CLAIMS

1. An immunoconjugate, comprising a PD-1 antibody or an antigen-binding fragment thereof, and an IL-2 variant, wherein the IL-2 variant comprises a mutation represented by the following mutation (i), and any one or any combination of the mutations (ii)-(iii):

(i) a mutation that makes the IL-2 variant have a reduced affinity for IL-2Rβ/γ as compared to wild-type IL-2;

(ii) a mutation that makes the IL-2 variant have a reduced affinity for IL-2Rα as compared to wild-type IL-2 or do not bind to IL-2Rα; and

(iii) a mutation that makes the IL-2 variant have increased stability, expression, and/or purity;

preferably,

(i) a mutation that makes the IL-2 variant have a reduced affinity for IL-2Rβ/γ as compared to wild-type IL-2, selected from the group consisting of any one or any combination of positions 12, 15, 16, 18, 19, 20, 23, 43, 69, 84, 87, 88, 91, 92, 95, 123, 126, 127, 129, and 130;

(ii) a mutation that makes the IL-2 variant have a reduced affinity for IL-2Rα as compared to wild-type IL-2 or do not bind to IL-2Rα, selected from the group consisting of any one or any combination of positions 38, 42, 45, 62, 65, 68, and 72; and

(iii) a mutation that makes the IL-2 variant have increased stability, expression, and/or purity as compared to wild-type IL-2, selected from the group consisting of any one or any combination of positions 11, 26, 27, 29, 30, 70, 71, 78, 82, and 132;

the positions described above are numbered according to a wild-type IL-2 amino acid sequence set forth in SEQ ID NO: 87.

2. The immunoconjugate according to claim 1, wherein:

the mutation (i) comprises any one or any combination of the following mutations: L12R, L12K, L12E, L12Q, E15Q, E15R, E15A, E15S, H16N, H16A, H16E, H16D, H16G, H16S, H16T, H16V, H16P, D20A, D20H, D20Y, D20N, M23A, M23R, M23K, M23G, M23S, M23T, M23V, M23P, S87K, S87A, D84S, D84L, D84N, D84V, D84H, D84Y, D84R, D84K, D84G, D84A, D84T, D84P, N88D, N88A, N88S, N88T, N88R, N88I, V91A, V91T, V91E, I92A, E95S, E95A, E95R, E95Q, E95G, E95T, E95V, E95P, E95H, E95N, T123A, T123E, T123K, T123Q, Q126D, Q126L, Q126A, Q126S, Q126T, Q126E, S127A, S127E, S127K, and S127Q; and/or,

the mutation (ii) comprises any one or any combination of the following mutations: R38A, R38D, R38E, E62Q, F42A, F42G, F42S, F42T, F42Q, F42E, F42N, F42D, F42R, F42K, Y45A, Y45G, Y45S, Y45T, Y45Q, Y45E, Y45N, Y45D, Y45R, Y45K, P65R, P65E, P65K, P65H, P65Y, P65Q, P65D, P65N, E68A, E68Q, E68K, E68R, L72G, L72A, L72S, L72T, L72Q, L72E, L72N, L72D, L72R, and L72K; and/or,

the mutation (iii) comprises at least one of the following groups of mutations: N26Q, N29S, N30S, N71Q, Q11C/L132C, L70C/P82C, and G27C/F78C;

preferably, the mutation (i) comprises at least one of the following groups of mutations:

N88D, N88R, D20A, D20N, V91T, Q126D, and H16A/D84S; and/or,
the mutation (ii) comprises at least one of the following groups of mutations: F42A, Y45A, L72G, R38E/F42A, F42A/L72G, F42A/Y45A, Y45A/L72G, and F42A/Y45A/L72G; and/or,
the mutation (iii) comprises at least one of the following groups of mutations: N26Q, N29S, N30S, N26Q/N29S/N71Q, N26Q/N29S, N26Q/N30S, N26Q/N30S/Q11C/L132C, N26Q/N29S/L70C/P82C, and N26Q/N30S/G27C/F78C.

