

suppress tumor progression through mechanisms such as angiogenesis inhibition.

Therefore, developing a treatment or combination therapy with higher efficacy is of great meaning.

SUMMARY

Through inventive efforts, the inventor conducted in-depth studies on combination therapies of anti-PD-1/anti-VEGFA antibodies and PARP inhibitors in the treatment of tumors, and found that the combined use of the two has good efficacy in treating and/or preventing tumors, particularly malignant tumors. The present ~~invention~~disclosure is detailed below.

One ~~aspect~~example of the present ~~invention~~disclosure relates to a therapeutic combination comprising at least one bispecific antibody and at least one PARP inhibitor,

wherein, the bispecific antibody comprises:

a first protein functional region targeting PD-1, and

a second protein functional region targeting VEGFA;

wherein the first protein functional region is an immunoglobulin, and the second protein functional region is a single chain antibody; or, the first protein functional region is a single chain antibody, and the second protein functional region is an immunoglobulin;

wherein,

the immunoglobulin comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 28-30, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 31-33, respectively; the single chain antibody comprises a heavy chain

variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 34-36, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 37-39, respectively;

or,

the immunoglobulin comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 34-36, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 37-39, respectively; the single chain antibody comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 28-30, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 31-33, respectively;

the immunoglobulin is of human IgG1 subtype;

wherein, according to the EU numbering system, the immunoglobulin comprises a heavy chain constant region having mutations at any 2 or 3 of positions 234, 235 and 237, and the affinity constant of the bispecific antibody for FcγRIIIa and/or C1q is reduced after the mutation as compared to that before the mutation; preferably, the affinity constant is measured by a Fortebio Octet system.

In one aspect, the present disclosure refer to a therapeutic combination comprising at least one bispecific antibody and at least one PARP inhibitor, wherein, the bispecific antibody comprises: a first protein functional region targeting PD-1, and a second protein functional region targeting VEGFA; wherein: the first protein functional region is an immunoglobulin, and the second protein functional region is a single-chain antibody; wherein the immunoglobulin comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 34-36, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 37-39, respectively; the single chain antibody comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 28-30, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 31-33, respectively; or, the first protein functional region is a single-chain antibody, and the second protein functional region is an immunoglobulin; wherein the immunoglobulin comprises a heavy chain

variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 28-30, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 31-33, respectively; the single chain antibody comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 34-36, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 37-39, respectively; the immunoglobulin is of human IgG1 subtype; according to the EU numbering system, the immunoglobulin comprises a heavy chain constant region having mutations at any 2 or 3 of positions 234, 235 and 237, and the affinity constant of the bispecific antibody for FcγRIIIa and/or C1q is reduced after the mutation as compared to that before the mutation; preferably, the affinity constant is measured by a Fortebio Octet system.

In one or more ~~embodiment~~examples of the present ~~invention~~disclosure, for the therapeutic combination, according to the EU numbering system, the heavy chain constant region of the immunoglobulin has the following mutations:

L234A and L235A; or

L234A and G237A; or

L235A and G237A;

or

L234A, L235A and G237A.

In the present ~~invention~~disclosure, letters before the position number represent amino acids before mutation, and letters after the position number represent amino acids after mutation, unless otherwise specified.

The present ~~invention~~disclosure further relates to a therapeutic combination comprising at least one bispecific antibody and at least one PARP inhibitor,

wherein, the bispecific antibody comprises:

a first protein functional region targeting PD-1, and

a second protein functional region targeting VEGFA;

wherein the first protein functional region is an immunoglobulin, and the second protein functional region is a single chain antibody; or, the first protein functional region is a single chain antibody, and the second protein functional region is an immunoglobulin;

wherein,

the immunoglobulin comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 28-30, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 31-33, respectively; the single chain antibody comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 34-36, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 37-39, respectively;

or,

the immunoglobulin comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs:

34-36, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 37-39, respectively; the single chain antibody comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 28-30, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 31-33, respectively; the immunoglobulin is of human IgG1 subtype.

The present ~~invention~~disclosure further relates to a therapeutic combination comprising at least one bispecific antibody and at least one PARP inhibitor,

wherein, the bispecific antibody comprises:

a first protein functional region targeting PD-1, and

a second protein functional region targeting VEGFA;

wherein the first protein functional region is an immunoglobulin, and the second protein functional region is a single chain antibody; or, the first protein functional region is a single chain antibody, and the second protein functional region is an immunoglobulin;

wherein,

the immunoglobulin comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 28-30, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 31-33, respectively; the single chain antibody comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 34-36, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences

set forth in SEQ ID NOs: 37-39, respectively;

or,

the immunoglobulin comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 34-36, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 37-39, respectively; the single chain antibody comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 28-30, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 31-33, respectively;

the immunoglobulin is of human IgG1 subtype;

wherein, according to an EU numbering system, the heavy chain constant region of the immunoglobulin has the following mutations:

L234A and L235A; or

L234A and G237A; or

L235A and G237A; or

L234A, L235A and G237A.

In one aspect, the present disclosure refers to a therapeutic combination comprising at least one bispecific antibody and at least one PARP inhibitor, wherein, the bispecific antibody comprises: a first protein functional region targeting PD-1, and a second protein functional region targeting VEGFA;

wherein: the first protein functional region is an immunoglobulin, and the second protein functional region is a single-chain antibody; wherein the immunoglobulin comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 34-36, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 37-39, respectively; the single chain antibody comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 28-30, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 31-33, respectively; or, the first protein

functional region is a single-chain antibody, and the second protein functional region is an immunoglobulin; wherein the immunoglobulin comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 28-30, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 31-33, respectively; the single chain antibody comprises a heavy chain variable region comprising HCDR1-HCDR3 having amino acid sequences set forth in SEQ ID NOs: 34-36, respectively, and a light chain variable region comprising LCDR1-LCDR3 having amino acid sequences set forth in SEQ ID NOs: 37-39, respectively; the immunoglobulin is of human IgG1 subtype; according to an EU numbering system, the immunoglobulin comprises a heavy chain constant region having the following mutations: L234A and L235A; or L234A and G237A; or L235A and G237A; or L234A, L235A and G237A.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the therapeutic combination, according to the EU numbering system, the heavy chain constant region of the immunoglobulin has or further has one or more mutations selected from:

N297A, D265A, D270A, P238D, L328E, E233D, H268D, P271G, A330R, C226S, C229S, E233P, P331S, S267E, L328F, A330L, M252Y, S254T, T256E, N297Q, P238S, P238A, A327Q, A327G, P329A, K322A, T394D, G236R, G236A, L328R, A330S, P331S, H268A, E318A and K320A.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the therapeutic

combination,

the heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 3; the heavy chain variable region of the single chain antibody has an amino acid sequence selected from SEQ ID NO: 5 and SEQ ID NO: 9, and the light chain variable region of the single chain antibody has an amino acid sequence selected from SEQ ID NO: 7, SEQ ID NO: 11 and SEQ ID NO: 17;

or,

the heavy chain variable region of the immunoglobulin has an amino acid sequence selected from SEQ ID NO: 5 and SEQ ID NO: 9, and the light chain variable region of the immunoglobulin has an amino acid sequence selected from SEQ ID NO: 7, SEQ ID NO: 11 and SEQ ID NO: 17; the heavy chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 3.

