CLAIMS

1. A bispecific antibody targeting programmed death receptor-1 (PD-1) and vascular endothelial growth factor (VEGF), comprising:

(a) a first binding functional region targeting PD-1; and

(b) a second binding functional region targeting VEGF;

wherein,

the first binding functional region targeting PD-1 is an anti-PD-1 antibody or an antigen-binding fragment thereof; and

the second binding functional region targeting VEGF comprises a variable domain of heavy chain of heavy-chain antibody (VHH) domain of complementarity-determining regions (CDRs) 1-3, wherein CDR1 comprises an amino acid sequence set forth in SEQ ID NO: 1, CDR2 comprises an amino acid sequence set forth in SEQ ID NO: 2 with or without one or two amino acid mutations, and CDR3 comprises an amino acid sequence set forth in SEQ ID NO: 3.

2. The bispecific antibody according to claim 1, wherein the amino acid mutation in CDR2 is at position 58 and/or position 65.

3. The bispecific antibody according to claim 2, wherein the amino acid mutation in CDR2 is N58Y and/or D65G mutation.

4. The bispecific antibody according to claim 1, wherein the VHH domain is a humanized VHH domain.

5. The bispecific antibody according to claim 1 or 3, wherein the second binding functional region targeting VEGF further comprises:

1) a first framework region domain FR1 comprising an amino acid sequence set forth in SEQ ID NO: 4 with or without one or two amino acid mutations; and/or

2) a second framework region domain FR2 comprising an amino acid sequence set forth in SEQ ID NO: 5 with or without one amino acid mutation; and/or

3) a third framework region domain FR3 comprising an amino acid sequence set forth in SEQ ID NO: 6 with or without 1-5 amino acid mutations; and/or

4) a fourth framework region domain FR4 comprising an amino acid sequence set forth in SEQ ID NO: 7 with or without one amino acid mutation;

preferably, the one or two amino acid mutations in FR1 are at position 1 and/or position 5; preferably Q1E and/or Q5L mutations.

preferably, the one amino acid mutation in FR2 is at position 49; preferably A49S mutation;

preferably, the 1-5 amino acid mutations in FR3 are at positions selected from the group consisting of positions 74, 82B, 83, 84, 89, and a combination thereof; preferably, the 1-5 amino acid mutations are selected from the group consisting of D74S, V(82B)S, K83R, P84A, M89V and a combination thereof;

preferably, the one amino acid mutation in FR4 is at position 108; preferably Q108L.

6. The bispecific antibody according to claim 5, wherein the second binding functional region targeting VEGF further comprises:

1) a first framework region domain FR1 comprising an amino acid sequence set forth in SEQ ID NO: 4 or SEQ ID NO: 9; and/or

2) a second framework region domain FR2 comprising an amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10; and/or

3) a third framework region domain FR3 comprising an amino acid sequence set forth in SEQ ID NO: 6 or SEQ ID NO: 11; and/or

4) a fourth framework region domain FR4 comprising an amino acid sequence set forth in

SEQ ID NO: 7 or SEQ ID NO: 12;

preferably, the second binding functional region targeting VEGF comprises:

1) a framework region combination of FR1 comprising an amino acid sequence set forth in SEQ ID NO: 4, FR2 comprising an amino acid sequence set forth in SEQ ID NO: 5, FR3 comprising an amino acid sequence set forth in SEQ ID NO: 6, and FR4 comprising an amino acid sequence set forth in SEQ ID NO: 7; or

2) a framework region combination of FR1 comprising an amino acid sequence set forth in SEQ ID NO: 9, FR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, FR3 comprising an amino acid sequence set forth in SEQ ID NO: 11, and FR4 comprising an amino acid sequence set forth in SEQ ID NO: 12; or

3) a framework region combination of FR1 comprising an amino acid sequence set forth in SEQ ID NO: 9, FR2 comprising an amino acid sequence set forth in SEQ ID NO: 5, FR3 comprising an amino acid sequence set forth in SEQ ID NO: 11, and FR4 comprising an amino acid sequence set forth in SEQ ID NO: 12.

7. The bispecific antibody according to claim 1, wherein the second binding functional region targeting VEGF comprises an amino acid sequence set forth in SEQ ID NO: 8 with or without 1-11 amino acid mutations;

preferably,

the amino acid mutation is at a position selected from the group consisting of positions 1, 5, 49, 58, 65, 74, 82B, 83, 84, 89, 108, and a combination thereof.

preferably, the amino acid mutation is selected from the group consisting of Q1E, Q5L, A49S, N58Y, D65G, D74S, V(82B)S, K83R, P84A, M89V, Q108L and a combination thereof;

preferably, the amino acid mutation is a mutation combination of

1) Q1E+Q5L+A49S+N58Y+D65G+D74S+V(82B)S+K83R+P84A+M89V+Q108L; or

2) Q1E+Q5L+N58Y+D65G+D74S+V(82B)S+K83R+P84A+M89V+Q108L.