3. The immunoconjugate according to claim 1 or 2, wherein the IL-2 variant further comprises a mutation at position 3 and/or a mutation at position 125; the mutation at position 3 is preferably T3A; the mutation at position 125 is preferably C125A or C125S.

4. The immunoconjugate according to any one of claims 1-3, wherein the IL-2 variant comprises any one of the following groups of mutations:
F42A/L72G/N88D,
F42A/L72G/N88R,
F42A/L72G/D20A,
F42A/L72G/D20N,
F42A/L72G/V91T,
F42A/R38E/V91T,
F42A/L72G/Q126D, and
F42A/L72G/H16A/D84S;
preferably, the IL-2 variant comprises any one of the following groups of mutations:
F42A/L72G/N88D/N26Q/N29S/N71Q,
F42A/L72G/N88R/N26Q/N29S/N71Q,
F42A/L72G/D20A/N26Q/N29S/N71Q,
F42A/L72G/D20N/N26Q/N29S/N71Q,
F42A/L72G/V91T/N26Q/N29S/N71Q,
F42A/L72G/Q126D/N26Q/N29S/N71Q, and
F42A/L72G/H16A/D84S/N26Q/N29S/N71Q;
more preferably, the IL-2 variant comprises any one of the following groups of mutations:
T3A/F42A/R38E/V91T/C125A,
T3A/F42A/L72G/N88D/N26Q/N29S/N71Q/C125A,
T3A/F42A/L72G/N88R/N26Q/N29S/N71Q/C125A,
T3A/F42A/L72G/D20A/N26Q/N29S/N71Q/C125A,
T3A/F42A/L72G/D20N/N26Q/N29S/N71Q/C125A,
T3A/F42A/L72G/V91T/N26Q/N29S/N71Q/C125A,
T3A/F42A/L72G/Q126D/N26Q/N29S/N71Q/C125A, and
T3A/F42A/L72G/H16A/D84S/N26Q/N29S/N71Q/C125A.

5. The immunoconjugate according to any one of claims 1-4, wherein the IL-2 variant

comprises the amino acid sequence set forth in any one of SEQ ID NOs: 60-68 or a sequence having at least 90% identity thereto.

6. The immunoconjugate according to any one of claims 1-5, wherein the PD-1 antibody or the antigen-binding fragment thereof comprises at least one immunoglobulin single variable domain, and the immunoglobulin single variable domain comprises
1) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 97, 100, and 101, or
2) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 4, 5, and 6;
preferably, the immunoglobulin single variable domain comprises
1-1) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 36, and 37,
1-2) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 97, 98, and 99, or
1-3) a CDR1 set forth in SEQ ID NO: 7 or 93, a CDR2 set forth in any one of SEQ ID NOs: 8, 38-40, and 94-95, and a CDR3 set forth in any one of SEQ ID NOs: 9, 41, 42, and 96;
preferably, the immunoglobulin single variable domain comprises the CDR1, CDR2, and CDR3 set forth in any one of 1) to 19):
1) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 38, and 41;
2) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 38, and 96;
3) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 94, and 41;
4) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 93, 94, and 41;
5) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 93, 94, and 96;
6) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 93, 95, and 96;
7) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 8, and 9;
8) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 8, and 42;
9) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 38, and 6;
10) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 39, and 9;
11) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 40, and 6;
12) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 8, and 41;
13) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 8, and 42;
14) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 38, and 41;
15) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 38, and 42;
16) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 39, and 41;
17) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 39, and 42;
18) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 40, and 41; and
19) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 40, and 42;
preferably, the immunoglobulin single variable domain comprises or consists of the following sequence: the amino acid sequence set forth in any one of SEQ ID NOs: 3, 13-15, 17-35, and 88-92; the amino acid sequence set forth in any one of SEQ ID NOs: 2 and 16; or an amino acid sequence having at least 90% sequence identity to any one of the aforementioned sequences.

