

INTERLEUKIN-2 AGENTS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/879,137, filed on July 26, 2019, and U.S. Provisional Application No. 62/983,061, filed February 28, 2020. The contents of the aforementioned applications are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 20, 2020, is named P2029-7028WO_SL.txt and is 1,728,528 bytes in size.

BACKGROUND

Interleukin-2 (IL-2) is a cytokine that regulates the activities of the immune system. It is produced by leukocytes, such as T cells, natural killer (NK) cells, dendritic cells, and macrophages, in response to antigenic or mitogenic stimulation. IL-2 is important for T cell proliferation, B cell stimulation, and other activities associated with immunity and tolerance. It is part of the body's adaptive immune response and discriminates between foreign and host antigens. IL-2 mediates its effects by binding to IL-2 receptors, which in turn activate downstream signaling events.

Human IL-2 is an-FDA approved drug for the treatment of diseases such as metastatic renal carcinoma and melanoma. The use of IL-2 in eligible patients is sometimes restricted due to the severe toxicity associated with IL-2 therapy, and only a small subset of eligible patients will actually receive therapy. The toxicities associated with IL-2 therapy can include severe fever, nausea, vomiting, vascular leak and serious hypotension. Despite these toxicities, however, IL-2 is typically effective for its approved indications.

For patients with various diseases and conditions that are amenable to treatment with IL-2, there continues to be an unmet need for novel IL-2-based agents that exhibit characteristics sufficient for the development of a safe and efficacious therapeutic.

SUMMARY

This disclosure provides, at least in part, IL-2 agents (*e.g.*, IL-2 variants, IL-2 fusion proteins, IL-2 complexes, and IL-2 conjugates) that comprise one or more amino acid alterations (*e.g.*, substitutions) in IL-2, and that comprise one or more of the structural or functional properties disclosed herein. In an ~~embodiment~~example, nucleic acid molecules encoding the IL-2 agents, expression vectors, host cells, compositions (*e.g.*, pharmaceutical compositions), kits, containers, and methods for making the IL-2 agents, are also provided. The IL-2 agents disclosed herein can be used (alone or

in combination with other agents or therapeutic modalities) to treat, prevent, and/or diagnose disorders, such as disorders and conditions disclosed herein.

The present disclosure is based, at least in part, on the discovery that a combination of mutations in IL-2 that stabilize the protein, reduce its affinity for CD122 (*e.g.*, CD122/CD132 heterodimer), and/or reduce or have no more than a minimal effect on its affinity for CD25, can be used to selectively enhance regulatory T cell (Treg) activity through the IL-2 pathway, and therefore achieve advantageous therapeutic effects for treating disorders and conditions such as autoimmune diseases. IL-2 agents comprising such mutations are suitable for treating conditions arising from abnormal immune responses, such as autoimmune diseases.

10 In one aspect, the present disclosure refers to an interleukin-2 (IL-2) variant, comprising:
 (i) the amino acid substitution H16L or H16N, and/or the amino acid substitution I92S, and
 (ii) the amino acid substitutions V69A, Q74P, and C125S,
 (iii) corresponding to human IL-2 (SEQ ID NO: 1031).

15 In another aspect, the present disclosure refers to an IL-2 fusion protein comprising the IL-2
 variant as disclosed herein.

In another aspect, the present disclosure refers to an IL-2 complex comprising the IL-2 variant
 as disclosed herein and an anti-IL-2 antibody molecule.

In another aspect, the present disclosure refers to an IL-2 conjugate comprising the IL-2
 variant as disclosed herein and a non-IL-2 moiety.

20 In another aspect, the present disclosure refers to a pharmaceutical composition comprising
 any of:

(i) the IL-2 variant as disclosed herein,
 (ii) the IL-2 fusion protein as disclosed herein,
 (iii) the IL-2 complex as disclosed herein, or
25 (iv) the IL-2 conjugate as disclosed herein; and
 a pharmaceutically acceptable carrier.

In another aspect, the present disclosure refers to a nucleic acid encoding any of:

(i) the IL-2 variant as disclosed herein,
 (ii) the IL-2 fusion as disclosed herein,
30 (iii) the IL-2 complex as disclosed herein, or
 (iv) the IL-2 conjugate as disclosed herein.

In another aspect, the present disclosure refers to a cell comprising the nucleic acid as
 disclosed herein.

In another aspect, the present disclosure refers to a method of producing an IL-2 variant,
35 comprising culturing the cell as disclosed herein under conditions that allow expression of the IL-2
 variant.

In yet another aspect, the present disclosure refers to a method of enhancing regulatory T cell (Treg) expansion, activity, survival, and/or proliferation, comprising contacting a Treg cell or a population of Treg cells *in vitro*, *ex vivo*, or *in vivo*, or administering to a subject in need thereof an effective amount of any of:

- 5 (i) the IL-2 variant as disclosed herein,
 (ii) the IL-2 fusion protein as disclosed herein,
 (iii) the IL-2 complex of as disclosed herein, or
 (iv) the IL-2 conjugate of as disclosed herein.

