

We claim:

1. An isolated monoclonal antibody, or an antigen-binding fragment thereof, wherein the antibody, or antigen-binding fragment thereof, specifically binds human natural killer cell inhibitory receptor group 2A (NKG2A) protein and exhibits at least one
5 of the following properties:

(a) reduces binding and/or interaction of HLA-E to human NKG2A protein;

(b) reverses NKG2A-mediated inhibitory signaling;

(c) does not bind, or binds with low affinity to, human NKG2C protein;

(d) binds to human or cynomolgus NKG2A protein;

10 (e) enhances natural killer cell response;

(f) enhances the functional activity of T cells;

(g) has reduced binding to human Fc gamma receptor (FcγR);

(h) induces and/or enhances an anti-tumor immune response; and/or

(i) has low immunogenicity in human subjects.

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2. The antibody, or an antigen-binding fragment thereof, of claim 1, wherein the antibody has one or more of the following properties:

(a) has an EC₅₀ value of about 0.6 nM or lower for binding to human NKG2A protein as measured by cell binding assay;

20 (b) has an EC₅₀ value of about 9.0 nM or higher for binding to human NKG2C protein as measured by cell binding assay;

(c) has an EC₅₀ value for binding to human NKG2A protein that is about 15-fold less than a second EC₅₀ value for binding to human NKG2C protein;

25 (d) has an IC₅₀ value of about 1.0 nM or lower as measured by cell blocking assay;

(e) binds to human NKG2A protein with a K_D of about 0.4 nM or lower as measured by Scatchard analysis;

(f) binds to human NKG2A protein with a K_D of about 61 nM or lower as measured by surface plasmon resonance;

5 (g) binds to cynomolgus NKG2A protein with a K_D of about 1.0 nM or lower as measured by Scatchard analysis;

(h) is internalized upon binding to NKG2A-expressing cells;

(i) increases interferon-gamma ($IFN\gamma$) production;

(j) wherein the half-life of the anti-NKG2A antibody:NKG2A protein complex is
10 about 40 seconds or longer; and/or

(k) exhibits internalization with an EC_{50} value of about 0.5 nM or lower.

3. The antibody, or antigen-binding fragment thereof, of claim 1 or 2, wherein the antibody reduces binding and/or interaction of HLA-E to human NKG2A protein.

15 4. The antibody, or antigen-binding fragment thereof, of claim 3, wherein the antibody binds to human NKG2A protein.

5. An isolated monoclonal antibody, or antigen-binding fragment thereof, that specifically binds to human NKG2A protein, wherein the antibody comprises:

(a) a heavy chain variable domain comprising amino acid sequences of SEQ ID
20 NOs: 10, 11, 12, and a light chain variable domain comprising the amino acid sequences of SEQ ID NOs: 13, 14, and 15;

(b) a heavy chain variable domain comprising amino acid sequences of SEQ ID NOs: 10, 11, and 12, and a light chain variable domain comprising amino acid sequences of SEQ ID NOs: 154, 14, and 15; or

25 (c) a heavy chain variable domain comprising amino acid sequences of SEQ ID NOs: 10, 11, and 12, and a light chain variable domain comprising amino acid sequences of SEQ ID NOs: 155, 14, and 15.

6. An isolated monoclonal antibody, or antigen-binding fragment thereof, that specifically binds to human NKG2A protein, and comprises heavy and light chain variable regions, wherein

5 (a) the heavy chain variable region comprises an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, about 100%, or 100% identical to the amino acid sequence of SEQ ID NO: 8 or SEQ ID NO: 167, and/or

10 (b) the light chain variable region comprises an amino acid sequence that is at least about 80%, at least about 85% at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, about 100%, or 100% identical to the amino acid sequence of SEQ ID NO: 9, SEQ ID NO: 164, or SEQ ID NO: 169.

7. The antibody, or antigen-binding fragment thereof, of claim 6, wherein
15 heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 8, and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 9.

8. The antibody, or antigen-binding fragment thereof, of claim 6, wherein
20 heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 8, and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 164.

9. The antibody, or antigen-binding fragment thereof, of claim 6, wherein
heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 167, and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 169.

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10. An isolated monoclonal antibody, or antigen-binding fragment thereof, that specifically binds to human NKG2A protein, wherein the heavy and light chains consist essentially of:

- (a) the amino acid sequences of SEQ ID NOs: 7 and 5, respectively;
- (b) the amino acid sequences of SEQ ID NOs: 7 and 19, respectively; or
- (c) the amino acid sequences of SEQ ID NOs: 35 and 36, respectively.