7. The immunoconjugate according to any one of claims 1-6, wherein the PD-1 antibody or the antigen-binding fragment thereof comprises a PD-1-binding domain 1 and/or a PD-1-binding domain 2; the PD-1-binding domain 1 comprises at least one said immunoglobulin single variable domain, and the PD-1-binding domain 2 comprises a heavy chain variable region (VH) and a light chain variable region (VL);
preferably, the PD-1-binding domain 2 is selected from the group consisting of a Fab, a Fab', an Fv, and an ScFv; more preferably, the PD-1-binding domain 2 is a Fab;
preferably, the VH comprises a HCDR1, a HCDR2, and a HCDR3 of the amino acid sequences set forth in SEQ ID NOs: 54-56, and the VL comprises a LCDR1, a LCDR2, and a LCDR3 of the amino acid sequences set forth in SEQ ID NOs: 57-59; more preferably, the VH comprises a heavy chain variable region from a Fab heavy chain of the amino acid sequence set forth in SEQ ID NO: 52, and the VL comprises a light chain variable region from a Fab light chain of the amino acid sequence set forth in SEQ ID NO: 53.

8. An immunoconjugate, comprising a PD-1 antibody or an antigen-binding fragment thereof, and an IL-2 or a variant thereof; wherein:
the PD-1 antibody or the antigen-binding fragment thereof comprises at least one immunoglobulin single variable domain, and the immunoglobulin single variable domain comprises
1) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 97, 100, and 101, or
2) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 4, 5, and 6;
optionally, the immunoglobulin single variable domain comprises
1-1) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 36, and 37,
1-2) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 97, 98, and 99, or
1-3) a CDR1 set forth in SEQ ID NO: 7 or 93, a CDR2 set forth in any one of SEQ ID NOs: 8, 38-40, and 94-95, and a CDR3 set forth in any one of SEQ ID NOs: 9, 41, 42, and 96.

9. The immunoconjugate according to claim 8, wherein the immunoglobulin single variable domain comprises the CDR1, CDR2, and CDR3 set forth in any one of 1) to 19):
1) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 38, and 41;
2) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 38, and 96;
3) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 94, and 41;
4) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 93, 94, and 41;
5) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 93, 94, and 96;
6) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 93, 95, and 96;
7) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 8, and 9;
8) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 8, and 42;
9) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 38, and 6;
10) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 39, and 9;

11) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 40, and 6;
12) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 8, and 41;
13) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 8, and 42;
14) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 38, and 41;
15) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 38, and 42;
16) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 39, and 41;
17) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 39, and 42;
18) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 40, and 41; and
19) a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOs: 7, 40, and 42.

10. The immunoconjugate according to claim 8, wherein the immunoglobulin single variable domain is modified by humanization, affinity maturation, T cell epitope removal, reduction of antibody deamidation, and/or reduction of antibody isomerization;
preferably, a heavy chain framework region of a human germline template used in the humanization modification is IGHV3-23 *01 or IGHV3-23*04.

11. The immunoconjugate according to any one of claims 8-10, wherein the immunoglobulin single variable domain comprises or consists of the following sequence:
the amino acid sequence set forth in any one of SEQ ID NOs: 3, 13-15, 17-35, and 88-92;
the amino acid sequence set forth in any one of SEQ ID NOs: 2 and 16; or
an amino acid sequence having at least 90% sequence identity to any one of the aforementioned sequences.

12. The immunoconjugate according to any one of claims 8-11, wherein the PD-1 antibody or the antigen-binding fragment thereof comprises a PD-1-binding domain 1 and/or a PD-1-binding domain 2; the PD-1-binding domain 1 comprises at least one said immunoglobulin single variable domain, and the PD-1-binding domain 2 comprises a heavy chain variable region (VH) and a light chain variable region (VL);
preferably, the PD-1-binding domain 2 is selected from the group consisting of a Fab, a Fab', an Fv, and an ScFv; more preferably, the PD-1-binding domain 2 is a Fab;
preferably, the VH comprises a HCDR1, a HCDR2, and a HCDR3 of the amino acid sequences set forth in SEQ ID NOs: 54-56, and the VL comprises a LCDR1, a LCDR2, and a LCDR3 of the amino acid sequences set forth in SEQ ID NOs: 57-59; more preferably, the VH comprises a heavy chain variable region from a Fab heavy chain of the amino acid sequence set forth in SEQ ID NO: 52, and the VL comprises a light chain variable region from a Fab light chain of the amino acid sequence set forth in SEQ ID NO: 53.

