

CLAIMS

What is claimed is:

1. An isolated anti-LAG-3 construct comprising a single-domain antibody (sdAb) specifically recognizing LAG-3, wherein the sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 amino acid substitutions.
2. The isolated anti-LAG-3 construct of claim 1, wherein the sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228; or a variant thereof comprising up to about 3 amino acid substitutions in the CDR regions.
3. The isolated anti-LAG-3 construct of claim 1 or 2, wherein the sdAb comprises any one of the following:
 - (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 191, or a variant thereof comprising up to about 3 amino acid substitutions;
 - (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 40, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192, or a variant thereof comprising up to about 3 amino acid substitutions;
 - (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193, or a variant thereof comprising up to about 3 amino acid substitutions;
 - (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 42, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID

- NO: 118, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194, or a variant thereof comprising up to about 3 amino acid substitutions;
- (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195, or a variant thereof comprising up to about 3 amino acid substitutions;
- (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 44, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196, or a variant thereof comprising up to about 3 amino acid substitutions;
- (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 45, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 121, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 197, or a variant thereof comprising up to about 3 amino acid substitutions;
- (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 122, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 198, or a variant thereof comprising up to about 3 amino acid substitutions;
- (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 54, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 130, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206, or a variant thereof comprising up to about 3 amino acid substitutions;
- (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 61, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 137, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 213, or a variant thereof comprising up to about 3 amino acid substitutions;

- (11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 70, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 146, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 222, or a variant thereof comprising up to about 3 amino acid substitutions;
 - (12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 72, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 148, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 224, or a variant thereof comprising up to about 3 amino acid substitutions; or
 - (13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 73, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 149, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 225, or a variant thereof comprising up to about 3 amino acid substitutions.
4. The isolated anti-LAG-3 construct of any one of claims 1-3, wherein the sdAb comprises any one of the following:
- (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 191;
 - (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 40; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192;
 - (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 41; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193;
 - (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 42; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194;
 - (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 43; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195;

- (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 44; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196;
 - (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 45; a CDR2 comprising the amino acid sequence of SEQ ID NO: 121; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 197;
 - (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46; a CDR2 comprising the amino acid sequence of SEQ ID NO: 122; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 198;
 - (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 54; a CDR2 comprising the amino acid sequence of SEQ ID NO: 130; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206;
 - (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 61; a CDR2 comprising the amino acid sequence of SEQ ID NO: 137; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 213;
 - (11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 70; a CDR2 comprising the amino acid sequence of SEQ ID NO: 146; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 222;
 - (12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 72; a CDR2 comprising the amino acid sequence of SEQ ID NO: 148; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 224; or
 - (13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 73; a CDR2 comprising the amino acid sequence of SEQ ID NO: 149; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 225.
5. The isolated anti-LAG-3 construct of any one of claims 1-4, wherein the sdAb comprises a V_HH domain comprising:
- (1) an FR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-38;
 - (2) an FR2 comprising the amino acid sequence of any one of SEQ ID NOs: 77-114;
 - (3) an FR3 comprising the amino acid sequence of any one of SEQ ID NOs: 153-190; and
 - (4) an FR4 comprising the amino acid sequence of any one of SEQ ID NOs: 229-266.
6. The isolated anti-LAG-3 construct of any one of claims 1-5, wherein the sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311, or a variant thereof having at least about 80% sequence identity to any one of SEQ ID NOs: 274-311.

7. The isolated anti-LAG-3 construct of claim 6, wherein the sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311, or a variant thereof comprising up to about 3 amino acid substitutions in the V_HH domain.
8. The isolated anti-LAG-3 construct of any one of claims 1-7, wherein the K_D of the binding between the sdAb and LAG-3 is about 10⁻⁷ M to about 10⁻¹² M.
9. The isolated anti-LAG-3 construct of claim 8, wherein the K_D of the binding between the sdAb and LAG-3 is about 10⁻⁹ M to about 10⁻¹¹ M.
10. The isolated anti-LAG-3 construct of any one of claims 1-9, wherein the sdAb cross-reacts with a LAG-3 from a non-human mammal.
11. The isolated anti-LAG-3 construct of any one of claims 1-10, wherein the sdAb specifically recognizing LAG-3 is camelid, chimeric, human, partially humanized, or fully humanized.
12. The isolated anti-LAG-3 construct of any one of claims 1-11, wherein the isolated anti-LAG-3 construct is a heavy chain-only antibody (HCAb) comprising the sdAb specifically recognizing LAG-3 fused to an Fc fragment.
13. The isolated anti-LAG-3 construct of claim 12, wherein the HCAb is monomeric or dimeric.
14. The isolated anti-LAG-3 construct of claim 12 or 13, wherein the Fc fragment is a human IgG1 (hIgG1) Fc, effectorless hIgG1 Fc, hIgG4 Fc, or hIgG4 Fc (S228P).
15. The isolated anti-LAG-3 construct of any one of claims 12-14, wherein the sdAb is fused to the Fc fragment via a peptide linker.
16. The isolated anti-LAG-3 construct of claim 15, wherein the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372.
17. The isolated anti-LAG-3 construct of any one of claims 12-16, wherein the HCAb comprises the amino acid sequence of any one of SEQ ID NOs: 312-349.
18. The isolated anti-LAG-3 construct of any one of claims 1-11, comprising: (a) a first antigen binding portion comprising the sdAb specifically recognizing LAG-3; and (b) a second antigen binding portion that specifically recognizes a second epitope.
19. The isolated anti-LAG-3 construct of claim 18, wherein the second antigen binding portion comprises a full-length antibody, a Fab, a Fab', a (Fab')₂, an Fv, a single chain Fv (scFv), an scFv-scFv, a minibody, a diabody, or a second sdAb.
20. The isolated anti-LAG-3 construct of claim 18 or 19, wherein the anti-LAG-3 construct is multispecific.
21. The isolated anti-LAG-3 construct of any one of claims 18-20, wherein the first antigen binding portion and the second antigen binding portion are fused to each other via a peptide linker.