In one or more **embodiment examples** of the present **invention disclosure**, the therapeutic combination is selected from any one of the following (1)-(12):

(1)

the heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 3; the heavy chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 5, and the light chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 7;

(2)

the heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 3; the heavy chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 5, and the light chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 11;

(3)

the heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 3; the heavy chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 5, and the light chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 17;

(4)

the heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 3; the heavy chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 9, and the light chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 7;

(5)

the heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID

NO: 3; the heavy chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 9, and the light chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 11;

(6)

the heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 3; the heavy chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 9, and the light chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 17;

(7)

the heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 5, and the light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 7; the heavy chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 3;

(8)

the heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 5, and the light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 11; the heavy chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 3;

(9)

the heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 5, and the light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 17; the heavy chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 3;

(10)

the heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 9, and the light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 7; the heavy chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 3;

(11)

the heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 9, and the light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 11; the heavy chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 3; and

(12)

the heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 9, and the light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID

NO: 17; the heavy chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region of the single chain antibody has an amino acid sequence set forth in SEQ ID NO: 3.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the therapeutic combination:

the immunoglobulin comprises a heavy chain having an amino acid sequence set forth in SEQ ID NO: 24, and a light chain having an amino acid sequence set forth in SEQ ID NO: 26.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the therapeutic combination, the bispecific antibody is in the form of IgG-scFv, i.e., the Morrison format.

In some **embodimentexamples** of the present **inventiondisclosure**, for the therapeutic combination,

the heavy chain constant region of the immunoglobulin is selected from a heavy chain constant region of human IgG1, IgG2, IgG3 or IgG4, and the immunoglobulin comprises a light chain constant region selected from a light chain constant region of human IgG1, IgG2, IgG3 or IgG4.

In some **embodimentexamples** of the present **inventiondisclosure**, for the therapeutic combination,

the heavy chain constant region of the immunoglobulin is human Ig gamma-1 chain C region or human Ig gamma-4 chain C region, and the light chain constant region of the immunoglobulin is human Ig kappa chain C region.

In some **embodimentexamples** of the present **inventiondisclosure**, the constant regions of the immunoglobulin are humanized. For example, the heavy chain constant region is Ig gamma-1 chain C region, **ACCESSION: P01857**, and the light chain constant region is Ig kappa chain C region,

ACCESSION: P01834; or

the heavy chain constant region of the immunoglobulin is Ig gamma-4 chain C region, ACCESSION: P01861.1, and the light chain constant region is Ig kappa chain C region, ACCESSION: P01834.

In one **embodiment example** of the present **invention disclosure**, the heavy chain constant region Ig gamma-1 chain C region (ACCESSION: P01857) has the following amino acid sequence:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT
KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS
RTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK (SEQ ID NO: 40)

In one **embodiment example** of the present **invention disclosure**, the heavy chain constant region Ig gamma-4 chain C region (ACCESSION: P01861.1) has the following amino acid sequence:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNT
KVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPE
VTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ
VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
QKSLSLSLGK (SEQ ID NO: 41)

In one **embodiment example** of the present **invention disclosure**, the light chain constant region Ig kappa chain C region (ACCESSION: P01834) has the following

amino acid sequence:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVT
HQGLSSPVTKSFNRGEC (SEQ ID NO: 42)

In some **embodimentexamples** of the present **inventiondisclosure**, for the therapeutic combination, the single chain antibody is linked to the C terminus of the heavy chain of the immunoglobulin. Since an immunoglobulin has two heavy chains, two single chain antibody molecules are linked to one immunoglobulin molecule. Preferably, the two single chain antibody molecules are identical.

In some **embodimentexamples** of the present **inventiondisclosure**, for the therapeutic combination, two single chain antibodies are present, and one terminus of each single chain antibody is linked to the C terminus or the N terminus of one of the two heavy chains of the immunoglobulin.

In some **embodimentexamples** of the present **inventiondisclosure**, a disulfide bond is present between the V_H and the V_L of the single chain antibody. Methods for introducing disulfide bonds between the V_H and V_L of an antibody are well known in the art, see, for example, US5,747,654; Rajagopal, et al., *Prot. Engin.*, 10(1997)1453-1459; Reiter et al., *Nat. Biotechnol.*, 14(1996)1239-1245; Reiter, et al., *Protein Engineering*, 8(1995)1323-1331; Webber, et al., *Molecular Immunology*, 32(1995)249-258; Reiter, et al., *Immunity*, 2(1995)281-287; Reiter, et al., *JBC*, 269(1994)18327-18331; Reiter, et al., *Inter. J. of Cancer*, 58(1994)142-149; and Reiter, et al., *Cancer Res.*, 54(1994)2714-2718, which are incorporated herein by reference.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the therapeutic combination, the first protein functional region is linked to the second protein functional region either directly or via a linker fragment; and/or

the heavy chain variable region of the single chain antibody is linked to the light chain variable region of the single chain antibody either directly or via a linker fragment.

In one or more **embodiment examples** of the present **invention disclosure**, for the therapeutic combination, the linker fragment is (GGGGS)_n; n is a positive integer, and preferably, n is 1, 2, 3, 4, 5 or 6.

In one or more **embodiment examples** of the present **invention disclosure**, for the therapeutic combination, the numbers of the first protein functional region and the second protein functional region are each independently 1, 2 or more.

In one or more **embodiment examples** of the present **invention disclosure**, for the therapeutic combination, the single chain antibody is linked to the C terminus of the heavy chain of the immunoglobulin.

In one aspect, ~~t~~The present **invention disclosure** further relates to a therapeutic combination comprising at least one bispecific antibody and at least one PARP inhibitor,

wherein, the bispecific antibody comprises:

a first protein functional region targeting PD-1, and

a second protein functional region targeting VEGFA;

the number of the first protein functional region is 1, and the number of the second protein functional region is 2;

wherein the first protein functional region is an immunoglobulin, and the second protein functional region is a single chain antibody;

the immunoglobulin comprises a heavy chain having an amino acid sequence set forth in SEQ ID NO: 24, and a light chain having an amino acid sequence set forth in SEQ ID NO: 26;

the single chain antibody comprises a heavy chain variable region having an amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region having an amino acid sequence set forth in SEQ ID NO:

17; or

the single chain antibody is linked to the C terminus of the heavy chain of the immunoglobulin;

the first protein functional region is linked to the second protein functional region via a first linker fragment; the heavy chain variable region of the single chain antibody is linked to the light chain variable region of the single chain antibody via a second linker fragment; the first linker fragment and the second linker fragment are identical or different;

preferably, amino acid sequences of the first linker fragment and the second linker fragment are independently selected from SEQ ID NO: 18 and SEQ ID NO: 19;

preferably, amino acid sequences of the first linker fragment and the second linker fragment are set forth in SEQ ID NO: 18.