5 11. An isolated monoclonal antibody, or antigen-binding fragment thereof, that competes for binding to NKG2A protein with or binds to the same epitope as any of the preceding antibodies.

10 12. An isolated monoclonal antibody, or antigen-binding fragment thereof, that, when bound to human NKG2A protein, specifically binds to the following amino acid residues as determined by hydrogen-deuterium exchange mass spectrometry (HDX-MS):

- (a) LSIDNEEMKF (SEQ ID NO: 156);
- (b) PSSWIGVFRNSSHHPW (SEQ ID NO: 157);
- 15 (c) LAFKHEIKDSDN (SEQ ID NO: 158); and
- (d) QVNRLKSAQQCGSSIYHC (SEQ ID NO: 159),

wherein the antibody blocks the binding of HLA-E to human NKG2A protein.

20 13. An isolated monoclonal antibody, or antigen-binding fragment thereof, that, when bound to human NKG2A, specifically binds to the following amino acid residues as determined by HDX-MS and/or fast photochemical oxidation of proteins (FPOP) epitope mapping:

- (a) LSIDNEEMKF (SEQ ID NO: 156)
- (b) PSSWIGVFRNSSHHPW (SEQ ID NO: 157)
- 25 (c) LAFKHEIKDSDN (SEQ ID NO: 158)
- (d) L; and

(e) QVNRLKSAQQCGSSIIYHC (SEQ ID NO: 159),

wherein the antibody blocks the binding of HLA-E to human NKG2A protein.

14. The antibody, or antigen-binding fragment thereof, of any one of the preceding claims, wherein the antibody is a full-length antibody.

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15. The antibody, or antigen-binding fragment thereof, of any one of the preceding claims, wherein the high-length antibody is an IgG1 antibody.

16. The antibody, or antigen-binding fragment thereof, of any one of claims 1-
10 13, wherein the antibody is an antibody fragment.

17. The antigen-binding fragment of claim 16, wherein the antibody fragment is a Fab, Fab', (Fab')₂, Fv, or scFv fragment.

18. The antibody, or antigen-binding fragment thereof, of any one of the preceding claims, wherein the antibody is a human, humanized, or chimeric antibody.

19. An isolated, full-length monoclonal antibody that binds specifically to human NKG2A protein, wherein

20 the heavy chain comprises an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, about 100%, or 100% identical to the amino acid sequence of SEQ ID NO: 7, and/or

the light chain comprises an amino acid sequence that is at least about 80%, at
25 least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, about 100%, or 100% identical to the amino acid sequence of SEQ ID NO: 5.

20. An isolated, full-length monoclonal antibody that specifically binds to human NKG2A protein, wherein the heavy chain comprises the amino acid sequence set forth in SEQ ID NO: 7, and the light chain comprise the amino acid sequence set forth in SEQ ID NO: 19.

5 21. An isolated, full-length monoclonal antibody that specifically binds to human NKG2A, wherein the heavy chain consists essentially of the amino acid sequence set forth in SEQ ID NO: 7, and the light chain consists essentially of the amino acid sequence set forth in SEQ ID NO: 19.

10 22. An isolated, full-length monoclonal antibody that specifically binds to human NKG2A protein, wherein the heavy chain comprises an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, about 100%, or 100% identical to the amino acid sequence of SEQ ID NO: 7, and

15 the light chain is an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, about 100%, or 100% identical to the amino acid sequence of SEQ ID NO: 19.

20 23. An isolated antibody, or antigen binding fragment thereof, that binds to human NKG2A protein and cross-competes for binding to human NKG2A protein with a reference antibody comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein:

(a) the VH comprises the amino acid sequences set forth in SEQ ID NO: 8 and the VL comprises the amino acid sequence set forth in SEQ ID NO: 9;

25 (b) the VH comprises the amino acid sequences set forth in SEQ ID NO: 8 and the VL comprising the amino acid sequence set forth in SEQ ID NO:164; or

(c) the VH comprises the amino acid sequences set forth in SEQ ID NO: 167 and the VL comprising the amino acid sequence set forth in SEQ ID NO: 169.

24. An isolated nucleic acid molecule encoding the heavy and/or light chain variable region of the antibody, or antigen binding fragment thereof, of any one of the preceding claims.

