VARIANT NUCLEIC ACID LIBRARIES FOR ADENOSINE RECEPTORS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/945,818 filed on December 9, 2019, which is incorporated by reference in its entirety.

BACKGROUND

[0002] G protein-coupled receptors (GPCRs) such as adenosine receptors are implicated in a wide variety of diseases. Raising antibodies to GPCRs has been difficult due to problems in obtaining suitable antigen because GPCRs are often expressed at low levels in cells and are very unstable when purified. Thus, there is a need for improved agents for therapeutic intervention which target GPCRs.

INCORPORATION BY REFERENCE

[0003] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF SUMMARY

[0003a] In one aspect, provided herein is 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.

[0003b] In another aspect, provided herein is 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.

[0003c] In another aspect, provided herein is 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.

[0003d] In another aspect, provided herein is use of the antibody or antibody fragment as described herein in the manufacture of a medicament for treating cancer.

[0003e] In another aspect, provided herein is use of the antibody or antibody fragment as described herein in the manufacture of a medicament for treating a neurological disease or disorder.

[0003f] In another aspect, provided herein is the antibody or antibody fragment as described herein for use in therapy.

[0004] Provided herein are methods 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 a 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 a 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 a 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 encoding for the adenosine A2A receptor antibody or antibody fragment thereof. Further provided herein are methods for generating a nucleic acid library encoding for an adenosine A2A receptor antibody or antibody fragment thereof, wherein the adenosine A2A receptor antibody or antibody fragment thereof is a single domain antibody. Further provided herein are methods for generating a nucleic acid library encoding for an adenosine A2A receptor antibody or antibody fragment thereof, wherein the single domain antibody comprises one heavy chain variable domain. Further provided herein are methods

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 10^5 non-identical nucleic acids.

4. 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.

5. 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.

6. 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.

7. 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.

8. The nucleic acid library of claim 1, wherein the variant immunoglobulin heavy chain when translated comprises any one of SEQ ID NO: 540-628.

9. The nucleic acid library of claim 1, wherein the variant immunoglobulin light chain when translated comprises any one of SEQ ID NO: 629-717.

10. 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.

11. The nucleic acid library of claim 10, wherein a length of the VH is about 90 to about 100 amino acids.

12. The nucleic acid library of claim 10, wherein a length of the VH is about 100 to about 400 amino acids.

13. The nucleic acid library of claim 10, wherein the library comprises at least 10^5 non-identical nucleic acids.

14. 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.

15. The antibody or antibody fragment of claim 14, 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.

16. The antibody or antibody fragment of claim 14, wherein the antibody or antibody fragment thereof is chimeric or humanized.

17. The antibody or antibody fragment of claim 14, 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.

18. The antibody or antibody fragment of claim 14, 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.

19. Use of the antibody or antibody fragment of claim 14 in the manufacture of a medicament for treating cancer.

20. Use of the antibody or antibody fragment of claim 14 in the manufacture of a medicament for treating a neurological disease or disorder.

21. The antibody or antibody fragment of claim 14 for use in therapy.