In one or more **embodiment examples** of the present **invention disclosure**, for the therapeutic combination, the bispecific antibody or an antigen-binding fragment thereof binds to FcγRI with an affinity constant less than about 10^{-6} M, for example, less than about 10^{-7} M, 10^{-8} M, 10^{-9} M or less; preferably, the affinity constant is measured by a Fortebio Octet system.

In one or more **embodiment examples** of the present **invention disclosure**, for the therapeutic combination, the bispecific antibody or an antigen-binding fragment thereof binds to C1q with an affinity constant less than about 10^{-9} M, for example, less than about 10^{-7} M, 10^{-8} M, 10^{-9} M or less; preferably, the affinity constant is measured by a Fortebio Octet system.

In some **embodiment examples** of the present **invention disclosure**, for the therapeutic combination, the bispecific antibody or the antigen-binding fragment thereof binds to a VEGFA protein and/or a PD-1 protein with a K_D less than 10^{-5} M, such as less than 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M or 10^{-10} M or

less; preferably, the K_D is measured by a Fortebio molecular interaction instrument.

In some **embodimentexamples** of the present **inventiondisclosure**, for the therapeutic combination,

the bispecific antibody or the antigen-binding fragment thereof binds to the VEGFA protein with an EC_{50} less than 1 nM, less than 0.5 nM, less than 0.2 nM, less than 0.15 nM or less than 0.14 nM; preferably, the EC_{50} is measured by indirect ELISA;

and/or,

the bispecific antibody or the antigen-binding fragment thereof binds to the PD-1 protein with an EC_{50} less than 1 nM, less than 0.5 nM, less than 0.2 nM, less than 0.17 nM, less than 0.16 nM or less than 0.15 nM; preferably, the EC_{50} is measured by indirect ELISA.

In one or more **embodimentexamples** of the present **inventiondisclosure**, the bispecific antibody is a monoclonal antibody.

In one or more **embodimentexamples** of the present **inventiondisclosure**, the bispecific antibody is a humanized antibody.

In some **embodimentexamples** of the present **inventiondisclosure**, for the therapeutic combination, the PARP inhibitor is selected from one or more of olaparib, rucaparib, niraparib, talazoparib, fluzoparib, veliparib ER, ABT-472, ABT-767, stenoparib, AST-6828, AG-PD, ANG-2864, ANG-3038, ANG-3186, AZD-5305, AZ-0108, AZD-2461, AMXI-5001, AMXI-2001, AMXI-3001, AMXI-7001, AMXI-9001, pamiparib, ZYTP-1, CK-102, XZ-120312, YHP-743, iobenguane I 131, rucaparib camsylate, CVL-218, CPH-101, CPH-102, CBX-11, CBX-15, minocycline, DB-207, DPS-102, E-7016, iobenguane I 131, MK-2512, HCX-014, HWH-340, IDX-1197, IDX-1197, senaparib, IMP-04100, IMP-04111, IMP-04149, IMP-04249, IMP-04307, IMP-04356, JPI-289, JPI-547, JPI-283,

fluzoparib, GT-1620, iobenguane I 131, DR-2313, MP-124, H-10, NT-125, BGP-15, NMSP-293, NMSP-118, NMSP-648, NMSP-914, DB-207, NUV-1156, NUV-1176, JPI-289, Stenoparib, OX-401, NU-1025, NU-1085, PLX-376, R-554, RBN-2397, RBN-012759, PJ-34, INO-1001, WW-46, BSI-401, iniparib, SOMCL-9112, SC-10914, HTMC-0435, SRX-3128, TSL-1502, PJ-34, CEP-8983, CK-102, THG-009, talazoparib SR, L-2286, mitoparib and WB-1340.

In some **embodimentexamples** of the present **inventiondisclosure**, the therapeutic combination further comprises one or more anti-tumor chemotherapeutics;

preferably, the anti-tumor chemotherapeutic is selected from a topoisomerase II (TOP2) inhibitor, and preferably, the topoisomerase II (TOP2) inhibitor is etoposide;

preferably, the anti-tumor chemotherapeutic is selected from a taxane, and preferably, the taxane is selected from paclitaxel, albumin-bound paclitaxel, liposome paclitaxel and docetaxel;

preferably, the anti-tumor chemotherapeutic is selected from a platinum-based drug, preferably, the platinum-based drug is selected from cisplatin, carboplatin, and oxaliplatin;

preferably, the anti-tumor chemotherapeutic is selected from one or more of gemcitabine, pemetrexed and capecitabine.

In some **embodimentexamples** of the present **inventiondisclosure**, the therapeutic combination is a fixed combination, e.g., in the form of a solid pharmaceutical composition or a liquid pharmaceutical composition; or

the therapeutic combination is a non-fixed combination, e.g., the bispecific antibody, the PARP inhibitor and the anti-tumor chemotherapeutic are each in the form of a pharmaceutical composition.

In some **embodimentexamples** of the present **inventiondisclosure**, for the therapeutic combination, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier and/or excipient.

The pharmaceutical composition of the present **inventiondisclosure** may be formulated into any dosage form known in the pharmaceutical field, such as tablet, pill, suspension, emulsion, solution, gel, capsule, powder, granule, elixir, troche, suppository, injection (including injection solution, sterile powder for injection and concentrated solution for injection), inhalant and spray. The preferred dosage form depends on the intended route of administration and therapeutic use. The pharmaceutical composition of the present **inventiondisclosure** should be sterile and stable under the conditions of manufacture and storage. One preferred dosage form is an injection. Such injections may be sterile solutions for injection. For example, sterile solutions for injection can be prepared by the following method: a necessary amount of the bispecific antibody of the present **inventiondisclosure** is added in an appropriate solvent, and optionally, other desired ingredients (including, but not limited to, pH regulators, surfactants, adjuvants, ionic strength enhancers, isotonic agents, preservatives, diluents, or any combination thereof) are added at the same time, followed by filtration and sterilization. In addition, sterile solutions for injection can be prepared as sterile lyophilized powders (e.g., by vacuum drying or lyophilizing) for ease of storage and use. Such sterile lyophilized powders may be dispersed in a suitable carrier (e.g., sterile pyrogen-free water) prior to use.

In addition, the bispecific antibody or the PARP inhibitor of the present **inventiondisclosure** may be present in the pharmaceutical composition in unit dose form for ease of administration. In some **embodimentexamples**, the unit dose is at

least 1 mg, at least 5 mg, at least 10 mg, at least 15 mg, at least 20 mg, at least 25 mg, at least 30 mg, at least 45 mg, at least 50 mg, at least 75 mg or at least 100 mg. When the pharmaceutical composition is in a liquid (e.g., injection) dosage form, it may comprise the bispecific antibody of the present ~~invention~~disclosure at a concentration of at least 0.1 mg/mL, such as at least 0.25 mg/mL, at least 0.5 mg/mL, at least 1 mg/mL, at least 2.5 mg/mL, at least 5 mg/mL, at least 8 mg/mL, at least 10 mg/mL, at least 15 mg/mL, at least 25 mg/mL, at least 50 mg/mL, at least 75 mg/mL or at least 100 mg/mL.