In yet another aspect, the present disclosure refers to a method of selectively activating the IL-2 signaling pathway in regulatory T cells (Tregs), comprising contacting a Treg cell or a population of Treg cells *in vitro*, *ex vivo*, or *in vivo*, or administering to a subject in need thereof an effective amount of any of:

- (i) the IL-2 variant as disclosed herein,
 (ii) the IL-2 fusion protein as disclosed herein,
15 (iii) the IL-2 complex of as disclosed herein, or
 (iv) the IL-2 conjugate of as disclosed herein.

In yet another aspect, the present disclosure refers to the use of any of

- (i) the IL-2 variant as disclosed herein,
 (ii) the IL-2 fusion protein as disclosed herein,
20 (iii) the IL-2 complex of as disclosed herein, or
 (iv) the IL-2 conjugate of as disclosed herein

in the manufacture of a medicament for inducing immune tolerance.

In yet another aspect, the present disclosure refers to the use of any of

- (i) the IL-2 variant as disclosed herein,
25 (ii) the IL-2 fusion protein as disclosed herein,
 (iii) the IL-2 complex of as disclosed herein, or
 (iv) the IL-2 conjugate of as disclosed herein

in the manufacture of a medicament for treating an autoimmune disease.

In yet another aspect, the present disclosure refers to the use of any of

- 30 (i) the IL-2 variant as disclosed herein,
 (ii) the IL-2 fusion protein as disclosed herein,
 (iii) the IL-2 complex of as disclosed herein, or
 (iv) the IL-2 conjugate of as disclosed herein

in the manufacture of a medicament for treating lupus nephritis in a subject.

35 In yet another aspect, the present disclosure refers to the use of any of

- (i) the IL-2 variant as disclosed herein,
 (ii) the IL-2 fusion protein as disclosed herein,

(iii) the IL-2 complex of as disclosed herein, or
(iv) the IL-2 conjugate of as disclosed herein
in the manufacture of a medicament for treating autoimmune hepatitis in a subject.

In yet another aspect, the present disclosure refers to the use of any of

(i) the IL-2 variant as disclosed herein,

(ii) the IL-2 fusion protein as disclosed herein,

(iii) the IL-2 complex of as disclosed herein, or

(iv) the IL-2 conjugate of as disclosed herein

in the manufacture of a medicament for treating nephrotic syndrome in a subject.

In yet another aspect, the present disclosure refers to a kit comprising any of:

(i) the IL-2 variant as disclosed herein,

(ii) the IL-2 fusion protein as disclosed herein,

(iii) the IL-2 complex of as disclosed herein, or

(iv) the IL-2 conjugate of as disclosed herein; and

instructions for use.

In yet another aspect, the present disclosure refers to the IL-2 variant as disclosed herein, the IL-2 fusion protein as disclosed herein, the IL-2 complex as disclosed herein, or the IL-2 conjugate as disclosed herein for use as a medicament.

Accordingly, in certain ~~aspect~~ examples, this disclosure provides an IL-2 agent, *e.g.*, an IL-2 agent having one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or all) of the following properties a)-x):

a) Expresses at a higher or increased level *in vitro* and/or *in vivo*, *e.g.*, increased by about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, or more, or by increased by about 0.5-fold, about 1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 5.5-fold, about 6-fold, about 6.5-fold, about 7-fold, about 7.5-fold, about 8-fold, about 8.5-fold, about 9-fold, about 9.5-fold, about 10-fold, or more, *e.g.*, relative to an IL-2 agent comprising a wild-type IL-2 or an IL-2 agent comprising a reference IL-2 variant, *e.g.*, as by an assay of protein concentration;

b) Aggregates at lower or decreased level *in vitro* and/or *in vivo*, *e.g.*, decreased by about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, or more, or decreased by about 0.5-fold, about 1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold,

- 5 about 5.5-fold, about 6-fold, about 6.5-fold, about 7-fold, about 7.5-fold, about 8-fold, about 8.5-fold, about 9-fold, about 9.5-fold, about 10-fold, or more *e.g.*, relative to an IL-2 agent comprising a wild-type IL-2 or an IL-2 agent comprising a reference IL-2 variant, *e.g.*, as determined by melting temperature analysis (*e.g.*, using fluorimetry), dynamic light scattering, and/or size-exclusion chromatography;
- c) Has enhanced or increased stability *in vitro* and/or *in vivo*, *e.g.*, increased by about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about

determined flow cytometry, a T regulatory cell proliferation or expansion assay *in vitro* or *in vivo*, and/or a T cell suppression assay;

- o) Modulates (*e.g.*, reduces (*e.g.*, inhibits, blocks, or neutralizes) or increases (*e.g.*, activates, initiates, or enhances) one or more biological activities of a T cell (*e.g.*, Treg), *in vitro*, *ex vivo*, or *in vivo*;
- p) Shows the same or similar binding affinity or specificity, or both, as an IL-2 agent described herein;
- q) Shows the same or similar binding affinity or specificity, or both, as an IL-2 agent comprising one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) alterations (*e.g.*, substitutions) described herein;
- r) Shows the same or similar binding affinity or specificity, or both, as an IL-2 agent comprising an amino acid sequence described herein;
- s) Shows the same or similar binding affinity or specificity, or both, as an IL-2 agent comprising an amino acid sequence encoded by a nucleotide sequence described herein;
- t) Inhibits, *e.g.*, competitively inhibits, the binding of a second IL-2 agent to an IL-2 receptor, wherein the second IL-2 agent is an IL-2 agent described herein,
- u) Competes for binding to an IL-2 receptor with a second IL-2 agent, wherein the second IL-2 agent is an IL-2 agent described herein;
- v) Has one or more biological properties of an IL-2 agent described herein;
- w) Has one or more structural properties of an IL-2 agent described herein; or
- x) Has one or more pharmacokinetic properties of an IL-2 agent described herein.

