

WHAT IS CLAIMED IS:

1. An IL-2 mutant comprising mutations at Y31/A73/H79; preferably, Y31V/A73L/H79Q; and more preferably further comprising mutation(s) at V91, D20 and/or H16, such as V91R, D20A or H16E ~~Q13, L18, G27, Y31, A73, H79, P82, I89, N90, V91, V93, F117 or R120~~ compared to wild-type IL-2.

~~2. The IL-2 mutant according to claim 1, wherein the IL-2 mutant comprises one or more mutation(s) of Q13L, L18I, G27W, Y31V, A73L, H79Q, P82L, I89L, N90Y, V91A, V93I, F117W or R120F.~~

~~3. The IL-2 mutant according to claim 1, wherein the IL-2 mutant comprises at least one group of mutation(s) in groups (a)–(h):~~

~~(a).mutations at Y31/A73/H79; preferably, Y31V/A73L/H79Q;~~

~~(b).a mutation at Q13; preferably, Q13L;~~

~~(c).a mutation at R120; preferably, R120F;~~

~~(d).mutations at L18/V91/F117; preferably, L18I/V91A/F117W;~~

~~(e).mutations at L18/I89/V93; preferably, L18I/I89L/V93I;~~

~~(f).mutations at G27/R120; preferably, G27W/R120F;~~

~~(g).mutations at P82/R120; preferably, P82L/R120F;~~

~~(h).mutations at N90/R120; preferably, N90Y/R120F.~~

~~4.2.~~ The IL-2 mutant according to claim 31, wherein the IL-2 mutant has an amino acid sequence as shown in any one of SEQ ID NOs: 2, 22, 29, 32 or 37 ~~to 9~~.

~~5.3.~~ The IL-2 mutant according to any one of claim 1 to 2, wherein the IL-2 mutant has a T_m value higher than that of the wild-type IL-2.

~~6. The IL-2 mutant according to any one of claims 1 to 3 and 5, wherein the IL-2 mutant further comprises one or more mutation(s) selected from the group consisting of mutation at H16, D20, N88, V91 or Q126, e.g., H16E, D20A, D20H, D20Y, N88A, N88I, N88G, N88R, N88D, V91R, V91K, Q126L or Q126F;~~

~~preferably, the IL-2 mutant further comprises at least one group of mutation(s) selected from groups (i)–(iv):~~

~~(i). a mutation at H16; preferably, H16E;~~

~~(ii). a mutation at D20; preferably, D20A;~~

~~(iii). a mutation at V91; preferably, V91R;~~

~~(iv). mutations at H16/V91; preferably, H16E/V91R.~~

~~7. The IL-2 mutant according to claim 6, wherein the IL-2 mutant comprises at least one group of mutation(s) in groups (a)–(n):~~

~~(a). mutations at H16/Y31/A73/H79; preferably, H16E/Y31V/A73L/H79Q;~~

~~(b). mutations at H16/R120, preferably, H16E/R120F;~~

~~(c). mutations at H16/L18/V91/F117; preferably, H16E/L18I/V91A/F117W;~~

~~(d). mutations at H16/L18/I89/V93; preferably, H16E/L18I/I89L/V93I;~~

~~(e). mutations at H16/G27/R120; preferably, H16E/G27W/R120F;~~

~~(f). mutations at H16/P82/R120; preferably, H16E/P82L/R120F;~~

(g). mutations at D20/Y31/A73/H79; preferably, D20A/Y31V/A73L/H79Q;

(h). mutations at D20/R120; preferably, D20A/R120F;

(i). mutations at V91/Y31/A73/H79; preferably, V91R/Y31V/A73L/H79Q;

(j). mutations at V91/Q13; preferably, V91R/Q13L;

5 (k). mutations at V91/R120; preferably, V91R/R120F;

(l). mutations at V91/L18/I89/V93; preferably, V91R/L18I/I89L/V93I;—

(m). — mutations — at — H16/V91/Y31/A73/H79; — preferably,
H16E/V91R/Y31V/A73L/H79Q;

10 (n). — mutations — at — H16/V91/L18/I89/V93; — preferably,
H16E/V91R/L18I/I89L/V93I.