25. The isolated nucleic acid of claim 24, wherein the nucleic acid molecule is complementary DNA (cDNA).

26. An expression vector comprising the nucleic acid molecule of claim 24 or 25.

27. A host cell transformed with the expression vector of claim 26.

28. An immunoconjugate comprising the antibody, or antigen-binding fragment thereof, according to any one of claims 1-23, linked to an agent.

29. A method of producing the antibody, or antigen-binding fragment thereof, comprising culturing the host cell of claim 27, wherein the antibody is produced.

30. The method of claim 29, further comprising recovering the antibody, or antigen-binding fragment thereof, from the host cell.

31. A bispecific molecule comprising the antibody, or antigen-binding fragment thereof, of any one of claims 1-23 linked to a second functional moiety.

32. A composition comprising the antibody, or antigen-binding fragment thereof, of any one of claims 1-23, or the bispecific molecule of claim 31, and a pharmaceutically acceptable carrier.

33. The composition of claim 32, further comprising an additional therapeutic agent.

34. The composition of claim 33, wherein the additional therapeutic agent is an anti-PD-1 antibody, anti-PD-L1 antibody, and/or an anti-CTLA-4 antibody.

35. The antibody, or antigen-binding fragment thereof, of any one of claims 1-23 for use as a medicament for treating cancer.

36. The antibody, or antigen-binding fragment thereof, of claim 35, wherein the cancer is bladder cancer, breast cancer, uterine/cervical cancer, ovarian cancer, prostate cancer, testicular cancer, esophageal cancer, gastrointestinal cancer, pancreatic

cancer, colorectal cancer, kidney cancer, head and neck cancer, lung cancer, stomach cancer, germ cell cancer, bone cancer, liver cancer, thyroid cancer, skin cancer, neoplasm of the central nervous system, lymphoma, leukemia, myeloma, sarcoma, endometrial, cervical, gastric, melanoma, renal, urothelial, glioblastoma multiform, or virus-related cancer.

37. The antibody, or antigen-binding fragment thereof, of claim 36, wherein the cancer is cervical, head and neck squamous cell carcinoma, pancreatic, non-small cell lung cancer-adenocarcinoma type, non-small cell lung cancer-squamous cell type, gastric, melanoma, colorectal, endometrial, ovarian, renal cell carcinoma, urothelial carcinoma, breast, small cell lung, glioblastoma multiforme, prostate cancer, or non-Hodgkin lymphoma.

38. The antibody, or antigen-binding fragment thereof, of any one of claims 1-23 for use in enhancing an immune response.

39. Use of the antibody, or antigen-binding fragment thereof, of any one of claims 1-23 in the manufacture of a medicament for treatment of cancer.

40. The antibody of any one of claims 1-23, the immunoconjugate of claim 28, the bispecific molecule of claim 31, or the composition of claim 33 or 34 for use in the treatment or delaying progression of cancer in a human subject, wherein an effective amount of the antibody, the immunoconjugate, the bispecific molecule, or the composition is to be administered to the human subject.

41. The antibody, the immunoconjugate, the bispecific molecule, or the composition for use of claim 40, wherein the cancer is bladder cancer, breast cancer, uterine/cervical cancer, ovarian cancer, prostate cancer, testicular cancer, esophageal cancer, gastrointestinal cancer, pancreatic cancer, colorectal cancer, kidney cancer, head and neck cancer, lung cancer, stomach cancer, germ cell cancer, bone cancer, liver cancer, thyroid cancer, skin cancer, neoplasm of the central nervous system, lymphoma, leukemia, myeloma, sarcoma, endometrial, cervical, gastric, melanoma, renal, urothelial, glioblastoma multiform, or virus-related cancer.

42. The antibody, the immunoconjugate, the bispecific molecule, or the composition for use of claim 41, wherein the cancer is cervical, head and neck squamous

cell carcinoma, pancreatic, non-small cell lung cancer-adenocarcinoma type, non-small cell lung cancer-squamous cell type, gastric, melanoma, colorectal, endometrial, ovarian, renal cell carcinoma, urothelial carcinoma, breast, small cell lung, glioblastoma multiforme, prostate cancer, or non-Hodgkin lymphoma.

5 43. The antibody, the immunoconjugate, the bispecific molecule, or the composition for use of claim 41 or 42, one or more additional therapeutic agent(s) is to be administered to the human subject.

