VARIANT NUCLEIC ACID LIBRARIES FOR SINGLE DOMAIN ANTIBODIES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/904,620 filed on September 23, 2019; U.S. Provisional Patent Application No. 62/935,603 filed on November 14, 2019; and U.S. Provisional Patent Application No. 62/945,761 filed on December 9, 2019, each of which is incorporated by reference in its entirety.

BACKGROUND

[0002] Antibodies possess the capability to bind with high specificity and affinity to biological targets. However, the design of therapeutic antibodies is challenging due to balancing of immunological effects with efficacy. Single domain antibodies such as VHH antibodies have several beneficial characteristics. Thus, there is a need to develop compositions and methods for generation of antibodies such as VHH antibodies for use in therapeutics.

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

[0004] In one aspect, provided herein is an antibody or antibody fragment comprising a CDRH1 comprising an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 152 or 155, a CDRH2 comprising an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 153 or 156, and a CDRH3 comprising an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 154 or 157. Further provided herein are antibodies or antibody fragments, further comprising a CDRL1 comprising an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 158 or 161, a CDRL2 comprising an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 159 or 162, and a CDRL3 comprising an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 160 or 163.

[0005] Provided herein are methods of treating cancer comprising administering the antibody or antibody fragment described herein.

[0005a] In one aspect, provided herein is a use of the antibody or antibody fragment as described herein in the manufacture of a medicament for treating cancer.

[0006] Provided herein are methods of treating a viral infection comprising administering the antibody or antibody fragment described herein.

[0006a] In one aspect, provided herein is a use of the antibody or antibody fragment as described herein in the manufacture of a medicament for treating a viral infection.

[0007] In one aspect, provided herein is a nucleic acid library comprising: a plurality of sequences comprising nucleic acids that when translated encode for an antibody or antibody fragment, 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) or a CDR1, CDR2, or CDR3 on a variable region of a light chain (VL); wherein the library comprises at least 30,000 variant sequences; and wherein the antibody or antibody fragments bind to its antigen with a K_D of less than 100 nM. Further provided herein are nucleic acid libraries, wherein the antibody is a single domain antibody. Further provided herein are nucleic acid libraries, wherein the single domain antibody is a VHH antibody. Further provided herein are nucleic acid libraries, wherein the antibody binds to TIGIT. Further provided herein are nucleic acid libraries, wherein the variable region of the heavy chain when translated comprises an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 84-100. Further provided herein are nucleic acid libraries, wherein the variable region of the light chain when translated comprises an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 101-117. Further provided herein are nucleic acid libraries, wherein the CDR1, CDR2, or CDR3 on the variable region of the heavy chain comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 67-83 or 118-128. Further provided herein are nucleic acid libraries, wherein the CDR1, CDR2, or CDR3 on the variable region of the light chain comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 129-137. Further provided herein are nucleic acid libraries, wherein the antibody binds to CD47. Further provided herein are nucleic acid libraries, wherein the antibody binds to CD3 epsilon. Further provided herein are nucleic acid libraries, wherein the variable region of the heavy chain when translated comprises an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 138-141. Further provided herein are nucleic acid libraries, wherein the variable region of the light chain when translated comprises an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 142-145. Further provided herein are nucleic acid libraries, wherein the nucleic acid library comprises at least 50,000 variant sequences. Further provided herein are nucleic acid libraries, wherein the nucleic acid library comprises at least 100,000 variant sequences. Further provided herein are nucleic acid libraries, wherein the nucleic acid library comprises at least 10^5 non-identical nucleic acids. Further provided herein are nucleic acid libraries, wherein the nucleic acid library has a theoretical diversity of at least 10^9 sequences.

[0008] In one aspect, provided herein is a nucleic acid library comprising: a plurality of sequences comprising nucleic acids that when translated encode for a single domain antibody, wherein each sequence of the plurality of sequences comprises a variant sequence encoding for 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 antibody or antibody fragments bind to its antigen with a K_D of less than 100 nM. Further provided herein are nucleic acid libraries, wherein a length of the VH when translated is about 90 to about 100 amino acids. Further provided herein are nucleic acid libraries, wherein a length of the VH when translated is about 100 to about 400 amino acids. Further provided herein are nucleic acid libraries, wherein a length of the VH is about 270 to about 300 base pairs. Further provided herein are nucleic acid libraries, wherein a length of the VH is about 300 to about 1200 base pairs. Further provided herein are nucleic acid libraries, wherein the single domain antibody is a VHH antibody. Further provided herein are nucleic acid libraries , wherein the antibody binds to TIGIT. Further provided herein are nucleic acid libraries, wherein the CDR1, CDR2, or CDR3 on the variable region of the heavy chain comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 67-83 or 118-128. Further provided herein are nucleic acid libraries, wherein the variable region of the heavy chain when translated comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 84-100. Further provided herein are nucleic acid libraries, wherein the CDR3 on the variable region of the heavy chain comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 101-117. Further provided herein are nucleic acid libraries, wherein the antibody binds to CD47. Further provided herein are nucleic acid libraries, wherein the antibody binds to CD3 epsilon. Further provided herein are nucleic acid libraries, wherein the variable region of the heavy chain when translated comprises an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 138-141. Further provided herein are nucleic acid libraries, wherein the nucleic acid library comprises at least 50,000 variant sequences. Further provided herein are nucleic acid libraries, wherein the nucleic acid library comprises at least 100,000 variant sequences. Further provided herein are nucleic acid libraries, wherein the nucleic acid library comprises at least 10^5 non-identical nucleic acids. Further provided herein are nucleic acid libraries, wherein the nucleic acid library has a theoretical diversity of at least 10^9 sequences.

