, P1-
068744_H50I_E100S, P1-068744_H50I_E102Y, P1-068744_E59Y_E102Y, P1-
068748_H31S, P1-068748_H32Y, P1-068748_D57K, P1-068748_D58Y, P1-
068748_D100S, P1-068748_H31S_H32Y, P1-068748_H32Y_D57K, P1-
068748_D57K_D58Y, P1-068748_D58Y_D100S, P1-068748_H31S_D57K, P1-
068748_H31S_D58Y, P1-068748_H31S_D100S, P1-068748_H32Y_D58Y, P1-
068748_H32Y_D100S, or P1-068748_D57K_D100S, optionally, in the case of P1-
061029 or its progeny wherein the VH comprises one or both of K16R and T84A
substitutions, and optionally, in the case of P1-061015 or its progeny wherein the VL
comprises a T85V substitution.

84. An isolated antibody that specifically binds to human VISTA (hVISTA), wherein the
antibody inhibits binding of hVISTA to a cell to which hVISTA binds, e.g., a cell
expressing human PSGL-1, wherein binding is determined according to the protocol
described in Example 31 herein, optionally wherein the anti-VISTA Ab is (a) an
antibody according to any one of claims 1-40, or (b) wherein the antibody comprises
the heavy and light chain CDRs of any one of antibodies: VISTA.4, P1-061016, 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, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744,
P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, 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, 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-
061029_F100fE_V102D, P1-061029_F100fE, P1-061029_V102D, P1-
061029_Y32E, P1-061029_Y32E_F100fE, P1-068744_E31S, P1-68744_H50I, P1-
68744_E59Y, P1-068744_E100S, P1-068744_E102Y, P1-068744_E31S_H50I, P1-
068744_H50I_E59Y, P1-068744_E59Y_E100S, P1-068744_E100S_E102Y, P1-
068744_E31S_E102Y P1-068744_E31S_E59Y, P1-068744_E31S_E100S, P1-
068744_H50I_E100S, P1-068744_H50I_E102Y, P1-068744_E59Y_E102Y, P1-
068748_H31S, P1-068748_H32Y, P1-068748_D57K, P1-068748_D58Y, P1-
068748_D100S, P1-068748_H31S_H32Y, P1-068748_H32Y_D57K, P1-
068748_D57K_D58Y, P1-068748_D58Y_D100S, P1-068748_H31S_D57K, P1-
068748_H31S_D58Y, P1-068748_H31S_D100S, P1-068748_H32Y_D58Y, P1-
068748_H32Y_D100S, or P1-068748_D57K_D100S, optionally, in the case of P1-
061029 or its progeny wherein the VH comprises one or both of K16R and T84A
substitutions, and optionally, in the case of P1-061015 or its progeny wherein the VL
comprises a T85V substitution.

85. The isolated antibody of claim 84, wherein the cell to which hVISTA binds is a human leukocyte, a human PBMC, or a human T cell, wherein the cell is stimulated for 72-96 hours with anti-CD3/CD28 bead stimulation, and wherein the hVISTA of the binding protocol is a VISTA multimer or a VISTA-Fc chimeric protein.
86. The isolated antibody of claim 84 or 85, wherein the antibody inhibits binding of hVISTA to the cell by at least 50%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99% or by 100%.
87. The isolated antibody of any one of claims 84-86, wherein the antibody inhibits binding of hVISTA on a first cell to human VSIG-3 on a second cell, wherein the second cell is a cell expressing human VSIG-3, and wherein binding of the first cell to the second cell is determined according to the protocol set forth in Example 31.
88. The isolated antibody of claim 87, wherein the first cell is a HEK293 cell ectopically expressing hVISTA; the second cell is a CHO cell ectopically expressing human

~~VSIG-3, and wherein the hVISTA expressed by the first cell is a VISTA multimer or VISTA-Fc chimeric protein.~~

~~89. The isolated antibody of claim 87 or 88, wherein the antibody inhibits binding of the first cell to the second cell by at least 50%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99% or by 100%.~~

~~90. An isolated antibody that binds specifically to human VISTA (hVISTA), wherein the antibody blocks (i.e., one way) or cross-blocks (i.e., both ways) binding of any one of antibodies VISTA.4, P1-061015, 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, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752, P1-068754, 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, 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, P1-061029-F100fE-V102D, P1-061029-F100fE, P1-061029-V102D, P1-061029-Y32E, P1-061029-Y32E-F100fE, P1-068744-E31S, P1-068744-H50I, P1-068744-E59Y, P1-068744-E100S, P1-068744-E102Y, P1-068744-E31S-H50I, P1-068744-H50I-E59Y, P1-068744-E59Y-E100S, P1-068744-E100S-E102Y, P1-068744-E31S-E102Y, P1-068744-E31S-E59Y, P1-068744-E31S-E100S, P1-068744-H50I-E100S, P1-068744-H50I-E102Y, P1-068744-E59Y-E102Y, P1-068748-H31S, P1-~~

~~068748_H32Y, P1-068748_D57K, P1-068748_D58Y, P1-068748_D100S, P1-068748_H31S_H32Y, P1-068748_H32Y_D57K, P1-068748_D57K_D58Y, P1-068748_D58Y_D100S, P1-068748_H31S_D57K, P1-068748_H31S_D58Y, P1-068748_H31S_D100S, P1-068748_H32Y_D58Y, P1-068748_H32Y_D100S, or P1-068748_D57K_D100S to hVISTA, as determined using competitive SPR epitope binning described in Example 32 herein.~~

~~91. An isolated anti-PSGL-1 antibody that inhibits binding of human VISTA (hVISTA) to a cell, wherein the cell is a cell to which hVISTA binds, e.g., a cell expressing human PSGL-1, and wherein binding is determined according to the protocol set forth in Example 31 herein.~~

~~92. The isolated anti-PSGL-1 antibody of claim 91, wherein the cell to which hVISTA binds is a human leukocyte, a human PBMC or a human T cell, wherein the cell is stimulated for 72-96 hours with anti-CD3/CD28 bead stimulation, and wherein hVISTA of the binding protocol is a VISTA multimer or VISTA-Fc chimeric protein.~~

~~93. The isolated anti-PSGL-1 antibody of claim 91 or 92, wherein the antibody inhibits binding of hVISTA to the cell by at least 50%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99% or by 100%.~~