8. The IL-2 mutant according to claim 7, wherein the IL-2 mutant has an amino acid sequence as shown in SEQ ID NOs: 22 to 27, SEQ ID NOs:29 to 30, SEQ ID NOs:32 to 35 or SEQ ID NOs:37 to 38.

15 9. The IL-2 mutant according to any one of claims 1 to 3 and 5, wherein the IL-2 mutant further comprises one or more mutation(s) selected from the group consisting of mutation at F42, Y45 or L72, preferably, F42A, Y45A or L72G.

10. An IL-2 mutant comprising one or more mutation(s) at H16, D20 or V91 compared to wild type IL-2; preferably, the IL-2 mutant comprises at least one group of mutation(s) selected from the groups (i)-(iv):

20 (i). a mutation at H16; preferably, H16E;

(ii). a mutation at D20; preferably, D20A;

(iii). a mutation at V91; preferably, V91R;

(iv). mutations at H16/V91; preferably, H16E/V91R.

11. The IL-2 mutant according to claim 10, wherein the IL-2 mutant has an amino acid sequence as shown in SEQ ID NO: 21, SEQ ID NO:28, SEQ ID NO:31 or
5 SEQ ID NO:36.

12. The IL-2 mutant according to any one of claims 1 to 3, 5 to 7 and 9 to 10, wherein the IL-2 mutant further comprises one or more mutation(s) selected from the group consisting of mutation at N26, N29, N30, N71, Q11, L132, L70, P82, G27 or
F28;

10 preferably, the IL-2 mutant further comprises one or more mutation(s) selected from the group consisting of N26Q, N29S, N30S, N71Q, Q11C, L132C, L70C, P82C, G27C or F78C;

more preferably, the IL-2 mutant further comprises at least one group of mutation(s) in groups (a)–(g):

15 (a). N26Q;

(b). N29S;

(c). N30S;

(d). N71Q;

(e). Q11C/L132C;

20 (f). L70C/P82C;

(g). G27C/F78C.

~~134~~. The IL-2 mutant according to any one of claims ~~6-1~~ to ~~8-3~~~~and 10 to 12~~, wherein the IL-2 mutant has a reduced binding ability to IL-2R $\beta\gamma$ subunit complex compared to the wild-type IL-2; preferably, the binding ability IL-2R $\beta\gamma$ subunit complex/binding ability IL-2 $\alpha\beta\gamma$ subunit complex decreases.

5 ~~145~~. The IL-2 mutant according to any one of claim ~~131~~ to 4, wherein the mutant has a reduced stimulation ability to non-regulatory T cells or NK (natural killer) cells compared to the wild-type IL-2; the stimulation can be selected from intracellular STAT5 phosphorylation or cell proliferation;

alternatively, the mutant preferentially stimulates regulatory T cells (Tregs) in
10 peripheral blood or T cell population compared to non-regulatory T cells or NK (natural killer) cells; said preferentially stimulating can be selected from preferentially stimulating STAT5 phosphorylation in regulatory T cells, preferentially stimulating regulatory T cell proliferation, increasing regulatory T cells to non-regulatory T cells ratio, or increasing regulatory T cells to NK cells ratio.

15 ~~615~~. The IL-2 mutant according to any one of claims 1 to ~~145~~, wherein the mutation comprises deletion, insertion or substitution, preferably substitution.

~~167~~. The IL-2 mutant according to any one of claims 1 to ~~156~~, wherein the wild-type IL-2 has an amino acid sequence as shown in SEQ ID NO: 60 or SEQ ID NO: 1.

20 ~~178~~. A fusion protein comprising a first polypeptide and a second polypeptide, wherein the first polypeptide is the IL-2 mutant according to any one of claims 1 to ~~167~~, and the second polypeptide is a non-IL-2 polypeptide.