13. The immunoconjugate according to any one of claims 8-12, wherein the IL-2 variant comprises any one or any combination of the following mutations (i)-(iii):

(i) a mutation that makes the IL-2 variant have a reduced affinity for IL-2Rβ/γ as compared to wild-type IL-2;

(ii) a mutation that makes the IL-2 variant have a reduced affinity for IL-2Rα as compared to wild-type IL-2 or do not bind to IL-2Rα; and

(iii) a mutation that makes the IL-2 variant have increased stability, expression, and/or purity;

optionally,

(i) the mutation that makes the IL-2 variant have a reduced affinity for IL-2Rβ/γ as compared to wild-type IL-2 is selected from the group consisting of any one or any combination of positions 12, 15, 16, 18, 19, 20, 23, 43, 69, 84, 87, 88, 91, 92, 95, 123, 126, 127, 129, and 130;

(ii) the mutation that makes the IL-2 variant have a reduced affinity for IL-2Rα as compared to wild-type IL-2 or do not bind to IL-2Rα is selected from the group consisting of any one or any combination of positions 38, 42, 45, 62, 65, 68, and 72;

(iii) the mutation that makes the IL-2 variant have increased stability, expression, and/or purity as compared to wild-type IL-2 is selected from the group consisting of any one or any combination of positions 11, 26, 27, 29, 30, 70, 71, 78, 82, and 132;

the positions described above are numbered according to a wild-type IL-2 amino acid sequence set forth in SEQ ID NO: 87;

optionally,

(i) comprises any one or any combination of the following mutations: L12R, L12K, L12E, L12Q, E15Q, E15R, E15A, E15S, H16N, H16A, H16E, H16D, H16G, H16S, H16T, H16V, H16P, D20A, D20H, D20Y, D20N, M23A, M23R, M23K, M23G, M23S, M23T, M23V, M23P, S87K, S87A, D84S, D84L, D84N, D84V, D84H, D84Y, D84R, D84K, D84G, D84A, D84T, D84P, N88D, N88A, N88S, N88T, N88R, N88I, V91A, V91T, V91E, I92A, E95S, E95A, E95R, E95Q, E95G, E95T, E95V, E95P, E95H, E95N, T123A, T123E, T123K, T123Q, Q126D, Q126L, Q126A, Q126S, Q126T, Q126E, S127A, S127E, S127K, and S127Q;

preferably, the mutation (i) comprises at least one of the following groups of mutations: N88D, N88R, D20A, D20N, V91T, Q126D, and H16A/D84S;

optionally,

the mutation (ii) comprises any one or any combination of the following mutations: R38A, R38D, R38E, E62Q, F42A, F42G, F42S, F42T, F42Q, F42E, F42N, F42D, F42R, F42K, Y45A, Y45G, Y45S, Y45T, Y45Q, Y45E, Y45N, Y45D, Y45R, Y45K, P65R, P65E, P65K, P65H, P65Y, P65Q, P65D, P65N, E68A, E68Q, E68K, E68R, L72G, L72A, L72S, L72T, L72Q, L72E, L72N, L72D, L72R, and L72K;

preferably, the mutation (ii) comprises at least one of the following groups of mutations: F42A, Y45A, L72G, R38E/F42A, F42A/L72G, F42A/Y45A, Y45A/L72G, and F42A/Y45A/L72G;

optionally, the mutation (iii) comprises the following mutations: N26Q, N29S, N30S, N71Q, Q11C/L132C, L70C/P82C, and G27C/F78C;

preferably, the mutation (iii) comprises at least one of the following groups of mutations: N26Q, N29S, N30S, N26Q/N29S/N71Q, N26Q/N29S, N26Q/N30S, N26Q/N30S/Q11C/L132C, N26Q/N29S/L70C/P82C, and N26Q/N30S/G27C/F78C;
optionally, the IL-2 variant further comprises a mutation at position 3 and/or a mutation at position 125; the mutation at position 3 is preferably T3A; the mutation at position 125 is preferably C125A or C125S.