8. The bispecific antibody according to claim 1, wherein the second binding functional region targeting VEGF comprises an amino acid sequence set forth in SEQ ID NO: 8, SEQ ID NO: 13 or SEQ ID NO: 14.

9. The bispecific antibody according to any one of claims 1 to 8, wherein the anti-PD-1 antibody or the antigen-binding fragment thereof is selected from the group consisting of a humanized antibody or an antigen-binding fragment thereof, a chimeric antibody or an antigen-binding fragment thereof, and a human antibody or an antigen-binding fragment thereof;

preferably, the anti-PD-1 antibody or the antigen-binding fragment thereof is an IgG antibody;

preferably, the anti-PD-1 antibody or the antigen-binding fragment thereof is IgG1, IgG2, or IgG4;

preferably, the first binding functional region targeting PD-1 comprises an amino acid sequence of light chain and heavy chain CDRs identical to that of nivolumab antibody or pembrolizumab antibody.

preferably, the first binding functional region targeting PD-1 comprises an amino acid sequence of heavy chain variable region identical to nivolumab antibody or pembrolizumab antibody;

preferably, the first binding functional region targeting PD-1 comprises an amino acid sequence of light chain variable region identical to nivolumab antibody or pembrolizumab antibody;

preferably, the first binding functional region targeting PD-1 comprises a heavy chain moiety, wherein the heavy chain moiety further comprises a domain selected from the group consisting of a heavy chain constant region CH1, a hinge region, a heavy chain constant region CH2, a heavy chain constant region CH3, and a combination thereof;

preferably, the first binding functional region targeting PD-1 further comprises a light chain

constant region;

preferably, the anti-PD-1 antibody comprises a heavy chain moiety and a light chain moiety, wherein the heavy chain moiety comprises, from N-terminus to C-terminus, a heavy chain variable region, a heavy chain constant region CH1, a hinge region, a heavy chain constant region CH2, and a heavy chain constant region CH3, and the light chain moiety comprises, from N-terminus to C-terminus, a light chain variable region and a light chain constant region;

preferably, the anti-PD-1 antibody comprises an Fc domain, wherein the Fc domain is an IgG1 Fc domain or a silent IgG1 mutation thereof.

preferably, the Fc domain comprises an amino acid sequence set forth in SEQ ID NO: 15 or at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence set forth in SEQ ID NO: 15;

preferably, the first binding functional region targeting PD-1 comprises a light chain and heavy chain combination of

1) a heavy chain comprising an amino acid sequence set forth in SEQ ID NO: 16, and/or a light chain comprising an amino acid sequence set forth in SEQ ID NO: 17; or

2) a heavy chain comprising an amino acid sequence set forth in SEQ ID NO: 18, and/or a light chain comprising an amino acid sequence set forth in SEQ ID NO: 19;

preferably, the second binding functional region is linked to the C-terminus or N-terminus of the first binding functional region;

preferably, the bispecific antibody is a bivalent, trivalent or tetravalent bispecific antibody; or

preferably, the VHH domain is directly or via a peptide linker linked to the C-terminus or N-terminus of the heavy chain moiety of the antibody or the antigen-binding fragment thereof;

preferably, the peptide linker is a flexible peptide linker;

preferably, the peptide linker comprises one or more amino acids;

preferably, the peptide linker comprises at least 5 amino acids;

preferably, the peptide linker comprises an amino acid sequence of $(GGGGS)_n$, wherein n is 1, 2, 3 or 4.

10. The bispecific antibody according to claim 1, wherein the bispecific antibody comprises a combination of a heavy chain set forth in SEQ ID NO: 20 and a light chain set forth in SEQ ID NO: 21, or comprises a combination of a heavy chain set forth in SEQ ID NO: 22 and a light chain set forth in SEQ ID NO: 23.

11. A polynucleotide encoding the bispecific antibody according to any one of claims 1 to 10.

12. An expression vector comprising the polynucleotide according to claim 11.

13. A host cell comprising the expression vector according to claim 12 or integrated with the polynucleotide according to claim 11 into the genome.

14. A pharmaceutical composition comprising the bispecific antibody according to any one of claims 1 to 10, and a pharmaceutically acceptable carrier.

15. Use of the bispecific antibody according to any one of claims 1 to 10 in the manufacture of a medicament for treating cancer;

preferably, the cancer is colorectal cancer.