[189](#). The fusion protein according to claim [178](#), wherein the second polypeptide is an Fc, a tumor-antigen-binding molecule or an IL-2 receptor subunit;

optionally, the Fc is a human IgG Fc, for example, a human IgG1 Fc;

preferably, the human IgG1 Fc comprises at least one group of mutation(s)

5 selected from groups (a) - (i):

(a). C220S;

(b). N297G;

(c). C220S and N297G;

(d). A327Q;

10 (e). L234A and L235A;

(f). A287C and L306C;

(g). A259C and V302C;

(h). R292C and V306C;

(i). V323C and I332C;

15 more preferably, the human IgG1 Fc has an amino acid sequence as shown in SEQ ID NO: 11;

optionally, the tumor-antigen comprises EDB-FN (extra domain of fibronectin), Muc1, p53, FAP, GD2, EpCAM, tenascin-C, CD20, CEA, MAdCAM-1 or WT1 (Wilms Tumor Protein 1); optionally, the tumor-antigen-binding molecule is an
20 antibody, such as scFv, sdFv, Fab, Fab', F(ab')₂ or Fv;

optionally, the IL-2 receptor subunit is an IL-2 receptor α subunit.

1910. The fusion protein according to any one of claims 17-8 to 189, wherein C-terminus of the first polypeptide is linked to N-terminus of the second polypeptide with or without a linker; or N-terminus of the first polypeptide is linked to C-terminus of the second polypeptide with or without a linker;

preferably, the linker is selected from: $(G_4S)_n$, $(GGNGT)_n$ or $(YGNGT)_n$, and the n is selected from 1, 2, 3, 4 or 5;

more preferably, the C-terminus of the first polypeptide is linked to the N-terminus of the second polypeptide with a linker $(G_4S)_3$.

2011. The fusion protein according to any one of claims 8 to 10~~claim 17~~, wherein the fusion protein comprises an amino acid sequence as shown in any one of SEQ ID NOs: 13, 40, 47, 50 or 55~~to 20 or SEQ ID NOs: 39 to 56~~.

~~21. A conjugate comprising the mutant of any one of claims 1 to 16 or the fusion protein of any one of claims 17 to 20, and further comprising a stabilizer, drug or tracer molecule conjugated to the mutant or the fusion protein; wherein the stabilizer can be selected from polyethylene glycol, such as monomethoxy polyethylene glycol.~~

~~22. An isolated nucleic acid fragment encoding the mutant of any one of claims 1 to 16 or the fusion protein of any one of claims 17 to 20.~~

~~23. A vector comprising the nucleic acid fragment of claim 22.~~

~~24. A host cell comprising the vector of claim 23; the host cell is a prokaryotic cell or a eukaryotic cell; the prokaryotic cell or the eukaryotic cell can be selected from *Escherichia coli*, yeast, insect cells or mammalian cells, and the mammalian~~

cells can be selected from a CHO cell line or a HEK293 cell line.

25. A method for preparing the mutant of any one of claims 1 to 16 or the fusion protein of any one of claims 17 to 20, wherein the method comprises culturing the host cell of claim 24 and isolating the IL-2 mutant or the fusion protein expressed by the host cell.

26. An IL-2 mutant or fusion protein prepared according to the method of claim 25.

27. A pharmaceutical composition comprising the mutant of any one of claims 1 to 16 or the fusion protein of any one of claims 17 to 20, the conjugate of claim 21, the nucleic acid fragment of claim 22, the vector of claim 23, the host cell of claim 24, or the mutant or fusion protein of claim 26; and a pharmaceutically acceptable carrier, diluent or adjuvant;