14. The immunoconjugate according to any one of claims 13, wherein the IL-2 variant comprises any one of the following groups of mutations:
F42A/L72G/N88D,
F42A/L72G/N88R,
F42A/L72G/D20A,
F42A/L72G/D20N,
F42A/L72G/V91T,
F42A/R38E/V91T,
F42A/L72G/Q126D, and
F42A/L72G/H16A/D84S;
preferably, the IL-2 variant comprises any one of the following groups of mutations:
F42A/L72G/N88D/N26Q/N29S/N71Q,
F42A/L72G/N88R/N26Q/N29S/N71Q,
F42A/L72G/D20A/N26Q/N29S/N71Q,
F42A/L72G/D20N/N26Q/N29S/N71Q,
F42A/L72G/V91T/N26Q/N29S/N71Q,
F42A/L72G/Q126D/N26Q/N29S/N71Q, and
F42A/L72G/H16A/D84S/N26Q/N29S/N71Q.

15. The immunoconjugate according to any one of claims 13 to 14, wherein the IL-2 variant comprises the amino acid sequence set forth in any one of SEQ ID NOs: 60-68 or a sequence having at least 90% identity thereto.

16. The immunoconjugate according to any one of the preceding claims, wherein the immunoconjugate also comprises an immunoglobulin Fc region;
optionally, the Fc region is an Fc region of human IgG1 or IgG4;
optionally, the Fc region of human IgG1 comprises one or more amino acid mutations that reduce binding to Fc receptors (FcRs) and/or reduce effector functions;
preferably, the Fc region of human IgG1 comprises one or more amino acid mutations that reduce binding to Fcγ receptors (FcγRs) and/or reduce ADCC effects;
more preferably, the Fc region of human IgG1 comprises any one or any combination of the L234A, L235A, and P329G mutations;
optionally, the Fc region comprises a first subunit and a second subunit, and the Fc region comprises a knob-into-hole modification that promotes the association of the first subunit

and the second subunit;
optionally, the first subunit of the Fc region comprises a knob modification, and the second subunit of the Fc region comprises a hole modification;
optionally,
the first subunit of the Fc region comprises a T366 mutation, and the second subunit comprises a mutation selected from the group consisting of T366, L368, and Y407 or any combination thereof;
the first subunit of the Fc region comprises an S354 or E356 mutation, and the second subunit comprises a Y349 mutation; or
the first subunit of the Fc region comprises an S354 mutation and a T366 mutation, and the second subunit comprises a Y349 mutation, a T366 mutation, an L368 mutation, and a Y407 mutation;
optionally,
the first subunit of the Fc region comprises the T366W mutation, and the second subunit comprises a mutation selected from the group consisting of T366S, L368A, and Y407V or any combination thereof;
the first subunit of the Fc region comprises the S354C or E356C mutation, and the second subunit comprises the Y349C mutation; or
the first subunit of the Fc region comprises the S354C/T366W mutations, and the second subunit comprises the Y349C/T366S/L368A/Y407V mutations.;
optionally,
the second subunit of the Fc region also comprises an H435 mutation and/or a Y436 mutation, preferably the H435R/Y436F, H435R, or H435K mutation(s);
optionally,
the first subunit and/or the second subunit of the Fc region also comprise(s) a C220 mutation, preferably the C220S mutation;
optionally, the Fc region of human IgG1 comprises a mutation that enhances FcRn-mediated recycling and/or increases half-life;
preferably, the Fc region of human IgG1 comprises a mutation selected from the group consisting of M252, S254, T256, and M428 or any combination thereof;
preferably, the Fc region of human IgG1 comprises the M252Y/M428V, M252Y/M428L, or M252Y/S254T/T256E mutations.