The bispecific antibody or the pharmaceutical composition of the present ~~invention~~disclosure may be administered by any suitable method known in the art, including, but not limited to, oral, buccal, sublingual, ocular, topical, parenteral, rectal, intrathecal, intracisternal, inguinal, intravesical, topical (e.g., powder, ointment, or drop), or nasal route. However, for many therapeutic uses, the preferred route/mode of administration is parenteral (such as intravenous injection, subcutaneous injection, intraperitoneal injection and intramuscular injection). Those skilled in the art will appreciate that the route and/or mode of administration will vary depending on the intended purpose. In a preferred ~~embodiment~~example, the bispecific antibody or the pharmaceutical composition of the present ~~invention~~disclosure is administered by intravenous infusion or injection.

The bispecific antibody or the pharmaceutical composition provided herein can be used alone or in combination, or used in combination with additional pharmaceutically active agents (e.g., a tumor chemotherapeutic). Such an additional pharmaceutically active agent may be administered prior to, concurrently with, or subsequent to the administration of the bispecific antibody of the present ~~invention~~disclosure or the pharmaceutical composition of the present ~~invention~~disclosure.

In the present **inventiondisclosure**, the administration regimen may be adjusted to achieve the optimal desired response (e.g., a therapeutic or prophylactic response). For example, the regimen may be a single dose, or multiple doses over a period of time, or the dose may be reduced or increased proportionally according to the emergency of the treatment. The bispecific antibody of the present **inventiondisclosure** is a bispecific antibody according to any **embodimentexample** of the present **inventiondisclosure**, i.e., an anti-PD-1/anti-VEGFA bispecific antibody. Another **aspectexample** of the present **inventiondisclosure** relates to a kit product comprising the therapeutic combination according to any **embodimentexample** of the present **inventiondisclosure**, and a package insert.

In one aspect, the present disclosure refers to a kit product, comprising: the therapeutic combination disclosed herein, and a package insert.

Yet another **aspectexample** of the present **inventiondisclosure** relates to use of the therapeutic combination according to any **embodimentexample** of the present **inventiondisclosure** or the kit product of the present **inventiondisclosure** in preparing a medicament for treating and/or preventing a malignant tumor;

preferably, the malignant tumor is selected from ovarian cancer, endometrial cancer, breast cancer, cervical cancer, fallopian tube cancer, peritoneal cancer, pancreatic cancer, colon cancer, rectal cancer, lung cancer, liver cancer, skin cancer, glioma, melanoma, lymphoma, renal tumor, prostate cancer, bladder cancer, gastrointestinal cancer, brain cancer, oesophageal cancer such as oesophageal squamous cancer, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) cancer, urothelial carcinoma, mesothelioma, gastric adenocarcinoma, gastroesophageal junction adenocarcinoma, leukemia, myeloma, thyroid cancer, head and neck cancer, bone cancer, biliary tract cancer and testicular cancer;

preferably, the tumor is a tumor with deficient repair function mediated

by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the tumor is a tumor without deficient repair function mediated by homologous recombination;

preferably, the lung cancer is non-small cell lung cancer or small cell lung cancer;

preferably, the non-small cell lung cancer is a non-small cell lung cancer with EGFR and/or ALK-sensitive mutation;

preferably, the non-small cell lung cancer is a non-small cell lung cancer without EGFR and/or ALK-sensitive mutation;

preferably, the liver cancer is hepatocellular carcinoma;

preferably, the renal cancer is renal cell carcinoma;

preferably, the breast cancer is triple negative breast cancer;

preferably, the urothelial carcinoma is bladder cancer;

preferably, the peritoneal cancer is primary peritoneal cancer;

preferably, the ovarian cancer is an ovarian cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the fallopian tube cancer is a fallopian tube cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the peritoneal cancer is a peritoneal cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the breast cancer is a breast cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation).

In one aspect, the present disclosure refers to use of the therapeutic combination disclosed herein or the kit product disclosed herein in preparing a medicament for treating and/or preventing a malignant tumor, wherein, preferably, the malignant tumor is selected from ovarian cancer, endometrial cancer, breast cancer, cervical cancer, fallopian tube cancer, peritoneal cancer, pancreatic cancer, colon cancer, rectal cancer, lung cancer, liver cancer, skin cancer, glioma, melanoma, lymphoma, renal tumor, prostate cancer, bladder cancer, gastrointestinal cancer, brain cancer, oesophageal cancer such as oesophageal squamous cancer, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) cancer, urothelial carcinoma, mesothelioma, gastric adenocarcinoma, gastroesophageal junction adenocarcinoma, leukemia,

myeloma, thyroid cancer, head and neck cancer, bone cancer, biliary tract cancer and testicular cancer; preferably, the tumor is a tumor with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation); preferably, the tumor is a tumor without deficient repair function mediated by homologous recombination; preferably, the lung cancer is non-small cell lung cancer or small cell lung cancer; preferably, the non-small cell lung cancer is a non-small cell lung cancer with EGFR and/or ALK-sensitive mutation; preferably, the non-small cell lung cancer is a non-small cell lung cancer without EGFR and/or ALK-sensitive mutation; preferably, the liver cancer is hepatocellular carcinoma; preferably, the renal cancer is renal cell carcinoma; preferably, the breast cancer is triple negative breast cancer; preferably, the urothelial carcinoma is bladder cancer; preferably, the peritoneal cancer is primary peritoneal cancer; preferably, the ovarian cancer is an ovarian cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation); preferably, the fallopian tube cancer is a fallopian tube cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation); preferably, the peritoneal cancer is a peritoneal cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation); preferably, the breast cancer is a breast cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation).

Yet another ~~aspect~~example of the present ~~invention~~disclosure relates to use of the

therapeutic combination according to any ~~embodiment~~example of the present ~~invention~~disclosure or the kit product of the present ~~invention~~disclosure in preparing the following medicament:

(1)

a medicament or an agent for detecting the level of VEGFA in a sample,
a medicament or an agent for blocking the binding of VEGFA to VEGFR2,

a medicament or an agent for down-regulating the activity or level of VEGFA,

a medicament or an agent for relieving the stimulation of VEGFA on vascular endothelial cell proliferation,

a medicament or an agent for inhibiting vascular endothelial cell proliferation, or

a medicament or an agent for blocking tumor angiogenesis;

and/or

(2)

a medicament or an agent for blocking the binding of PD-1 to PD-L1,

a medicament or an agent for down-regulating the activity or level of PD-1,

a medicament or an agent for relieving the immunosuppression of PD-1 in an organism,

a medicament or an agent for promoting IFN- γ secretion in T lymphocytes, or

a medicament or an agent for promoting IL-2 secretion in T lymphocytes.