In an ~~embodiment~~example, the IL-2 agent is expressed at a higher or increased level *in vitro* and/or *in vivo*, *e.g.*, increased by about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, or more, or by increased by about 0.5-fold, about 1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 5.5-fold, about 6-fold, about 6.5-fold, about 7-fold, about 7.5-fold, about 8-fold, about 8.5-fold, about 9-fold, about 9.5-fold, about 10-fold, or more, *e.g.*, relative to an IL-2 agent comprising a wild-type IL-2 or an IL-2 agent comprising a reference IL-2 variant, *e.g.*, as by an assay of protein concentration. In an ~~embodiment~~example, the IL2-agent aggregates at lower or decreased level *in vitro* and/or *in vivo*, *e.g.*, decreased by about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, or more, or decreased by about 0.5-fold, about 1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 5.5-fold, about 6-fold, about 6.5-fold, about 7-fold, about 7.5-fold, about 8-fold, about 8.5-fold, about 9-fold, about 9.5-fold, about 10-fold, or more *e.g.*,

relative to an IL-2 agent comprising a wild-type IL-2 or an IL-2 agent comprising a reference IL-2 variant, *e.g.*, as determined by melting temperature analysis (*e.g.*, using fluorimetry), dynamic light scattering, and/or size-exclusion chromatography.

In an ~~embodiment~~example, the IL-2 agent has enhanced or increased stability *in vitro* and/or *in vivo*, *e.g.*, increased by about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, or more, or increased by about 0.5-fold, about 1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 5.5-fold, about 6-fold, about 6.5-fold, about 7-fold, about 7.5-fold, about 8-fold, about 8.5-fold, about 9-fold, about 9.5-fold, about 10-fold, or more, *e.g.*, relative to an IL-2 agent comprising a wild-type IL-2 or an IL-2 agent comprising a reference IL-2 variant, *e.g.*, as determined by expression in yeast surface display, expression in mammalian cells, chromatography, circular dichroism or related spectroscopic technical, and/or melting temperature analysis (*e.g.*, using fluorimetry).

In an ~~embodiment~~example the IL-2 agent as enhanced or increased half-life *in vitro* and/or *in vivo*, *e.g.*, increased by about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, or more, or greater than about 0.5-fold, about 1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 5.5-fold, about 6-fold, about 6.5-fold, about 7-fold, about 7.5-fold, about 8-fold, about 8.5-fold, about 9-fold, about 9.5-fold, about 10-fold, or more, *e.g.*, relative to an IL-2 agent comprising a wild-type IL-2 or an IL-2 agent comprising a reference IL-2 variant, *e.g.*, as determined by ELISA, flow cytometry, and/or mass spectrometry.

In an ~~embodiment~~example, the IL-2 agent has a lower, reduced or decreased rate or level of turnover and/or clearance *in vivo*, *e.g.*, decreased by about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, or more, or decreased by about 0.5-fold, about 1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 5.5-fold, about 6-fold, about 6.5-fold, about 7-fold, about 7.5-fold, about 8-fold, about 8.5-fold, about 9-fold, about 9.5-fold, about 10-fold, or more, *e.g.*, relative to an IL-2 agent comprising a wild-type IL-2 or an IL-2 agent comprising a reference IL-2 variant, *e.g.*, as determined by ELISA, flow cytometry, and/or mass spectrometry.

In an ~~embodiment~~example, the IL-2 agent has reduced or decreased or substantially unchanged binding affinity for CD25 (*e.g.*, human CD25), *e.g.*, decreased by about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about

40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, or more (*e.g.*, about 1% to about 20%, about 2% to about 15%, or about 5% to about 10%), or decreased or increased by no more than about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or about 50%, or decreased by about 0.5-fold, about 1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 5.5-fold, about 6-fold, about 6.5-fold, about 7-fold, about 7.5-fold, about 8-fold, about 8.5-fold, about 9-fold, about 9.5-fold, about 10-fold, or more, or decreased or increased by no more than about 0.5-fold, about 1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold, *e.g.*, relative to an IL-2 agent comprising a wild-type IL-2 or an IL-2 agent comprising a reference IL-2 variant *e.g.*, as determined by yeast surface display, bio-layer interferometry (*e.g.* Octet binding), and/or surface plasmon resonance (*e.g.* Biacore). In an embodiment example, the reduction or decrease of binding affinity for CD25 is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% lower than the reduction or decrease of binding affinity for CD25. In an embodiment example, the binding affinity for CD25 is not substantially reduced or decreased.