17. The immunoconjugate according to any one of the preceding claims, wherein the PD-1 antibody or the antigen-binding fragment thereof is located at the N-terminus of the Fc region, and the IL-2 or the variant thereof is located at the N-terminus or C-terminus of the Fc region;
preferably, the PD-1 antibody or the antigen-binding fragment thereof is located at the N-terminus of the Fc, and the IL-2 or the variant thereof is located at the C-terminus of the Fc region.

18. The immunoconjugate according to any one of claims 16 to 17, wherein the IL-2 or the variant thereof is located in a polypeptide chain where the first subunit of the Fc region is present; preferably, the IL-2 or the variant thereof is linked to the first subunit of the Fc region directly or by a linker.

19. The immunoconjugate according to any one of the preceding claims, wherein the valence ratio of the PD-1-binding domains to the IL-2 or the variant thereof in the PD-1 antibody or the antigen-binding fragment thereof is between 4:1 and 1:2, preferably 4:1, 3:1, 2:1, or 1:1.

20. The immunoconjugate according to any one of the preceding claims, wherein the immunoconjugate also comprises a linker;
preferably, the linker is a $(G_xS)_y$ linker, wherein x is selected from the group consisting of integers of 1-5, and y is selected from the group consisting of integers of 1-6;
more preferably, x is 4, and y is 1, 2, or 3.

21. The immunoconjugate according to any one of the preceding claims, comprising, from N-terminus to C-terminus, a polypeptide chain combination set forth in any one of the following I)-VI):
I) a first polypeptide chain: [PD-1-binding domain 1]-[the first subunit of the Fc region]-[linker 1]-[IL-2 or the variant thereof], and
a second polypeptide chain: [PD-1-binding domain 1]-[the second subunit of the Fc region];
II) a first polypeptide chain: [PD-1-binding domain 1]-[linker 2]-[PD-1-binding domain 1]-[the first subunit of the Fc region]-[linker 1]-[IL-2 or the variant thereof], and
a second polypeptide chain: [PD-1-binding domain 1]-[linker 2]-[PD-1-binding domain 1]-[the second subunit of the Fc region];
III) a first polypeptide chain: [the Fab heavy chain of PD-1-binding domain 2]-[the first subunit of the Fc region]-[linker 1]-[IL-2 or the variant thereof],
a second polypeptide chain: [PD-1-binding domain 1]-[the second subunit of the Fc region], and
a third polypeptide chain: [the Fab light chain of PD-1-binding domain 2];
IV) a first polypeptide chain: [the Fab heavy chain of PD-1-binding domain 2]-[the first subunit of the Fc region]-[linker 1]-[IL-2 or the variant thereof],
a second polypeptide chain: [PD-1-binding domain 1]-[linker 2]-[PD-1-binding domain 1]-[the second subunit of the Fc region], and
a third polypeptide chain: [the Fab light chain of PD-1-binding domain 2];
V) a first polypeptide chain: [PD-1-binding domain 1]-[linker 2]-[the Fab heavy chain of PD-1-binding domain 2]-[the first subunit of the Fc region]-[linker 1]-[IL-2 or the variant thereof],
a second polypeptide chain: [PD-1-binding domain 1]-[linker 2]-[the Fab light chain of