In the *in vitro* experiment of the present ~~invention~~disclosure, the anti-VEGFA antibody and the anti-VEGFA/anti-PD-1 bispecific antibody both can inhibit HUVEC cell proliferation, and both the anti-PD-1 antibody and

the anti-VEGFA/anti-PD-1 bispecific antibody can promote the secretion of IFN- γ and/or IL-2 and activate an immune response.

Yet another **aspectexample** of the present **inventiondisclosure** relates to a method for treating and/or preventing a malignant tumor, comprising: administering to a subject in need an effective amount of the therapeutic combination according to any **embodimentexample** of the present **inventiondisclosure** or the kit product of the present **inventiondisclosure**;
preferably, the malignant tumor is selected from ovarian cancer, endometrial cancer, breast cancer, cervical cancer, fallopian tube cancer, peritoneal cancer, pancreatic cancer, colon cancer, rectal cancer, lung cancer, liver cancer, skin cancer, glioma, melanoma, lymphoma, renal tumor, prostate cancer, bladder cancer, gastrointestinal cancer, brain cancer, oesophageal cancer such as oesophageal squamous cancer, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) cancer, urothelial carcinoma, mesothelioma, gastric adenocarcinoma, gastroesophageal junction adenocarcinoma, leukemia, myeloma, thyroid cancer, head and neck cancer, bone cancer, biliary tract cancer and testicular cancer;
preferably, the tumor is a tumor with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);
preferably, the tumor is a tumor without deficient repair function mediated by homologous recombination;
preferably, the lung cancer is non-small cell lung cancer or small cell lung cancer;
preferably, the non-small cell lung cancer is a non-small cell lung cancer with EGFR and/or ALK-sensitive mutation;

preferably, the non-small cell lung cancer is a non-small cell lung cancer without EGFR and/or ALK-sensitive mutation;

preferably, the liver cancer is hepatocellular carcinoma;

preferably, the renal cancer is renal cell carcinoma;

preferably, the breast cancer is triple negative breast cancer;

preferably, the urothelial carcinoma is bladder cancer;

preferably, the peritoneal cancer is primary peritoneal cancer;

preferably, the ovarian cancer is an ovarian cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the fallopian tube cancer is a fallopian tube cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the peritoneal cancer is a peritoneal cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the breast cancer is a breast cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation).

In some **embodimentexamples** of the present **inventiondisclosure**, for the method for treating and/or preventing the malignant tumor, the administration is performed before or after surgery and/or before or after radiotherapy.

In some **embodimentexamples** of the present **inventiondisclosure**, for the method for treating and/or preventing the malignant tumor, the bispecific antibody is administered at a unit dose of 0.1-100 mg per kg body weight, preferably 5-50 mg or 5-15 mg per kg body weight, administered once every 3 days, 4 days, 5 days, 6 days, 10 days, 1 week, 2 weeks or 3 weeks,

and/or

administered by intravenous drip infusion or intravenous injection.

In some **embodimentexamples** of the present **inventiondisclosure**, for the method for treating and/or preventing the malignant tumor, the PARP inhibitor is administered at a unit dose of 0.1-100 mg per kg body weight, preferably 5-50 mg or 5-15 mg per kg body weight,

administered once every 3 days, 4 days, 5 days, 6 days, 10 days, 1 week, 2 weeks or 3 weeks,

and/or

administered by intravenous drip infusion or intravenous injection.

In some **embodimentexamples** of the present **inventiondisclosure**, for the method for treating and/or preventing the malignant tumor, the bispecific antibody and the PARP inhibitor are administered simultaneously or non-simultaneously.

A typical non-limiting range of a therapeutically or prophylactically effective amount of the bispecific antibody and/or the PARP inhibitor of the present **inventiondisclosure** is 0.02-100 mg/kg, such as 0.1-50 mg/kg, 0.1-25 mg/kg, or 1-10 mg/kg. It should be noted that the dose may vary with the type and severity of the symptom to be treated. Furthermore, those skilled in the art will appreciate that for any particular patient, the particular dose regimen may be adjusted over time according to the needs of the patient and the professional judgment of the physician; the dose ranges provided herein are for illustrative purpose only and do not limit the use or scope of the pharmaceutical composition of the present **inventiondisclosure**.

In the present **inventiondisclosure**, the subject may be a mammal, such as a human.

Yet another **aspectexample** of the present **inventiondisclosure** relates to a method *in vivo* or *in vitro*, selected from:

(1)

a method for detecting the level of VEGFA in a sample,
a method for blocking the binding of VEGFA to VEGFR2,
a method for down-regulating the activity or level of VEGFA,
a method for relieving the stimulation of VEGFA on vascular endothelial cell proliferation,
a method for inhibiting vascular endothelial cell proliferation, or
a method for blocking tumor angiogenesis;
and/or

(2)

a method for blocking the binding of PD-1 to PD-L1,
a method for down-regulating the activity or level of PD-1,
a method for relieving the immunosuppression of PD-1 in an organism,
a method for promoting IFN- γ secretion in T lymphocytes, or
a method for promoting IL-2 secretion in T lymphocytes.

In one or more **embodiment examples** of the present **invention disclosure**, the therapeutic combination or kit product is for use in treating and/or preventing a malignant tumor;

preferably, the malignant tumor is selected from ovarian cancer, endometrial cancer, breast cancer, cervical cancer, fallopian tube cancer, peritoneal cancer, pancreatic cancer, colon cancer, rectal cancer, lung cancer, liver cancer, skin cancer, glioma, melanoma, lymphoma, renal tumor, prostate cancer, bladder cancer, gastrointestinal cancer, brain cancer, oesophageal cancer such as oesophageal squamous cancer, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) cancer, urothelial carcinoma, mesothelioma, gastric adenocarcinoma, gastroesophageal junction adenocarcinoma, leukemia,

function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation).

In one aspect, the present disclosure refers to the therapeutic combination disclosed herein or the kit product disclosed herein for use in treating and/or preventing a malignant tumor, wherein, preferably, the malignant tumor is selected from ovarian cancer, endometrial cancer, breast cancer, cervical cancer, fallopian tube cancer, peritoneal cancer, pancreatic cancer, colon cancer, rectal cancer, lung cancer, liver cancer, skin cancer, glioma, melanoma, lymphoma, renal tumor, prostate cancer, bladder cancer, gastrointestinal cancer, brain cancer, oesophageal cancer such as oesophageal squamous cancer, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) cancer, urothelial carcinoma, mesothelioma, gastric adenocarcinoma, gastroesophageal junction adenocarcinoma, leukemia, myeloma, thyroid cancer, head and neck cancer, bone cancer, biliary tract cancer and testicular cancer; preferably, the tumor is a tumor with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation); preferably, the tumor is a tumor without deficient repair function mediated by homologous recombination; preferably, the lung cancer is non-small cell lung cancer or small cell lung cancer; preferably, the non-small cell lung cancer is a non-small cell lung cancer with EGFR and/or ALK-sensitive mutation; preferably, the non-small cell lung cancer is a non-small cell lung cancer without EGFR and/or ALK-sensitive mutation; preferably, the liver cancer is hepatocellular carcinoma; preferably, the renal cancer is renal cell carcinoma; preferably, the breast cancer is triple negative breast cancer; preferably, the urothelial carcinoma is bladder cancer; preferably, the peritoneal cancer is primary peritoneal cancer; preferably, the ovarian cancer is an ovarian cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation); preferably, the fallopian tube cancer is a fallopian tube cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation); preferably, the peritoneal cancer is a peritoneal cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation); preferably, the breast cancer is a breast cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation).