22. The isolated anti-LAG-3 construct of claim 21, wherein the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372.
23. The isolated anti-LAG-3 construct of any one of claims 18-22, wherein the second antigen binding portion comprises a full-length antibody consisting of two heavy chains and two light chains.
24. The isolated anti-LAG-3 construct of claim 23, wherein the Fc fragment of the full-length antibody is human IgG1 (hIgG1) Fc, effectorless hIgG1 Fc, hIgG4 Fc, or hIgG4 Fc (S228P).
25. The isolated anti-LAG-3 construct of claim 23 or 24, wherein the N-terminus of the sdAb specifically recognizing LAG-3 is fused to the C-terminus of a heavy chain of the full-length antibody.
26. The isolated anti-LAG-3 construct of claim 23 or 24, wherein the C-terminus of the sdAb specifically recognizing LAG-3 is fused to the N-terminus of a heavy chain of the full-length antibody.
27. The isolated anti-LAG-3 construct of claim 23 or 24, wherein the N-terminus of the sdAb specifically recognizing LAG-3 is fused to the C-terminus of a light chain of the full-length antibody.
28. The isolated anti-LAG-3 construct of claim 23 or 24, wherein the C-terminus of the sdAb specifically recognizing LAG-3 is fused to the N-terminus of a light chain of the full-length antibody.
29. The isolated anti-LAG-3 construct of any one of claims 18-28, wherein the second antigen binding portion specifically recognizes an immune checkpoint molecule selected from the group consisting of PD-1, 4-1BB, PD-L1, TIM-3, TIGIT, CTLA-4, VISTA, B7-1, B7-H3, CD47, OX40 and GITR.
30. The isolated anti-LAG-3 construct of claim 29, wherein the second antigen binding portion specifically recognizes PD-1.
31. The isolated anti-LAG-3 construct of claim 30, wherein the second antigen binding portion comprises:
 - (i) HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 356, and LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 357; or
 - (ii) HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 373, and LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 374.
32. The isolated anti-LAG-3 construct of claim 30, wherein the second antigen binding portion is a full-length antibody comprising:
 - (i) a heavy chain comprising the amino acid sequence of SEQ ID NO: 356, and a light chain comprising the amino acid sequence of SEQ ID NO: 357; or
 - (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 373, and a light chain comprising the amino acid sequence of SEQ ID NO: 374.
33. An isolated anti-LAG-3 construct comprising an sdAb specifically recognizing LAG-3, wherein the sdAb comprises CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 274-311.

34. An isolated anti-LAG-3 construct that specifically binds to LAG-3 competitively with the isolated anti-LAG-3 construct of any one of claims 1-33.
35. A pharmaceutical composition comprising the isolated anti-LAG-3 construct of any one of claims 1-34, and a pharmaceutically acceptable carrier.
36. A method of treating an individual having a LAG-3-related disease, comprising administering to the individual an effective amount of the pharmaceutical composition of claim 35.
37. The method of claim 36, wherein the LAG-3-related disease is cancer.
38. The method of claim 37, wherein the cancer is colon cancer.
39. The method of claim 37 or 38, wherein the individual is a human.
40. An isolated nucleic acid encoding the isolated anti-LAG-3 construct of any one of claims 1-34.
41. A vector comprising the isolated nucleic acid of claim 40.
42. An isolated host cell comprising the isolated nucleic acid of claim 40, or the vector of claim 41.
43. A kit comprising the isolated anti-LAG-3 construct of any one of claims 1-34, the pharmaceutical composition of claim 35, the isolated nucleic acid of claim 40, the vector of claim 41, or the isolated host cell of claim 42.
44. A method of producing an anti-LAG-3 construct, comprising: (a) culturing the isolated host cell of claim 42 under conditions effective to express the encoded anti-LAG-3 construct; and (b) obtaining the expressed anti-LAG-3 construct from the host cell.