PD-1-binding domain 2]-[the second subunit of the Fc region], and
a third polypeptide chain: [the Fab light chain of PD-1-binding domain 2]; and
VI) a first polypeptide chain: [IL-2 or the variant thereof]-[linker 3]-[the first subunit of the Fc region], and
a second polypeptide chain: [PD-1-binding domain 1]-[linker 2]-[PD-1-binding domain 1]-[the second subunit of the Fc region];
wherein - represents a peptide bond; the linkers are polypeptides capable of fulfilling the function of linking, and any two of linker 1, linker 2, and linker 3 may be identical or different; preferably, the linkers are $(G_xS)_y$ linkers, wherein x is selected from the group consisting of integers of 1-5, and y is selected from the group consisting of integers of 1-6; more preferably, linker 1 is $(G_4S)_3$, linker 2 is $(G_4S)_2$, and linker 3 is G_4S;
any two [PD-1-binding domain 1]s may be identical or different and are selected from the group consisting of any of the PD-1-binding domains according to claims 1 to 3;
preferably, [PD-1-binding domain 2] comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises a HCDR1, a HCDR2, and a HCDR3 of the amino acid sequences set forth in SEQ ID NOs: 54-56, and the VL comprises a LCDR1, a LCDR2, and a LCDR3 of the amino acid sequences set forth in SEQ ID NOs: 57-59; preferably, the VH comprises a heavy chain variable region from a Fab heavy chain of the amino acid sequence set forth in SEQ ID NO: 52, and the VL comprises a light chain variable region from a Fab light chain of the amino acid sequence set forth in SEQ ID NO: 53.

22. The immunoconjugate according to any one of the preceding claims, comprising a polypeptide chain combination set forth in the following amino acid sequences:
SEQ ID NOs: 73 and 74;
SEQ ID NOs: 75 and 76;
SEQ ID NOs: 77 and 78;
SEQ ID NOs: 79 and 80;
SEQ ID NOs: 43 and 44;
SEQ ID NOs: 45 and 46;
SEQ ID NOs: 47, 44, and 48;
SEQ ID NOs: 47, 46, and 48;
SEQ ID NOs: 49, 50, and 48; or
SEQ ID NOs: 51 and 46;
SEQ ID NOs: 102 and 103.

23. A nucleic acid molecule, encoding the immunoconjugate according to any one of claims 1 to 22.

24. A host cell, comprising the nucleic acid molecule according to claim 23.

25. A method for preparing the immunoconjugate according to any one of claims 1 to 22, comprising culturing the host cell according to claim 24 and expressing the immunoconjugate, and optionally comprising isolating or purifying the immunoconjugate.

26. A pharmaceutical composition, comprising the immunoconjugate according to any one of claims 1-22, and one or more pharmaceutically acceptable carriers, diluents, or excipients.

27. Use of the immunoconjugate according to any one of claims 1 to 22, the nucleic acid molecule according to claim 23, or the pharmaceutical composition according to claim 26 in the manufacture of a medicament for at least one of the following (1)-(5):
(1) treating a cancer;
(2) activating cytotoxic T cells (CTLs) ;
(3) promoting the proliferation of $CD4^+$ and/or $CD8^+$ T cells;
(4) making cytotoxic T cells (CTLs) with high PD-1 antibody expression have higher cell activation efficiency than cytotoxic T cells (CTLs) with low or no PD-1 antibody expression;
(5) making $CD4^+$ and/or $CD8^+$T cells with high PD-1 antibody expression have higher cell proliferation efficiency than $CD4^+$ and/or $CD8^+$T cells with low or no PD-1 antibody expression;
preferably, the cancer is selected from the group consisting of lung cancer, prostate cancer, breast cancer, head and neck cancer, esophageal cancer, stomach/gastric cancer, colon cancer, colorectal cancer, bladder cancer, cervical cancer, uterine cancer, ovarian cancer, liver cancer, melanoma, renal cancer, squamous cell cancer, and hematological cancer.

28. Use of the immunoconjugate according to any one of claims 1 to 21, or the nucleic acid molecule according to claim 22, or the pharmaceutical composition according to claim 25 in the manufacture of a medicament for activating cytotoxic T cells (CTLs) in a subject;
preferably, the subject has a cancer;
more preferably, the cancer is selected from the group consisting of lung cancer, prostate cancer, breast cancer, head and neck cancer, esophageal cancer, stomach/gastric cancer, colon cancer, colorectal cancer, bladder cancer, cervical cancer, uterine cancer, ovarian cancer, liver cancer, melanoma, renal cancer, squamous cell cancer, and hematological cancer;
preferably, the cytotoxic T cells (CTLs) specifically express PD-1.