In an embodiment example, the IL-2 agent binds to CD25 (*e.g.*, human CD25) with low affinity, *e.g.*, with a dissociation constant (KD) of about 5-500 pM, *e.g.*, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 105, about 110, about 115, about 120, about 125, about 130, about 135, about 140, about 145, about 150, about 200, about 250, about 300, about 350, about 400, about 450, or about 500 pM, or *e.g.*, about 10 pM to about 490 pM, about 20 pM to about 480 pM, about 30 pM to about 470 pM, about 40 pM to about 460 pM, about 50 pM to about 450 pM, about 60 pM to about 440 pM, about 70 pM to about 430 pM, about 80 pM to about 420 pM, about 90 pM to about 410 pM, about 100 pM to about 400 pM, about 110 pM to about 390 pM, about 120 pM to about 380 pM, about 130 pM to about 370 pM, about 140 pM to about 360 pM, about 150 pM to about 350 pM, about 160 pM to about 340 pM, about 170 pM to about 330 pM, about 180 pM to about 320 pM, about 190 pM to about 310 pM, about 200 pM to about 300 pM, about 210 pM to about 290 pM, about 220 pM to about 280 pM, about 230 pM to about 270 pM, about 240 pM to about 260 pM, or *e.g.*, about 5 pM to about 450 pM, about 5 pM to about 400 pM, about 5 pM to about 350 pM, about 5 pM to about 300 pM, about 5 pM to about 250 pM, about 5 pM to about 200 pM, about 5 pM to about 150 pM, about 5 pM to about 100 pM, about 5 pM to about 50 pM, or *e.g.*, about 10 pM to about 500 pM, about 20 pM to about 500 pM, about 50 pM to about 500 pM, about 100 pM to about 500 pM, about 150 pM to about 500 pM, about 200 pM to about 500 pM, about 250 pM to about 500 pM, about 300 pM to about 500 pM, about 350 pM to about 500 pM, about 400 pM to about 500 pM, about 450 pM to about 500 pM, or *e.g.*, greater than about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 105, about 110,

about 115, about 120, about 125, about 130, about 135, about 140, about 145, about 150, about 200, about 250, about 300, about 350, about 400, about 450, or about 500 pM, *e.g.* as determined yeast surface display.

In an ~~embodiment~~example, the IL-2 agent binds to CD25 (*e.g.*, human CD25) with low affinity, *e.g.*, with a dissociation constant (KD) of about 0.1-10 nM, *e.g.*, about 0.1, about 0.2, about 0.3, about 0.4, about 0.5, about 0.6, about 0.7, about 0.8, about 0.9, about 1, about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 6, about 7, about 8, about 9, or about 10 nM, or *e.g.*, about 0.1 to about 9 nM, about 0.1 to about 8 nM, about 0.1 to about 7 nM, or about 0.1 to about 6 nM, *e.g.*, about 0.1 to about 5 nM, about 0.1 to about 4 nM, about 0.1 to about 3 nM, about 0.1 to about 2 nM, about 0.1 to about 1 nM, or about 0.1 to about 0.5 nM, or *e.g.*, about 0.1 to about 10 nM, about 0.5 to about 10 nM, about 1 to about 10 nM, about 1.5 to about 10 nM, about 2 to about 10 nM, about 2.5 to about 10 nM, about 3 to about 10 nM, about 3.5 to about 10 nM, about 4 to about 10 nM, about 4.5 to about 10 nM, about 5 to about 10 nM, about 5.5 to about 10 nM, about 6 to about 10 nM, about 6.5 to about 10 nM, about 7 to about 10 nM, about 7.5 to about 10 nM, about 8 to about 10 nM, about 8.5 to about 10 nM, about 9 to about 10 nM, or about 9.5 to about 10 nM, or *e.g.*, about 0.1 to about 9.5 nM, about 0.5 to about 9 nM, about 1 to about 8.5 nM, about 1.5 to about 8 nM, about 2 to about 7.5 nM, about 2.5 to about 7 nM, about 3 to about 6.5 nM, about 3.5 to about 6 nM, about 4 to about 5.5 nM, or about 4.5 to about 5 nM, or *e.g.*, greater than about 0.1, about 0.2, about 0.3, about 0.4, about 0.5, about 0.6, about 0.7, about 0.8, about 0.9, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10 nM, *e.g.*, as determined by bio-layer interferometry (*e.g.*, Octet binding) and/or surface plasmon resonance (*e.g.* Biacore).

