

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.
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.
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.
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%

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

- 5 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, P1-10 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
15 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 P1-20 061015 or the VL of P1-061015 modified by a T85V substitution.
15. The isolated antibody of any one of claims 1-14, wherein the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 182, 183, or 184.
- 25 16. The isolated antibody of any one of claims 1-14, wherein the antibody comprises the LC of P1-061015, or the LC of P1-061015 modified by a T85V substitution.
17. The isolated antibody of any one of claims 1-16, 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.

18. The isolated antibody of any one of claims 1-17, wherein the antibody competes or cross-competes for binding to hVISTA with one or more antibodies 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, P1-5 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, P1-10 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 by a competitive bio-layer interferometry (BLI) epitope binning assay or as determined using competitive surface plasmon resonance (SPR) epitope binning.
19. The isolated antibody of any one of claims 1-18, wherein the antibody bins to epitope Group A, as determined by a competitive bio-layer interferometry (BLI) epitope binning assay.
20. The isolated antibody of any one of claims 1-19, 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 as determined by yeast mutational analysis.
21. An isolated antibody (Ab) according to any one of claims 1-20 that specifically binds to hVISTA under acidic conditions, wherein the Ab:
- 25 o inhibits the interaction between hVISTA and (a) T cells and/or (b) PSGL-1 and/or (c) VSIG-3;
- o enhances T cell activation, enhances T cell proliferation; enhances IFN- γ production from T cells; and/or stimulates T cell receptor mediated NF-kB signaling;

- o contacts hVISTA through one or more energetically important contact residues Y37, T39, R54, F62, H66, V117, I119 or S124, as determined by yeast surface display and NGS; and wherein numbering is that of mature hVISTA (SEQ ID NO: 2);
 - 5 o binds to the histidine-rich β -sheet extension of hVISTA;
 - o contacts H121, H122 and/or H123 of mature hVISTA;
 - 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
 - 10 SEQ ID NO: 1;
 - o competes for binding to hVISTA with P1-061015, P1-068744, P1-068748, P1-061029, P1-068761, P1-068767 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;
 - 15 o has a mean residence time (MRT) of at least 100, 200, 300, 400, 500, 600 or 700 hours;
 - 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
 - 20 spleen in a subject to which the antibody was administered.
22. An isolated antibody (Ab) according to any one of claims 1-21 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, wherein the Ab:
- 25 o specifically binds to hVISTA 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;
 - o lacks significant binding to hVISTA at a histidine rich region of the ECD or a polypeptide comprising amino acid residues 35-127 of SEQ ID NO: 2, at
 - 30 physiological pH or neutral pH;

- o specifically binds to cyno VISTA at a histidine rich region of the ECD, at acidic pH;
- o lacks significant binding to cyno VISTA at a histidine rich region of the ECD, at physiological pH or neutral pH;
- 5 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;
- 10 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;
- 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
- 15 NO: 1 and PSGL-1 tyrosines Y46 and Y48), wherein PSGL-1 is with or without sialyl 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;
- o stimulates T cell activation by, e.g., enhancing T cell proliferation; enhancing
- 20 IFN- γ production from T cells; and/or stimulating T cell receptor mediated NF- κ B 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;
- 25 o contacts hVISTA through one or more energetically important contact residues Y37, T39, R54, F62, H66, V117, I119 or S124, as determined using a yeast surface display and NGS assay; and wherein numbering is that of mature hVISTA (SEQ ID NO: 2);
- o binds to Region 1 of hVISTA: ₅₇LGPVDKGHDVTF₆₈ (SEQ ID NO: 566);
- 30 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;
- o binds to the histidine-rich β -sheet extension of hVISTA, as determined by crystallography;
 - 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 by crystallography; 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.
23. The isolated antibody of any one of claims 1-22, wherein the antibody has an isoelectric point (pI) between 6.5 and 6.8, as measured by icIEF.
24. The isolated antibody of any one of claims 1-23, which is an IgG antibody.
25. The isolated antibody of claim 23, which is an IgG1, IgG2 or IgG4 antibody (IgG4 optionally with S228P).
26. The isolated antibody of any one of claims 1-25, which is an antibody drug conjugate (ADC) or an activatable antibody.
27. An isolated nucleic acid encoding an antibody of any one of claims 1-26.
28. An isolated nucleic acid encoding the heavy chain and/or the light chain of an antibody of any one of claims 1-26.
29. A composition comprising an isolated nucleic acid encoding the heavy chain of an antibody of any one of claims 1-26 and a nucleic acid encoding the light chain of the antibody.
30. A cell comprising the isolated nucleic acid of any one of claims 27-29.
31. A method of preparing an antibody, comprising culturing the cell of claim 30 in conditions under which the antibody is expressed.
32. A composition comprising an isolated antibody, nucleic acid, composition or cell of any one of claims 1-26 and a pharmaceutically acceptable carrier.
33. Use of the isolated antibody of any one of claims 1-26 in the manufacture of a medicament for treating cancer, wherein a therapeutically effective amount of the

isolated antibody is to be administered to the subject, wherein the isolated antibody stimulates an immune response and/or is a VISTA antagonist antibody.

34. Use of the isolated antibody of any one of claims 1-26 in the manufacture of a medicament for treating an infectious disease, wherein a therapeutically effective amount of the isolated antibody is to be administered to the subject, 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 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 to the subject, wherein the antibody inhibits an immune response, or is a VISTA agonist.

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