[0009] In one aspect, provided herein is a method for generating a nucleic acid library encoding for a single domain antibody 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 sequences 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 sequences 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 sequences 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 single domain antibody, and wherein at least about 70% of the variant nucleic acids encode for a single domain antibody that binds to its antigen with a K_D of less than 100 nM. Further provided herein are methods for generating a nucleic acid library, wherein the single domain antibody comprises one heavy chain variable domain. Further provided herein are methods for generating a nucleic acid library, wherein the single domain antibody is a VHH antibody. Further provided herein are methods for generating a nucleic acid library, wherein the single domain antibody binds to TIGIT. Further provided herein are methods for generating a nucleic acid library, wherein the single domain antibody comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 84-100 or 138-141. Further provided herein are methods for generating a nucleic acid library, wherein the single domain antibody binds to CD47. Further provided herein are methods for generating a nucleic acid library, wherein the nucleic acid library comprises at least 50,000 variant sequences. Further provided herein are methods for generating a nucleic acid library, wherein the nucleic acid library comprises at least 100,000 variant sequences. Further provided herein are methods for generating a nucleic acid library, wherein the nucleic acid library comprises at least 10^5 non-identical nucleic acids. Further provided herein are methods for generating a nucleic acid library, wherein the nucleic acid library comprises at least one sequence encoding for the single domain antibody that binds to an antigen with a K_D of less than 75 nM. Further provided herein are methods for generating a nucleic acid library, wherein the nucleic acid library comprises at least one sequence encoding for the single domain antibody that binds to an antigen with a K_D of less than 50 nM. Further provided herein are methods for generating a nucleic acid library, wherein the nucleic acid library comprises at least one sequence encoding for the single domain antibody that binds to an antigen with a K_D of less than 25 nM. Further provided herein are methods for generating a nucleic acid library, wherein the nucleic acid library comprises at least one sequence encoding for the single domain antibody that binds to an antigen with a K_D of less than 10 nM. Further provided herein are methods for generating a nucleic acid library, wherein the nucleic acid library has a theoretical diversity of at least 10^9 sequences.

[0009a] In one aspect, provided herein is the antibody or antibody fragment as described herein for use in therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] **Figure 1** presents a diagram of steps demonstrating an exemplary process workflow for gene synthesis as disclosed herein.

[0011] **Figure 2** illustrates an example of a computer system.

CLAIMS

WHAT IS CLAIMED IS:

1. An antibody or antibody fragment comprising a CDRH1 comprising an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 152 or 155, a CDRH2 comprising an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 153 or 156, and a CDRH3 comprising an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 154 or 157.

2. The antibody or antibody fragment of claim 1, further comprising a CDRL1 comprising an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 158 or 161, a CDRL2 comprising an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 159 or 162, and a CDRL3 comprising an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 160 or 163.

3. Use of the antibody or antibody fragment of any one of claims 1-2 in the manufacture of a medicament for treating cancer.

4. Use of the antibody or antibody fragment of any one of claims 1-2 in the manufacture of a medicament for treating a viral infection.

5. A nucleic acid library comprising: a plurality of sequences comprising nucleic acids that when translated encode for an antibody or antibody fragment, 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) or a CDR1, CDR2, or CDR3 on a variable region of a light chain (VL); wherein the library comprises at least 30,000 variant sequences; and wherein the antibody or antibody fragments bind to its antigen with a K_D of less than 100 nM.

6. The nucleic acid library of claim 5, wherein the antibody is a single domain antibody.

7. The nucleic acid library of claim 6, wherein the single domain antibody is a VHH antibody.

8. The nucleic acid library of claim 5, wherein the antibody binds to TIGIT.

9. The nucleic acid library of claim 5, wherein the variable region of the heavy chain when translated comprises an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 84-100.

10. The nucleic acid library of claim 5, wherein the variable region of the light chain when translated comprises an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 101-117.

11. The nucleic acid library of claim 5, wherein the CDR1, CDR2, or CDR3 on the variable region of the heavy chain comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 67-83 or 118-128.