In one or more ~~embodiment~~examples of the present ~~invention~~disclosure, the therapeutic combination or kit product is for use in:

(1)

detecting the level of VEGFA in a sample,
blocking the binding of VEGFA to VEGFR2,
down-regulating the activity or level of VEGFA,
relieving the stimulation of VEGFA on vascular endothelial cell proliferation,
inhibiting vascular endothelial cell proliferation, or
blocking tumor angiogenesis;
and/or

(2)

blocking the binding of PD-1 to PD-L1,
down-regulating the activity or level of PD-1,
relieving the immunosuppression of PD-1 in an organism,
promoting IFN- γ secretion in T lymphocytes, or
promoting IL-2 secretion in T lymphocytes.

Yet another aspect of the present ~~invention~~disclosure relates to use of the bispecific antibody in the therapeutic combination ~~according to any embodiment of the present invention~~disclosed herein in preparing a medicament for treating and/or preventing a malignant tumor, wherein the malignant tumor is selected from ovarian cancer, fallopian tube cancer, peritoneal cancer and breast cancer;
preferably, the ovarian cancer is an ovarian cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1

mutation and/or a BRCA2 mutation);

preferably, the fallopian tube cancer is a fallopian tube cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the peritoneal cancer is a peritoneal cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the breast cancer is a breast cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation).

Yet another **aspectexample** of the present **inventiondisclosure** relates to a method for treating and/or preventing a malignant tumor, comprising: administering to a subject in need an effective amount of the bispecific antibody in the therapeutic combination according to any **embodimentexample** of the present **inventiondisclosure**, wherein the malignant tumor is selected from ovarian cancer, fallopian tube cancer, peritoneal cancer and breast cancer;

preferably, the ovarian cancer is an ovarian cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the fallopian tube cancer is a fallopian tube cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the peritoneal cancer is a peritoneal cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the breast cancer is a breast cancer with deficient repair

function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation).

Yet another aspect of the present ~~invention~~disclosure relates to the bispecific antibody in the therapeutic combination ~~according to any embodiment of the present invention~~disclosed herein for use in treating and/or preventing a malignant tumor, wherein the malignant tumor is selected from ovarian cancer, fallopian tube cancer, peritoneal cancer and breast cancer;

preferably, the ovarian cancer is an ovarian cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the fallopian tube cancer is a fallopian tube cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the peritoneal cancer is a peritoneal cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation);

preferably, the breast cancer is a breast cancer with deficient repair function mediated by homologous recombination (e.g., a BRCA1 mutation and/or a BRCA2 mutation).

Antibody drugs, especially monoclonal antibodies, have achieved good efficacy in the treatment of various diseases. Conventional methods for acquiring these therapeutic antibodies include immunizing animals with an antigen and acquiring antibodies targeting the antigen in the immunized animals, or modifying those antibodies with lower affinity for the antigen by affinity maturation.

The variable regions of the light chain and the heavy chain determine the

binding to antigens; the variable region of each chain comprises three hypervariable regions called complementarity determining regions (CDRs) (CDRs of the heavy chain (H Chain) are HCDR1, HCDR2, and HCDR3, and CDRs of the light chain (L Chain) are LCDR1, LCDR2, and LCDR3, which are named by Kabat et al.; see Bethesda M.d., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication, (1-3) 1991: 91-3242).

Preferably, CDRs may also be defined by the IMGT numbering system; see Ehrenmann, Francois, Quentin Kaas, and Marie-Paule Lefranc. "IMGT/3Dstructure-DB and IMGT/DomainGapAlign: a database and a tool for immunoglobulins or antibodies, T cell receptors, MHC, IgSF and MhcSF." *Nucleic acids research*, 38.suppl_1 (2009): D301-D307.

The amino acid sequences of the CDR regions of the monoclonal antibody sequences in (1)-(13) below were analyzed by technical means well known to those skilled in the art, for example by VBASE2 database and especially according to the IMGT definition, and the results are as follows:

(1) Bevacizumab

The heavy chain variable region has an amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region has an amino acid sequence set forth in SEQ ID NO: 3.

The 3 CDRs of the heavy chain variable region have the following amino acid sequences:

HCDR1: GYTFTNYG (SEQ ID NO: 28)

HCDR2: INTYTGEP (SEQ ID NO: 29)

HCDR3: AKYPHYYGSSHWFYFDV (SEQ ID NO: 30)

the 3 CDRs of the light chain variable region have the following amino acid sequences:

LCDR1: QDISNY (SEQ ID NO: 31)

LCDR2: FTS (SEQ ID NO: 32)

LCDR3: QQYSTVPWT (SEQ ID NO: 33)

(2) 14C12, 14C12H1L1 or 14C12H1L1(M)

The 3 CDRs of the heavy chain variable region have the following amino acid sequences:

HCDR1: GFAFSSYD (SEQ ID NO: 34)

HCDR2: ISGGGRYT (SEQ ID NO: 35)

HCDR3: ANRYGEAWFAY (SEQ ID NO: 36)

the 3 CDRs of the light chain variable region have the following amino acid sequences:

LCDR1: QDINTY (SEQ ID NO: 37)

LCDR2: RAN (SEQ ID NO: 38)

LCDR3: LQYDEFPLT (SEQ ID NO: 39)

(3) VP101(hG1WT) or VP101(hG1DM)

The 9 CDRs of the heavy chain have the following amino acid sequences:

HCDR1: GYTFTNYG (SEQ ID NO: 28)

HCDR2: INTYTGEP (SEQ ID NO: 29)

HCDR3: AKYPHYYGSSHWFYFDV (SEQ ID NO: 30)

HCDR4: GFAFSSYD (SEQ ID NO: 34)

HCDR5: ISGGGRYT (SEQ ID NO: 35)

HCDR6: ANRYGEAWFAY (SEQ ID NO: 36)

HCDR7: QDINTY (SEQ ID NO: 37)

HCDR8: RAN (SEQ ID NO: 38)

HCDR9: LQYDEFPLT (SEQ ID NO: 39)

the 3 CDRs of the light chain variable region have the following amino acid sequences:

LCDR1: QDISNY (SEQ ID NO: 31)

LCDR2: FTS (SEQ ID NO: 32)

LCDR3: QQYSTVPWT (SEQ ID NO: 33).