In an ~~embodiment~~example, the IL-2 agent has reduced or decreased binding affinity for CD122/CD132 heterodimer (*e.g.*, human CD122/CD132 heterodimer), *e.g.*, decreased by about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, or more (*e.g.*, about 1% to about 50%, about 2% to about 40%, about 3% to about 30%, about 4% to about 20%, or about 5% to about 10%, about 1% to about 40%, about 1% to about 30%, about 1% to about 20%, about 1% to about 10%, about 40% to about 50%, about 30% to about 50%, about 20% to about 50%, about 10% to about 50%, about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 10% to about 30%, or 20% to about 40%), or decreased by about 0.5-fold, about 1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 5.5-fold, about 6-fold, about 6.5-fold, about 7-fold, about 7.5-fold, about 8-fold, about 8.5-fold, about 9-fold, about 9.5-fold, about 10-fold, or more (*e.g.*, about 0.5-fold to about 5-fold, about 1-fold to about 4-fold, or about 2-fold to about 3-fold), *e.g.*, relative to an IL-2 agent comprising a wild-type IL-2 or an IL-2 agent comprising a reference IL-2 variant *e.g.*, as determined by yeast surface display, bio-layer interferometry (*e.g.* Octet binding), and/or surface plasmon resonance (*e.g.* Biacore). In an

~~embodiment~~example, the reduction or decrease of binding affinity for CD122/CD132 heterodimer is at least 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5-fold higher than the reduction or decrease of binding affinity for CD25. In an ~~embodiment~~example, the binding affinity for CD25 is not substantially reduced or decreased.

5 In an ~~embodiment~~example, the IL-2 agent binds to CD122/CD132 heterodimer (*e.g.*, human CD122/CD132 heterodimer) with low affinity, *e.g.*, with a dissociation constant (KD) of about 0.2-20 nM, *e.g.*, about 0.2, about 0.3, about 0.4, about 0.5, about 0.6, about 0.7, about 0.8, about 0.9, about 1, about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17,
10 about 18, or about 20 nM, or *e.g.*, about 0.2 to about 19 nM, about 0.2 to about 18 nM, about 0.2 to about 17 nM, or about 0.2 to about 16 nM, *e.g.*, about 0.2 to about 15 nM, about 0.1 to about 4 nM, about 0.1 to about 3 nM, about 0.1 to about 2 nM, about 0.1 to about 1 nM, or about 0.1 to about 0.5 nM, or *e.g.*, about 0.1 to about 10 nM, about 0.5 to about 10 nM, about 1 to about 10 nM, about 1.5 to about 10 nM, about 2 to about 10 nM, about 2.5 to about 10 nM, about 3 to about 10 nM, about 3.5 to
15 about 10 nM, about 4 to about 10 nM, about 4.5 to about 10 nM, about 5 to about 10 nM, about 5.5 to about 10 nM, about 6 to about 10 nM, about 6.5 to about 10 nM, about 7 to about 10 nM, about 7.5 to about 10 nM, about 8 to about 10 nM, about 8.5 to about 10 nM, about 9 to about 10 nM, or about 9.5 to about 10 nM, or *e.g.*, about 0.1 to about 9.5 nM, about 0.5 to about 9 nM, about 1 to about 8.5 nM, about 1.5 to about 8 nM, about 2 to about 7.5 nM, about 2.5 to about 7 nM, about 3 to about 6.5 nM,
20 about 3.5 to about 6 nM, about 4 to about 5.5 nM, or about 4.5 to about 5 nM, or *e.g.*, greater than about 0.2, about 0.3, about 0.4, about 0.5, about 0.6, about 0.7, about 0.8, about 0.9, about 1, about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, or about 20 nM, *e.g.*, as determined by yeast surface display.

25 In an ~~embodiment~~example, the IL-2 agent binds to CD122/CD132 heterodimer (*e.g.*, human CD122/CD132 heterodimer) with low affinity, *e.g.*, with a dissociation constant (KD) of about 0.2-300 nM, *e.g.*, about 0.2 nM, about 0.5 nM, about 1 nM, about 2 nM, about 5 nM, about 10 nM, about 15 nM, about 20 nM, about 25 nM, about 30 nM, about 40 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, about 100 nM, about 110 nM, about 120 nM, about 130 nM, about
30 140 nM, about 150 nM, about 160 nM, about 170 nM, about 180 nM, about 190 nM, about 200 nM, about 210 nM, about 220 nM, about 230 nM, about 240 nM, about 250 nM, about 260 nM, about 270 nM, about 280 nM, about 290 nM, or about 300 nM, or *e.g.*, about 0.2 to about 280 nM, about 0.2 to about 260 nM, about 0.2 to about 240 nM, about 0.2 to about 220 nM, about 0.2 to about 200 nM, about 0.2 to about 180 nM, about 0.2 to about 160 nM, about 0.2 to about 140 nM, about 0.2 to about
35 120 nM, about 0.2 to about 100 nM, about 0.2 to about 80 nM, about 0.2 to about 60 nM, about 0.2 to about 40 nM, about 0.2 to about 20 nM, or *e.g.*, about 0.5 to about 300 nM, about 1 to about 300 nM, about 5 to about 300 nM, about 10 to about 300 nM, about 20 to about 300 nM, about 40 to about 300 nM, about 60 to about 300 nM, about 80 to about 300 nM, about 100 to about 300 nM, about 120 to

