

CLAIMS

WHAT IS CLAIMED IS:

1. A nucleic acid library, comprising: a plurality of nucleic acids, wherein each of the nucleic acids encodes for a sequence that when translated encodes for an adenosine A2A receptor binding immunoglobulin, wherein the adenosine A2A receptor binding immunoglobulin comprises a variant of an adenosine A2A receptor binding domain, wherein the adenosine A2A receptor binding domain is a ligand for the adenosine A2A receptor, and wherein the nucleic acid library comprises at least 10,000 variant immunoglobulin heavy chains and at least 10,000 variant immunoglobulin light chains.
2. The nucleic acid library of claim 1, wherein the nucleic acid library comprises at least 50,000 variant immunoglobulin heavy chains and at least 50,000 variant immunoglobulin light chains.
3. The nucleic acid library of claim 1, wherein the nucleic acid library comprises at least 100,000 variant immunoglobulin heavy chains and at least 100,000 variant immunoglobulin light chains.
4. The nucleic acid library of claim 1, wherein the nucleic acid library comprises at least 10^5 non-identical nucleic acids.
5. The nucleic acid library of claim 1, wherein a length of the immunoglobulin heavy chain when translated is about 90 to about 100 amino acids.
6. The nucleic acid library of claim 1, wherein a length of the immunoglobulin heavy chain when translated is about 100 to about 400 amino acids.
7. The nucleic acid library of claim 1, wherein the variant immunoglobulin heavy chain when translated comprises at least about 90% sequence identity to any one of SEQ ID NO: 540-628.
8. The nucleic acid library of claim 1, wherein the variant immunoglobulin light chain when translated comprises at least about 90% sequence identity to any one of SEQ ID NO: 629-717.
9. The nucleic acid library of claim 1, wherein the variant immunoglobulin heavy chain when translated comprises any one of SEQ ID NO: 540-628.
10. The nucleic acid library of claim 1, wherein the variant immunoglobulin light chain when translated comprises any one of SEQ ID NO: 629-717.
11. A nucleic acid library comprising a plurality of nucleic acids, wherein each nucleic acid of the plurality of nucleic acids encodes for a sequence that when translated encodes for an antibody or antibody fragment thereof, wherein the antibody or antibody fragment thereof comprises a variable region of a heavy chain (VH) that comprises an adenosine A2A receptor binding domain, wherein each nucleic acid of the plurality of nucleic acids comprises a sequence encoding for a

sequence variant of the adenosine A2A receptor binding domain, and wherein the antibody or antibody fragment binds to its antigen with a K_D of less than 100 nM.

12. The nucleic acid library of claim 11, wherein a length of the VH is about 90 to about 100 amino acids.
13. The nucleic acid library of claim 11, wherein a length of the VH is about 100 to about 400 amino acids.
14. The nucleic acid library of claim 11, wherein a length of the VH is about 270 to about 300 base pairs.
15. The nucleic acid library of claim 11, wherein a length of the VH is about 300 to about 1200 base pairs.
16. The nucleic acid library of claim 11, wherein the library comprises at least 10^5 non-identical nucleic acids.
17. A nucleic acid library comprising: a plurality of nucleic acids, wherein each of the nucleic acids encodes for a sequence that when translated encodes for an adenosine A2A receptor single domain antibody, wherein each sequence of the plurality of sequences comprises a variant sequence encoding for a CDR1, CDR2, or CDR3 on a variable region of a heavy chain (VH); wherein the library comprises at least 30,000 variant sequences; and wherein the adenosine A2A receptor single domain antibody binds to its antigen with a K_D of less than 100 nM.
18. The nucleic acid library of claim 17, wherein a length of the VH when translated is about 90 to about 100 amino acids.
19. The nucleic acid library of claim 17, wherein a length of the VH when translated is about 100 to about 400 amino acids.
20. The nucleic acid library of claim 17, wherein a length of the VH is about 270 to about 300 base pairs.
21. The nucleic acid library of claim 17, wherein a length of the VH is about 300 to about 1200 base pairs.
22. The nucleic acid library of claim 17, wherein the variant library comprises a variant sequence encoding for a CDR1, CDR2, and CDR3.
23. The nucleic acid library of claim 17, wherein the VH when translated comprises at least 90% sequence identity to any one of SEQ ID NO: 540-628.
24. The nucleic acid library of claim 17, wherein the VH when translated comprises any one of SEQ ID NO: 540-628.
25. An antibody or antibody fragment that binds adenosine A2A receptor, comprising an immunoglobulin heavy chain and an immunoglobulin light chain:

- a. wherein the immunoglobulin heavy chain comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 540-628; and
 - b. wherein the immunoglobulin light chain comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 629-717.
26. The antibody or antibody fragment of claim 25, wherein the immunoglobulin heavy chain comprises an amino acid sequence at least about 95% identical to that set forth in any one of SEQ ID NOs: 540-628; and wherein the immunoglobulin light chain comprises an amino acid sequence at least about 95% identical to that set forth in any one of SEQ ID NOs: 629-717.
27. The antibody or antibody fragment of claim 25, wherein the immunoglobulin heavy chain comprises an amino acid sequence as set forth in any one of SEQ ID NOs: 540-628; and wherein the immunoglobulin light chain comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 629-717.
28. The antibody or antibody fragment of claim 25, wherein the antibody is a monoclonal antibody, a polyclonal antibody, a bi-specific antibody, a multispecific antibody, a grafted antibody, a human antibody, a humanized antibody, a synthetic antibody, a chimeric antibody, a camelized antibody, a single-chain Fvs (scFv), a single chain antibody, a Fab fragment, a F(ab')₂ fragment, a Fd fragment, a Fv fragment, a single-domain antibody, an isolated complementarity determining region (CDR), a diabody, a fragment comprised of only a single monomeric variable domain, disulfide-linked Fvs (sdFv), an intrabody, an anti-idiotypic (anti-Id) antibody, or ab antigen-binding fragments thereof.
29. The antibody or antibody fragment of claim 25, wherein the antibody or antibody fragment thereof is chimeric or humanized.
30. The antibody or antibody fragment of claim 25, wherein the antibody has an EC₅₀ less than about 25 nanomolar in a cAMP assay.
31. The antibody or antibody fragment of claim 25, wherein the antibody has an EC₅₀ less than about 20 nanomolar in a cAMP assay.
32. The antibody or antibody fragment of claim 25, wherein the antibody has an EC₅₀ less than about 10 nanomolar in a cAMP assay.
33. An antibody or antibody fragment, wherein the antibody or antibody fragment comprises a complementarity determining region (CDR) comprising an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 6-539.
34. An antibody or antibody fragment, wherein the antibody or antibody fragment comprises a variable heavy chain complementarity determining region (CDRH) comprising an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 6-272.

35. An antibody or antibody fragment, wherein the antibody or antibody fragment comprises a variable light chain complementarity determining region (CDRH) comprising an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 273-539.
36. An antibody or antibody fragment, wherein the antibody or antibody fragment comprises a sequence of any one of SEQ ID NOs: 6-539 and wherein the antibody is a monoclonal antibody, a polyclonal antibody, a bi-specific antibody, a multispecific antibody, a grafted antibody, a human antibody, a humanized antibody, a synthetic antibody, a chimeric antibody, a camelized antibody, a single-chain Fvs (scFv), a single chain antibody, a Fab fragment, a F(ab')₂ fragment, a Fd fragment, a Fv fragment, a single-domain antibody, an isolated complementarity determining region (CDR), a diabody, a fragment comprised of only a single monomeric variable domain, disulfide-linked Fvs (sdFv), an intrabody, an anti-idiotypic (anti-Id) antibody, or ab antigen-binding fragments thereof.
37. A method of treating cancer, comprising administering the antibody or antibody fragment of any one of claims 25-36.
38. A method of treating a neurological disease or disorder, comprising administering the antibody or antibody fragment of any one of claims 25-36.
39. A method for generating a nucleic acid library encoding for an adenosine A2A receptor antibody or antibody fragment thereof comprising:
- (a) providing predetermined sequences encoding for:
 - i. a first plurality of polynucleotides, wherein each polynucleotide of the first plurality of polynucleotides encodes for at least 1000 variant sequence encoding for CDR1 on a heavy chain;
 - ii. a second plurality of polynucleotides, wherein each polynucleotide of the second plurality of polynucleotides encodes for at least 1000 variant sequence encoding for CDR2 on a heavy chain;
 - iii. a third plurality of polynucleotides, wherein each polynucleotide of the third plurality of polynucleotides encodes for at least 1000 variant sequence encoding for CDR3 on a heavy chain; and
 - (b) mixing the first plurality of polynucleotides, the second plurality of polynucleotides, and the third plurality of polynucleotides to form the nucleic acid library of variant nucleic acids encoding for the adenosine A2A receptor antibody or antibody fragment thereof, and wherein at least about 70% of the variant nucleic acids encode for an antibody or antibody fragment that binds to adenosine A2A receptor with a K_D of less than 100 nM.
40. The method of claim 39, wherein the adenosine A2A receptor antibody or antibody fragment thereof is a single domain antibody.

41. The method of claim 40, wherein the single domain antibody comprises one heavy chain variable domain.
42. The method of claim 40, wherein the single domain antibody is a VHH antibody.
43. The method of claim 39, wherein the nucleic acid library comprises at least 50,000 variant sequences.
44. The method of claim 39, wherein the nucleic acid library comprises at least 100,000 variant sequences.
45. The method of claim 39, wherein the nucleic acid library comprises at least 10^5 non-identical nucleic acids.
46. The method of claim 39, wherein the nucleic acid library comprises at least one sequence encoding for the adenosine A2A receptor antibody or antibody fragment that binds to adenosine A2A receptor with a K_D of less than 75 nM.
47. The method of claim 39, wherein the nucleic acid library comprises at least one sequence encoding for the adenosine A2A receptor antibody or antibody fragment that binds to adenosine A2A receptor with a K_D of less than 50 nM.
48. The method of claim 39, wherein the nucleic acid library comprises at least one sequence encoding for the adenosine A2A receptor antibody or antibody fragment that binds to adenosine A2A receptor with a K_D of less than 10 nM.
49. The method of claim 48, wherein the nucleic acid library comprises at least 500 variant sequences.
50. The method of claim 39, wherein the nucleic acid library comprises at least five sequences encoding for the antibody or antibody fragment that binds to adenosine A2A receptor with a K_D of less than 75 nM.
51. A protein library encoded by the nucleic acid library of any one of claims 1-24, wherein the protein library comprises peptides.
52. The protein library of claim 51, wherein the protein library comprises immunoglobulins.
53. The protein library of claim 51, wherein the protein library comprises antibodies.
54. The protein library of claim 51, wherein the protein library is a peptidomimetic library.
55. A vector library comprising the nucleic acid library of any one of claims 1-24.
56. A cell library comprising the nucleic acid library of any one of claims 1-24.
57. A cell library comprising the protein library of any one of 51-54.