For the antibody VP101(hG1DM) of the present ~~invention~~disclosure, amino acid mutations are introduced into the non-variable region of VP101(hG1WT). According to the EU numbering system, amino acid mutations are introduced at positions 234 and 235:

VP101 (hG1DM) was obtained by introducing a leucine-to-alanine point mutation at position 234 (L234A) and a leucine-to-alanine point mutation at position 235 (L235A) in the hinge region of the heavy chain.

In the present ~~invention~~disclosure, unless otherwise defined, the scientific and technical terms used herein have the meanings generally understood by those skilled in the art. In addition, the laboratory operations of cell culture, molecular genetics, nucleic acid chemistry and immunology used herein are the routine procedures widely used in the corresponding fields. Meanwhile, in order to better understand the present ~~invention~~disclosure, the definitions and explanations of the relevant terms are provided below.

As used herein, when referring to the amino acid sequence of VEGFA protein, it includes, but is not limited to, the full length of the VEGFA protein (GenBank ID: NP_001165097.1), as well as a fusion protein of VEGFA, such as a fragment fused to an Fc protein fragment of mouse or human IgG (mFc or hFc). However, those skilled in the art will appreciate that in the amino acid sequence of the VEGFA protein, mutations or variations (including but not limited to, substitutions, deletions and/or additions) may naturally occur or can be artificially

introduced without affecting biological functions thereof. Therefore, in the present **inventiondisclosure**, the term "VEGFA protein" shall include all such sequences, including their natural or artificial variants. In addition, when describing a sequence fragment of the VEGFA protein, it also includes the corresponding sequence fragments in their natural or artificial variants. In one **embodimentexample** of the present **inventiondisclosure**, the VEGFA protein has an amino acid sequence set forth in the underlined part of SEQ ID NO: 33 (a total of 302 amino acids excluding the last 6 His).

As used herein, when referring to the amino acid sequence of VEGFR2 protein (also known as KDR), it includes, but is not limited to, the full length of the VEGFR2 protein (GenBank ID: NP_002244), or the extracellular fragment VEGFR2-ECD of VEGFR2, or a fragment comprising VEGFR2-ECD, and it also includes a fusion protein of VEGFR2-ECD, such as a fragment fused to an Fc protein fragment of mouse or human IgG (mFc or hFc). However, those skilled in the art will appreciate that in the amino acid sequence of the VEGFR2 protein, mutations or variations (including but not limited to, substitutions, deletions and/or additions) may naturally occur or can be artificially introduced without affecting biological functions thereof. Therefore, in the present **inventiondisclosure**, the term "VEGFR2 protein" shall include all such sequences, including their natural or artificial variants. In addition, when describing a sequence fragment of the VEGFR2 protein, it also includes the corresponding sequence fragments in their natural or artificial variants. In one **embodimentexample** of the present **inventiondisclosure**, the extracellular fragment VEGFR2-ECD of VEGFR2 has an amino acid sequence set forth in SEQ ID NO: 34 (766 amino acids).

As used herein, unless otherwise specified, the VEGFR is VEGFR1 and/or VEGFR2; specific protein sequence thereof is a sequence known

in the prior art, and reference may be made to the sequence disclosed in the existing literature or GenBank. For example, VEGFR1 (VEGFR1, NCBI Gene ID: 2321); VEGFR2 (VEGFR2, NCBI Gene ID: 3791).

As used herein, when referring to the amino acid sequence of PD-1 protein (programmed cell death protein 1), it includes the full length of the PD-1 protein (NCBI GenBank: NP_005009.2), or the extracellular fragment PD-1ECD of PD-1 or a fragment comprising PD-1ECD, and it also includes a fusion protein of PD-1ECD, such as a fragment fused to an Fc protein fragment of a mouse or human IgG (mFc or hFc). However, it will be appreciated by those skilled in the art that in the amino acid sequence of PD-1 protein, mutations or variations (including but not limited to substitutions, deletions and/or additions) may naturally occur or can be artificially introduced without affecting biological functions thereof. Therefore, in the present ~~invention~~disclosure, the term "PD-1 protein" shall include all such sequences, including their natural or artificial variants. In addition, when describing a sequence fragment of the PD-1 protein, it also includes the corresponding sequence fragments in their natural or artificial variants.

As used herein, the term EC₅₀ refers to the concentration for 50% of maximal effect, i.e., the concentration that can cause 50% of the maximal effect.

As used herein, the term "antibody" refers to an immunoglobulin molecule that generally consists of two pairs of polypeptide chains (each pair consisting of one "light" (L) chain and one "heavy" (H) chain). In a general sense, the heavy chain can be interpreted as a polypeptide chain with a larger molecular weight in an antibody, and the light chain refers to a polypeptide chain with a smaller molecular weight in an antibody. Light chains can be classified into κ and λ light chains. Heavy chains are

MHC, IgSF and MhcSF." *Nucleic acids research*, 38.suppl_1 (2009): D301-D307). In particular, the heavy chain may further comprise more than 3 CDRs, such as 6, 9, or 12. For example, in the bispecific antibody of the present ~~invention~~disclosure, the heavy chain may be an scFv with the C-terminus of the heavy chain of IgG antibody linked to another antibody, and in this case, the heavy chain comprises 9 CDRs. The term "antibody" is not limited by any specific method for producing the antibody. For example, the antibody includes, in particular, a recombinant antibody, a monoclonal antibody and a polyclonal antibody. The antibody can be antibodies of different isotypes, such as IgG (e.g., subtypes IgG1, IgG2, IgG3 or IgG4), IgA1, IgA2, IgD, IgE or IgM.

As used herein, the term "antigen binding fragment", also known as the "antigen binding portion", refers to a polypeptide comprising the fragment of a full-length antibody, which maintains the ability to specifically bind to the same antigen to which the full-length antibody binds, and/or competes with the full-length antibody for the specific binding to the antigen. See generally, *Fundamental Immunology*, Ch. 7 (Paul, W., ed., 2nd edition, Raven Press, N.Y. (1989)). An antigen-binding fragment of an antibody can be produced by recombinant DNA technique or by enzymatic or chemical cleavage of the intact antibody. In some cases, the antigen binding fragment includes Fab, Fab', F (ab')₂, Fd, Fv, dAb, and complementarity determining region (CDR) fragment, single chain antibody fragment (e.g., scFv), chimeric antibody, diabody and a polypeptide that comprises at least a portion of an antibody sufficient to impart specific antigen binding ability to the polypeptide.