 44. The antibody, the immunoconjugate, the bispecific molecule, or the composition for use of claim 43, wherein the one or more additional therapeutic agent(s)
10 is a chemotherapeutic agent, a radiotherapeutic agent, and/or an immunotherapeutic agent.

 45. The antibody, the immunoconjugate, the bispecific molecule, or the composition for use of claim 44, wherein the immunotherapeutic agent is an anti-PD-1 antibody, an anti-PD-L1 antibody, and/or an anti-CTLA-4 antibody.

15 46. The antibody, the immunoconjugate, the bispecific molecule, or the composition for use of claim 45, wherein the anti-PD-1 antibody is nivolumab, and the anti-CTLA-4 antibody is ipilimumab.

 47. The antibody of any one of claims 1-23, the immunoconjugate of claim 28, the bispecific molecule of claim 31, or the composition of claim 33, 34, or 35 for use in
20 stimulating an immune response in a human subject, wherein an effective amount of the antibody, the immunoconjugate, the bispecific molecule, or the composition is to be administered to the human subject.

 48. The antibody, the immunoconjugate, the bispecific molecule, or the composition for use of claim 47, wherein the human subject has a tumor and an anti-
25 tumor immune response is stimulated.

 49. The antibody, the immunoconjugate, the bispecific molecule, or the composition for use of claim 47, wherein the human subject has a chronic viral infection and an anti-viral immune response is stimulated.

50. Use of the antibody of any one of claims 1-23, the immunoconjugate of claim 28, the bispecific molecule of claim 31, or the composition of claim 33 or 34 in the manufacture of a medicament for the treating or delaying progression of cancer in a human subject, wherein an effective amount of the medicament is to be administered to the human subject.

51. The use of claim 50, wherein the cancer is bladder cancer, breast cancer, uterine/cervical cancer, ovarian cancer, prostate cancer, testicular cancer, esophageal cancer, gastrointestinal cancer, pancreatic cancer, colorectal cancer, kidney cancer, head and neck cancer, lung cancer, stomach cancer, germ cell cancer, bone cancer, liver cancer, thyroid cancer, skin cancer, neoplasm of the central nervous system, lymphoma, leukemia, myeloma, sarcoma, endometrial, cervical, gastric, melanoma, renal, urothelial, glioblastoma multiform, or virus-related cancer.

52. The use of claim 51, wherein the cancer is cervical, head and neck squamous cell carcinoma, pancreatic, non-small cell lung cancer-adenocarcinoma type, non-small cell lung cancer-squamous cell type, gastric, melanoma, colorectal, endometrial, ovarian, renal cell carcinoma, urothelial carcinoma, breast, small cell lung, glioblastoma multiforme, prostate cancer, or non-Hodgkin lymphoma.

53. The use of claim 51 or 52, wherein one or more additional therapeutic agent(s) is to be administered to the human subject.

54. The use of claim 53, wherein the one or more additional therapeutic agent(s) is a chemotherapeutic agent, a radiotherapeutic agent, and/or an immunotherapeutic agent.

55. The use of claim 54, wherein the immunotherapeutic agent is an anti-PD-1 antibody, an anti-PD-L1 antibody, and/or an anti-CTLA-4 antibody.

56. The use of claim 55, wherein the anti-PD-1 antibody is nivolumab, and the anti-CTLA-4 antibody is ipilimumab.

57. Use of the antibody of any one of claims 1-23, the immunoconjugate of claim 28, the bispecific molecule of claim 31, or the composition of claim 33, 34, or 35 in the manufacture of a medicament for stimulating an immune response in a human subject,

wherein an effective amount of the medicament is to be administered to the human subject.

58. The use of claim 57, wherein the human subject has a tumor and an anti-tumor immune response is stimulated.

5 59. The use of claim 57, wherein the human subject has a chronic viral infection and an anti-viral immune response is stimulated.

60. A method of detecting presence of NKG2A protein in a sample comprising
contacting the sample with the antibody, or antigen-binding fragment thereof, of
any one of claims 1-23, under conditions that allow for formation of a complex between
10 the antibody, or antigen-binding fragment thereof, and NKG2A protein, and
detecting the formation of the complex.

61. The method of claim 60, wherein the antibody, or antigen-binding
fragment thereof, forms a complex more quickly with NKG2A protein than with NKG2C
protein.

15 62. The method of claim 61, where the antibody, or antigen-binding fragment
thereof, forms a complex 15 more quickly with NKG2A protein than with NKG2C
protein.