about 300 nM, about 140 to about 300 nM, about 160 to about 300 nM, about 180 to about 300 nM, about 200 to about 300 nM, about 220 to about 300 nM, about 240 to about 300 nM, about 260 to about 300 nM, about 280 to about 300 nM, or *e.g.*, about 0.5 to about 280 nM, about 1 to about 260 nM, about 5 to about 240 nM, about 10 to about 220 nM, about 20 to about 200 nM, about 40 to about 180 nM, about 60 to about 160 nM, about 80 to about 140 nM, about 100 to about 120 nM, or *e.g.*, greater than about 0.2, about 0.5, about 1, about 2, about 5, about 10, about 15, about 20 nM, about 25 nM, about 30 nM, about 40 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, about 100 nM, about 110 nM, about 120 nM, about 130 nM, about 140 nM, about 150 nM, about 160 nM, about 170 nM, about 180 nM, about 190 nM, about 200 nM, about 210 nM, about 220 nM, about 230 nM, about 240 nM, about 250 nM, about 260 nM, about 270 nM, about 280 nM, about 290 nM, or greater than about 300 nM, *e.g.*, as determined by biolayer interferometry (*e.g.* Octet binding) and/or surface plasmon resonance (*e.g.* Biacore).

In an ~~embodiment~~example, the IL-2 agent selectively activates IL-2 signaling in T regulatory cells *in vitro* and/or *in vivo*, *e.g.*, having an T helper EC50/Treg EC50 ratio greater than about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 150, about 200, about 250, about 300, about 350, about 400, about 450, about 500, about 600, about 700, about 800, about 900, about 1000, about 1500, about 2000, about 2500, or about 3000, or more, or *e.g.*, greater than 1 and about 1 to 2, about 2 to 3, about 3 to 4, about 4 to 5, greater than 1 and about 1 to 10, greater than 1 and about 1 to 20, greater than 1 and about 1 to 30, greater than 1 and about 1 to 40, greater than 1 and about 1 to 50, about 2 to 10, about 2 to 20, about 2 to 30, about 2 to 40, 2 to 50, about 5 to 10, about 5 to 20, about 5 to 30, about 5 to 40, about 5 to 50, about 10 to 20, about 10 to 30, about 10 to 40 about 10 to 50, about 20 to 40, about 20 to 50, about 50 to 100, about 100 to 200, about 200 to 500, about 500 to 1000, about 1000 to 2000, or about 1000 to 3000, relative to an IL-2 agent comprising a wild-type IL-2 or an IL-2 agent comprising a reference IL-2 variant *e.g.*, as determined flow cytometry. In an ~~embodiment~~example, the T helper cell is a CD45+CD3+CD4+Foxp3- cell, *e.g.*, determined by flow cytometry. In an ~~embodiment~~example, the Treg is CD45+CD3+CD4+Foxp3+ cell, *e.g.*, determined by flow cytometry.

In an ~~embodiment~~example, the IL-2 agent selectively activates IL-2 signaling in T regulatory cells *in vitro* and/or *in vivo*, *e.g.*, having an NK cell EC50/Treg EC50 ratio greater than about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 150, about 200, about 250, about 300, about 350, about 400, about 450, about 500,

about 600, about 700, about 800, about 900, about 1000, about 1500, about 2000, about 2500, or about 3000, or more, or *e.g.*, greater than 1 and about 1 to 2, about 2 to 3, about 3 to 4, about 4 to 5, greater than 1 and about 1 to 10, greater than 1 and about 1 to 20, greater than 1 and about 1 to 30, greater than 1 and about 1 to 40, greater than 1 and about 1 to 50, about 2 to 10, about 2 to 20, about 2 to 30, about 2 to 40, about 2 to 50, about 5 to 10, about 5 to 20, about 5 to 30, about 5 to 40, about 5 to 50, about 10 to 20, about 10 to 30, about 10 to 40, about 10 to 50, about 20 to 40, about 20 to 50, about 50 to 100, about 100 to 200, about 200 to 500, about 500 to 1000, about 1000 to 2000, or about 1000 to 3000, relative to an IL-2 agent comprising a wild-type IL-2 or an IL-2 agent comprising a reference IL-2 variant *e.g.*, as determined flow cytometry. In an ~~embodiment~~example, the NK cell is a CD45+CD3- cell that is CD56+ and/or CD16+, *e.g.*, determined by flow cytometry. In an ~~embodiment~~example, the NK cell is a CD45+CD3-CD56+ cell, *e.g.*, determined by flow cytometry. In an ~~embodiment~~example, the Treg is CD45+CD3+CD4+Foxp3+ cell, *e.g.*, determined by flow cytometry.

In an ~~embodiment~~example, the IL-2 agent has enhanced or increased potency and/or ability to induce or promote T regulatory cell activity, *e.g.*, having an EC50 for Tregs that is lower by about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100% or more, or *e.g.*, decreased by about 0.5-fold, about 1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 5.5-fold, about 6-fold, about 6.5-fold, about 7-fold, about 7.5-fold, about 8-fold, about 8.5-fold, about 9-fold, about 9.5-fold, about 10-fold or more *e.g.*, relative to an IL-2 agent comprising a wild-type IL-2 or an IL-2 agent comprising a reference IL-2 variant *e.g.*, as determined flow cytometry, a T regulatory cell proliferation or expansion assay *in vitro* or *in vivo*, and/or a T cell suppression assay.