preferably, the pharmaceutical composition is a pharmaceutical composition for injection, e.g. for intravenous or subcutaneous injection; more preferably, the pharmaceutical composition per dose comprises an effective amount of fusion protein to be administrated to a subject; most preferably, the effective amount is 0.001-10 mpk, such as 0.001 mpk, 0.002 mpk, 0.003 mpk, 0.004 mpk, 0.005 mpk, 0.006 mpk, 0.007 mpk, 0.008 mpk, 0.009 mpk, 0.01 mpk, 0.02 mpk, 0.03 mpk, 0.04 mpk, 0.05 mpk, 0.06 mpk, 0.07 mpk, 0.08 mpk, 0.09 mpk, 0.1 mpk, 0.2 mpk, 0.3 mpk, 0.4 mpk, 0.5 mpk, 0.6 mpk, 0.7 mpk, 0.8 mpk, 0.9 mpk, 1 mpk, 2 mpk, 3 mpk, 4 mpk, 5 mpk, 6 mpk, 7 mpk, 8 mpk, 9 mpk or 10 mpk.

28. Use of the mutant of any one of claims 1 to 16 or the fusion protein of

any one of claims ~~17-8~~ to ~~2011~~, ~~the conjugate of claim 21, the nucleic acid fragment of claim 22, the vector of claim 23, the host cell of claim 24, or the mutant or fusion protein of claim 26~~ in the manufacture of a medicament for treating disease;

preferably, the medicament is a medicament for injection, e.g. for intravenous or
5 subcutaneous injection;

preferably, the medicament per dose comprises an effective amount of fusion protein to be administrated to a subject; most preferably, the effective amount is 0.001-10 mpk, such as 0.001 mpk, 0.002 mpk, 0.003 mpk, 0.004 mpk, 0.005 mpk, 0.006 mpk, 0.007 mpk, 0.008 mpk, 0.009 mpk, 0.01 mpk, 0.02 mpk, 0.03 mpk, 0.04
10 mpk, 0.05 mpk, 0.06 mpk, 0.07 mpk, 0.08 mpk, 0.09 mpk, 0.1 mpk, 0.2 mpk, 0.3 mpk, 0.4 mpk, 0.5 mpk, 0.6 mpk, 0.7 mpk, 0.8 mpk, 0.9 mpk, 1 mpk, 2 mpk, 3 mpk, 4 mpk, 5 mpk, 6 mpk, 7 mpk, 8 mpk, 9 mpk or 10 mpk;

preferably, the medicament is used for treating an autoimmune disease, proliferative disease, or viral infection;

15 more preferably, the autoimmune disease comprises rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, cutaneous lupus erythematosus, lupus nephritis, IgA nephropathy, Sjogren's syndrome, polymyositis, dermatomyositis, scleroderma, psoriasis, plaque psoriasis, alopecia areata, multiple sclerosis, amyotrophic lateral sclerosis, inflammatory bowel disease, ulcerative colitis, Crohn's
20 disease, graft-versus-host disease, organ transplant rejection, autoimmune hepatitis, type I diabetes, autoimmune vasculitis, eczema or asthma;

more preferably, the proliferative disease comprises neoplasm, solid tumor,

hematological tumor, malignant ascites or malignant pleural effusion; wherein the solid tumor can be selected from benign or malignant, primary or metastatic, the malignant solid tumor can be selected from be a cancer or a sarcoma, for example, epithelial cell carcinoma, endothelial cell carcinoma, squamous cell carcinoma, teratoma, lung tumor, papillomavirus-induced cancer, adenocarcinoma, carcinoma, melanoma, angiosarcoma, neuroblastoma, metastatic lung cancer, non-small cell lung cancer, small cell lung cancer, breast cancer, Merkel cell cancer, ovarian cancer, renal cell cancer, metastatic renal cancer, head and neck cancer, bladder cancer, non-muscle invasive bladder cancer; hematological tumor can be selected from leukemia, lymphoma, multiple myeloma, such as B-cell lymphoma, T-cell lymphoma, cutaneous T-cell lymphoma, T-cell large granular lymphocytic leukemia;

more preferably, the viral infection is selected from HIV infection, novel coronavirus infection or HPV viral infection.