As used herein, the term "Fd fragment" refers to an antibody fragment consisting of V_H and C_{H1} domains; the term "Fv fragment" refers to an antibody fragment consisting of the V_L and V_H domains of a single arm of

an antibody; the term "dAb fragment" refers to an antibody fragment consisting of a V_H domain (Ward et al., *Nature*, 341 (1989):544-546); the term "Fab fragment" refers to an antibody fragment consisting of V_L, V_H, C_L and C_{H1} domains; the term "F(ab')₂ fragment" refers to an antibody fragment comprising two Fab fragments linked by disulfide bridges in the hinge region.

In some cases, the antigen binding fragment of the antibody is a single chain antibody (e.g., scFv) in which the V_L and V_H domains are paired to form a monovalent molecule via a linker that enables the production of a single polypeptide chain (see, e.g., Bird et al., *Science* 242 (1988):423-426 and Huston et al., *Proc. Natl. Acad. Sci. USA*, 85 (1988):5879-5883). Such scFv molecules may have a general structure: NH₂-V_L-linker-V_H-COOH or NH₂-V_H-linker-V_L-COOH. An appropriate linker in prior art consists of GGGGS amino acid sequence repeats or a variant thereof. For example, a linker having the amino acid sequence (GGGGS)₄ can be used, and variants thereof can also be used (Holliger et al., *Proc. Natl. Acad. Sci. USA*, 90 (1993):6444-6448). Other linkers useful in the present ~~invention~~disclosure are described by Alfthan et al., *Protein Eng.* 8 (1995): 725-731, Choi et al., *Eur. J. Immunol.* 31 (2001): 94-106, Hu et al., *Cancer Res.* 56 (1996): 3055-3061, Kipriyanov et al., *J. Mol. Biol.* 293 (1999): 41-56, and Roovers et al., *Cancer Immunol.* (2001).

In some cases, the antigen binding fragment of the antibody is a diabody, that is, a bivalent antibody, in which the V_H and V_L domains are expressed on a single polypeptide chain. However, the linker used is too short to allow the pairing of the two domains on the same chain. Thus the domains are forced to pair with the complementary domains on the other chain and two antigen binding sites are generated (see, e.g., Holliger P. et al., *Proc. Natl. Acad. Sci. USA* 90 (1993):6444-6448, and Poljak R.J. et al.,

are not limited to retroviruses (including lentiviruses), adenoviruses, adeno-associated viruses, herpes viruses (such as herpes simplex virus), poxviruses, baculoviruses, papillomaviruses, and papovaviruses (such as SV40). A vector may comprise a variety of elements that control expression, including, but not limited to, promoter sequences, transcription initiation sequences, enhancer sequences, selection elements and reporter genes. In addition, the vector may further comprise a replication initiation site.

As used herein, the term "host cell" refers to cells to which vectors can be introduced, including, but not limited to, prokaryotic cells such as *Escherichia coli* or *Bacillus subtilis*, fungal cells such as yeast cells or *Aspergillus*, insect cells such as S2 drosophila cells or Sf9, or animal cells such as fibroblasts, CHO cells, COS cells, NSO cells, HeLa cells, BHK cells, HEK 293 cells or human cells.

As used herein, the term "specifically bind" refers to a non-random binding reaction between two molecules, such as a reaction between an antibody and an antigen it targets. In some **embodiment examples**, an antibody that specifically binds to an antigen (or an antibody that is specific for an antigen) means that the antibody binds to the antigen with an affinity (K_D) less than about 10^{-5} M, such as less than about 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M or 10^{-10} M or less. In some **embodiment examples** of the present **invention disclosure**, the term "target" refers to specific binding.

As used herein, the term " K_D " refers to a dissociation equilibrium constant for a specific antibody-antigen interaction, which is used to describe the binding affinity between the antibody and the antigen. A smaller dissociation equilibrium constant indicates a stronger antibody-antigen binding and a higher affinity between the antibody and the antigen. Generally, an antibody binds to an antigen with a

aluminum hydroxide), Freund's adjuvant (e.g., complete Freund's adjuvant and incomplete Freund's adjuvant), *Corynebacterium parvum*, lipopolysaccharide, cytokine, etc. The Freund's adjuvant is the most commonly used adjuvant in animal experiments. The aluminum hydroxide adjuvant is used more frequently in clinical trials.

As used herein, the term "effective amount" refers to an amount sufficient to obtain or at least partially obtain a desired effect. For example, a prophylactically effective amount (e.g., for a disease associated with PD-1 binding to PD-L1 or overexpression of VEGF, such as a tumor) is an amount sufficient to prevent, stop or delay the onset of the disease (e.g., a disease associated with PD-L1 binding to PD-L1 or overexpression of VEGF, such as a tumor); a therapeutically effective amount is an amount sufficient to cure or at least partially stop the disease and its complications in a patient suffering from the disease. It is undoubtedly within the ability of those skilled in the art to determine such an effective amount. For example, the amount effective for therapeutic purpose will depend on the severity of the disease to be treated, the overall state of the patient's own immune system, the general condition of the patient such as age, body weight and gender, the route of administration, and other treatments given concurrently, etc.

Beneficial Effects

The present ~~invention~~disclosure achieves any one or more of the following technical effects (1) to (6):

(1) In the present ~~invention~~disclosure, the modifications at the Fc fragment of the antibody completely eliminate the binding activity of VP101(hG1WT) to F_C receptors FcγRI and FcγRIIIa_F158, thereby completely eliminating the ADCC activity.

(2) In the present **inventiondisclosure**, the modifications at the Fc fragment of the antibody completely eliminate the binding activity of VP101(hG1WT) to complement C1q, thereby eliminating the CDC activity.

(3) The bispecific antibody of the present **inventiondisclosure** can specifically bind to VEGFA, effectively block the binding of VEGFA to VEGFR2, and specifically relieve the promotion effect of VEGFA on the immune suppression and the angiogenesis.

(4) The bispecific antibody of the present **inventiondisclosure** can specifically bind to PD-1, effectively block the binding of PD-1 to PD-L1, and specifically relieve the immunosuppression of PD-1 to the organism and activate immune responses.

(5) The combined use of PARPi and the bispecific antibody of the present **inventiondisclosure** has significantly superior efficacy in tumors, particularly breast or ovarian cancer, to the PARPi or the bispecific antibody alone.

(6) The PARPi and the bispecific antibody of the present **inventiondisclosure** have a synergistic effect in treating or preventing tumors.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1: Affinity constant assay of VP101(hG1DM) to FcγRI. The antibody concentrations for the curve pairs from top to bottom are 50 nM, 25 nM, 12.5 nM, 6.25 nM and 3.12 nM, respectively.

FIG. 2: Affinity constant assay of bevacizumab to FcγRI. The antibody concentrations for the curve pairs from top to bottom are 50 nM, 25 nM, 12.5 nM, 6.25 nM and 3.12 nM, respectively.

FIG. 3: Affinity constant assay of nivolumab to FcγRI. The antibody concentrations for the curve pairs from top to bottom are 50 nM, 25 nM, 12.5 nM, 6.25 nM and 3.12 nM, respectively.

FIG. 4: Affinity constant assay of VP101(hG1WT) to FcγRI. The