In an ~~embodiment~~example, the IL-2 agent as reduced or decreased potency and/or ability to induce or promote T regulatory cell activity, *e.g.*, having an EC50 for Tregs that is higher by about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100% or more, or *e.g.*, decreased by about 0.5-fold, about 1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 5.5-fold, about 6-fold, about 6.5-fold, about 7-fold, about 7.5-fold, about 8-fold, about 8.5-fold, about 9-fold, about 9.5-fold, about 10-fold, about 50-fold, about 100-fold, about 200-fold, about 500-fold, about 1000-fold, about 2000-fold, about 5000-fold, about 10,000-fold, about 15,000-fold, about 20,000-fold or more *e.g.*, relative to an IL-2 agent comprising a wild-type IL-2 or an IL-2 agent comprising a reference IL-2 variant *e.g.*, as determined flow cytometry, a T regulatory cell proliferation or expansion assay *in vitro* or *in vivo*, and/or a T cell suppression assay. In an ~~embodiment~~example, the IL-2 agent has reduced or decreased potency and/or ability to induce or promote T regulatory cell activity, *e.g.*, having an EC50 for Tregs that is higher by about

100-fold or more, relative to an IL-2 agent comprising a wild-type IL-2 or an IL-2 agent comprising a reference IL-2 variant (*e.g.*, as determined flow cytometry, a T regulatory cell proliferation or expansion assay *in vitro* or *in vivo*, and/or a T cell suppression assay), and does not activate, or does not significantly activate, NK cells.

5 In an ~~embodiment~~example, the IL-2 agent modulates (*e.g.*, reduces (*e.g.*, inhibits, blocks, or neutralizes) or increases (*e.g.*, activates, initiates, or enhances) one or more biological activities of a T cell (*e.g.*, Treg), *in vitro*, *ex vivo*, or *in vivo*.

In an ~~embodiment~~example, the IL-2 agent shows the same or similar binding affinity or specificity, or both, as an IL-2 agent described herein.

10 In an ~~embodiment~~example, the IL-2 agent shows the same or similar binding affinity or specificity, or both, as an IL-2 agent comprising one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) alterations (*e.g.*, substitutions) described herein.

In an ~~embodiment~~example, the IL-2 agent shows the same or similar binding affinity or specificity, or both, as an IL-2 agent comprising an amino acid sequence described herein.

15 In an ~~embodiment~~example, the IL-2 agent shows the same or similar binding affinity or specificity, or both, as an IL-2 agent comprising an amino acid sequence encoded by a nucleotide sequence described herein.

In an ~~embodiment~~example, the IL-2 agent inhibits, *e.g.*, competitively inhibits, the binding of a second IL-2 agent to an IL-2 receptor, wherein the second IL-2 agent is an IL-2 agent described
20 herein.

In an ~~embodiment~~example, the IL-2 agent competes for binding to an IL-2 receptor with a second IL-2 agent, wherein the second IL-2 agent is an IL-2 agent described herein.

In an ~~embodiment~~example, the IL-2 agent has one or more biological properties of an IL-2 agent described herein.

25 In an ~~embodiment~~example, the IL-2 agent has one or more structural properties of an IL-2 agent described herein.

In an ~~embodiment~~example, the IL-2 agent has one or more pharmacokinetic properties of an IL-2 agent described herein.

30 In an ~~embodiment~~example, the interleukin-2 (IL-2) agent comprises a human IL-2 variant comprising an amino acid alteration (*e.g.*, substitution) at one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or all) position(s) chosen from: T3, H16, I28, K35, R38, F42, E68, V69, Q74, D84, S87, N88, I92, C125, Q126, or a combination thereof, *e.g.*, corresponding to wild-type human IL-2. In another ~~embodiment~~example, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position V69, Q74, or a combination thereof. In an ~~embodiment~~example, the IL-2 agent comprises an
35 amino acid alteration (*e.g.*, substitution) at positions V69 and Q74. In an ~~embodiment~~example, the IL-2 agent comprises the amino acid substitution V69A. In an ~~embodiment~~example, the IL-2 agent comprises the amino acid substitution Q74P. In an ~~embodiment~~example, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position H16, I92, D84, or a combination thereof. In an
40 ~~embodiment~~example, the IL-2 agent comprises an

amino acid alteration (*e.g.*, substitution) at position H16, optionally wherein the amino acid substitution is H16N, H16L, or H16D. In an embodimentexample, the IL-2 agent comprises the amino acid substitution H16N. In an embodimentexample, the IL-2 agent comprises the amino acid substitution H16L. In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position at I92, optionally wherein the amino acid substitution is I92S. In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position D84, optionally wherein the amino acid substitution is D84V. In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position K35, R38, F42, E68, or a combination thereof. In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position K35, optionally wherein the amino acid substitution is K35E. In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position R38, optionally wherein the amino acid substitution is R38E, R38N or R38Q. In an embodimentexample, the IL-2 agent comprises the amino acid substitution R38N. In an embodimentexample, the IL-2 agent comprises the amino acid substitution R38Q. In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position F42, optionally wherein the amino acid substitution is F42K or F42Q. In an embodimentexample, the IL-2 agent comprises the amino acid substitution F42Q.