~~2914. A method for treating autoimmune diseases, proliferative diseases, or viral infection, wherein the method comprises a step of administering to a subject an effective amount of the IL-2 mutant of any one of claims 1 to 167, the fusion protein of any one of claims 17 8 to 2011, the conjugate of claim 21, the nucleic acid fragment of claim 22, the vector of claim 23, the host cell of claim 24, the mutant or fusion protein of claim 26, or the pharmaceutical composition of claim 2712;~~

~~preferably, the step of administering is performed via injection, e.g. intravenous or subcutaneous injection;~~

~~preferably, the effective amount is 0.001-10 mpk, such as 0.001 mpk, 0.002 mpk, 0.003 mpk, 0.004 mpk, 0.005 mpk, 0.006 mpk, 0.007 mpk, 0.008 mpk, 0.009 mpk,~~

~~0.01 mpk, 0.02 mpk, 0.03 mpk, 0.04 mpk, 0.05 mpk, 0.06 mpk, 0.07 mpk, 0.08 mpk, 0.09 mpk, 0.1 mpk, 0.2 mpk, 0.3 mpk, 0.4 mpk, 0.5 mpk, 0.6 mpk, 0.7 mpk, 0.8 mpk, 0.9 mpk, 1 mpk, 2 mpk, 3 mpk, 4 mpk, 5 mpk, 6 mpk, 7 mpk, 8 mpk, 9 mpk or 10 mpk;~~

5 ~~preferably, the autoimmune disease comprises rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, cutaneous lupus erythematosus, lupus nephritis, IgA nephropathy, Sjogren's syndrome, polymyositis, dermatomyositis, scleroderma, psoriasis, plaque psoriasis, alopecia areata, multiple sclerosis, amyotrophic lateral sclerosis, inflammatory bowel disease, ulcerative colitis, Crohn's~~
 10 ~~disease, graft versus host disease, organ transplant rejection, autoimmune hepatitis, type I diabetes, autoimmune vasculitis, eczema or asthma;~~

~~preferably, the proliferative disease comprises neoplasm, solid tumor, hematological tumor, malignant ascites or malignant pleural effusion; wherein the solid tumor is optionally selected from benign or malignant, primary or metastatic, the~~
 15 ~~malignant solid tumor is optionally selected from be a cancer or a sarcoma, for example, epithelial cell carcinoma, endothelial cell carcinoma, squamous cell carcinoma, teratoma, lung tumor, papillomavirus-induced cancer, adenocarcinoma, carcinoma, melanoma, angiosarcoma, neuroblastoma, metastatic lung cancer, non-small cell lung cancer, small cell lung cancer, breast cancer, Merkel cell cancer,~~
 20 ~~ovarian cancer, renal cell cancer, metastatic renal cancer, head and neck cancer, bladder cancer, non-muscle invasive bladder cancer; the hematological tumor is optionally selected from leukemia, lymphoma, multiple myeloma, such as B-cell lymphoma, T-cell lymphoma, cutaneous T-cell lymphoma, T-cell large granular~~

~~lymphocytic leukemia.~~

30. ~~A method for preferentially stimulating a T cell population or regulatory T cells in peripheral blood, wherein the method comprises a step of contacting the T cell population or peripheral blood with the IL-2 mutant of any one of claims 1 to 16, the fusion protein of any one of claims 17 to 20, the conjugate of claim 21, the nucleic acid fragment of claim 22, the vector of claim 23, the host cell of claim 24, the mutant or fusion protein of claim 26, or the pharmaceutical composition of claim 27; preferably, said preferentially stimulating comprises:~~

(a) ~~preferentially stimulating STAT5 phosphorylation of regulatory T cells compared to non-regulatory T cells or NK cells;~~

(b) ~~preferentially stimulating proliferation of regulatory T cells compared to non-regulatory T cells or NK cells; and/or~~

(c) ~~increasing regulatory T cells to non-regulatory T cells ratio, or increasing regulatory T cells to NK cells ratio.~~