12. The nucleic acid library of claim 5, wherein the CDR1, CDR2, or CDR3 on the variable region of the light chain comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 129-137.

13. The nucleic acid library of claim 5, wherein the antibody binds to CD47.

14. The nucleic acid library of claim 5, wherein the antibody binds to CD3 epsilon.

15. The nucleic acid library of claim 5, wherein the variable region of the heavy chain when translated comprises an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 138-141.

16. The nucleic acid library of claim 5, wherein the variable region of the light chain when translated comprises an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 142-145.

17. The nucleic acid library of claim 5, wherein the nucleic acid library comprises at least 50,000 variant sequences.

18. The nucleic acid library of claim 5, wherein the nucleic acid library comprises at least 100,000 variant sequences.

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

20. The nucleic acid library of claim 5, wherein the nucleic acid library has a theoretical diversity of at least 10^9 sequences.

21. A nucleic acid library comprising: a plurality of sequences comprising nucleic acids that when translated encode for a single domain antibody, wherein each sequence of the plurality of sequences comprises a variant sequence encoding for 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 antibody or antibody fragments bind to its antigen with a K_D of less than 100 nM.

22. The nucleic acid library of claim 21, wherein a length of the VH when translated is about 90 to about 100 amino acids.

23. The nucleic acid library of claim 21, wherein a length of the VH when translated is about 100 to about 400 amino acids.

24. The nucleic acid library of claim 21, wherein a length of the VH is about 270 to about 300 base pairs.

25. The nucleic acid library of claim 21, wherein a length of the VH is about 300 to about 1200 base pairs.

26. The nucleic acid library of claim 21, wherein the single domain antibody is a VHH antibody.

27. The nucleic acid library of claim 21, wherein the antibody binds to TIGIT.

28. The nucleic acid library of claim 21, wherein the CDR1, CDR2, or CDR3 comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 67-83 or 118-128.

29. The nucleic acid library of claim 21, wherein the variable region of the heavy chain when translated comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 84-100.

30. The nucleic acid library of claim 21, wherein the CDR3 on the variable region of the heavy chain comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 101-117.

31. The nucleic acid library of claim 21, wherein the antibody binds to CD47.

32. The nucleic acid library of claim 21, wherein the antibody binds to CD3 epsilon.

33. The nucleic acid library of claim 21, wherein the variable region of the heavy chain when translated comprises an amino acid sequence at least about 90% identical to that set forth in SEQ ID NOs: 138-141.

34. The nucleic acid library of claim 21, wherein the nucleic acid library comprises at least 50,000 variant sequences.

35. The nucleic acid library of claim 21, wherein the nucleic acid library comprises at least 100,000 variant sequences.

36. The nucleic acid library of claim 21, wherein the nucleic acid library comprises at least 10^5 non-identical nucleic acids.

37. The nucleic acid library of claim 21, wherein the nucleic acid library has a theoretical diversity of at least 10^9 sequences.

38. A method for generating a nucleic acid library encoding for a single domain antibody 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 sequences 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 sequences 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 sequences 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 single domain antibody, and wherein at least about 70% of the variant nucleic acids encode for a single domain antibody that binds to its antigen with a K_D of less than 100 nM.

39. The method of claim 38, wherein the single domain antibody comprises one heavy chain variable domain.

40. The method of claim 38, wherein the single domain antibody is a VHH antibody.

41. The method of claim 38, wherein the single domain antibody binds to TIGIT.

42. The method of claim 38, wherein the single domain antibody comprises an amino acid sequence at least about 90% identical to that set forth in any one of SEQ ID NOs: 84-100 or 138-141.

43. The method of claim 38, wherein the single domain antibody binds to CD47.

44. The method of claim 38, wherein the nucleic acid library comprises at least 50,000 variant sequences.

45. The method of claim 38, wherein the nucleic acid library comprises at least 100,000 variant sequences.

46. The method of claim 38, wherein the nucleic acid library comprises at least 10^5 non-identical nucleic acids.

47. The method of claim 38, wherein the nucleic acid library comprises at least one sequence encoding for the single domain antibody that binds to an antigen with a K_D of less than 75 nM.

48. The method of claim 38, wherein the nucleic acid library comprises at least one sequence encoding for the single domain antibody that binds to an antigen with a K_D of less than 50 nM.

49. The method of claim 38, wherein the nucleic acid library comprises at least one sequence encoding for the single domain antibody that binds to an antigen with a K_D of less than 25 nM.

50. The method of claim 38, wherein the nucleic acid library comprises at least one sequence encoding for the single domain antibody that binds to an antigen with a K_D of less than 10 nM.

51. The method of claim 38, wherein the nucleic acid library has a theoretical diversity of at least 10^9 sequences.

52. The antibody or antibody fragment of any one of claims 1-2 for use in therapy.