In an embodimentexample, the IL-2 agent comprises one or more (*e.g.*, two, three, four, or all) of (i)-(v):

(i) one or more (*e.g.*, two, three, four, five, six, or seven) amino acid alterations (*e.g.*, substitutions) that reduce, or are identified to reduce, its affinity for CD122 (*e.g.*, CD122/CD132 heterodimer), *e.g.*, an alteration (*e.g.*, substitution) at position H16 (*e.g.*, H16L, H16N, or H16D), I28 (*e.g.*, I28T or I28F), D84 (*e.g.*, D84V), S87 (*e.g.*, S87R), N88 (*e.g.*, N88S, N88L, or N88D), I92 (*e.g.*, I92S), and/or Q126 (*e.g.*, Q126T, Q126K, or Q126R);

(ii) one or more (*e.g.*, two) amino acid alterations (*e.g.*, substitutions) that increase, or are identified to increase, the stability of the IL-2 agent, *e.g.*, an alteration (*e.g.*, substitution) at position V69 (*e.g.*, V69A) and/or Q74 (*e.g.*, Q74P);

(iii) one or more (*e.g.*, two, three, or four) amino acid alterations (*e.g.*, substitutions) that reduce, or are identified to reduce, its affinity for CD25, *e.g.*, an alteration (*e.g.*, substitution) at position K35 (*e.g.*, K35E), R38 (*e.g.*, R38E, R38N, or R38Q), F42 (*e.g.*, F42K or F42Q), and/or E68 (*e.g.*, E68Q or E68N); or

(iv) one or more amino acid alterations (*e.g.*, substitutions) that reduce, or are identified to reduce, O-glycosylation of the IL-2 agent, *e.g.*, an alteration (*e.g.*, substitution) at position T3 (*e.g.*, T3A); or

(v) one or more amino acid alterations (*e.g.*, substitutions) that reduce, or are identified to reduce, incorrect disulfide pairing and/or aggregation (*e.g.*, to improve stability) of the IL-2 agent, *e.g.*, an alteration (*e.g.*, substitution) at position C125 (*e.g.*, C125S).

IL-2 agent does not comprise (ii), (iv), and (iv). In an embodimentexample, the IL-2 agent does not comprise (iii), (iv), and (v).

In an embodimentexample, the IL-2 agent does not comprise (i), (ii), (iii), and (iv). In an embodimentexample, the IL-2 agent does not comprise (i), (ii), (iii), and (v). In an embodimentexample, the IL-2 agent does not comprise (i), (ii), (iv), and (v). In an embodimentexample, the IL-2 agent does not comprise (i), (iii), (iv), and (v). In an embodimentexample, the IL-2 agent does not comprise (ii), (iii), (iv), and (v).

In an embodimentexample, the IL-2 agent does not comprise (i), (ii), (iii), (iv), and (v).

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution):

- (i) at position V69 and Q74, and/or at position K35; and
- (ii) at position H16, I92, or D84; and optionally
- (iii) at position R38, F42, E68, or a combination thereof.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution):

- (i) at position V69 and Q74, and/or at position K35; and
- (ii) at position H16, I92, or D84; and
- (iii) at position R38, F42, E68, or a combination thereof.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution):

- (i) at position V69 and Q74, and/or at position K35; and
- (ii) at position H16, I92, or D84; or
- (iii) at position R38, F42, E68, or a combination thereof.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution):

- (i) at position V69 and Q74; and/or at position K35; and
- (ii) at position H16, I92, D84, or a combination thereof, and
- (iii) at position R38, F42, E68, or a combination thereof.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position V69, Q74, and H16, optionally wherein the amino acid substitution is V69A, Q74P, and H16N or H16L, respectively, optionally wherein the amino acid substitutions are V69A, Q74P, and H16L. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions V69A, Q74P, and H16L.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position V69, Q74, and I92, optionally wherein the amino acid substitution is V69A, Q74P, and I92S, respectively. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions V69A, Q74P, and I92S.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position V69, Q74, and D84, optionally wherein the amino acid substitution is V69A, Q74P, and D84V, respectively. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions V69A, Q74P, and D84V.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position V69, Q74, and R38, optionally wherein the amino acid substitution is V69A, Q74P, and R38Q, respectively.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position V69, Q74, and F42, optionally wherein the amino acid substitution is V69A, Q74P, and F42Q, respectively. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions V69A, Q74P, and F42Q.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position V69, Q74, and R38, optionally wherein the amino acid substitution is V69A, Q74P, and R38N, respectively. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions V69A, Q74P, and R38N.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position V69, Q74, and R38, optionally wherein the amino acid substitution is V69A, Q74P, and R38E, respectively. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions V69A, Q74P, and R38E.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position V69, Q74, K35, and H16, optionally wherein the amino acid substitution is V69A, Q74P, K35E, and H16N or H16L, respectively. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions V69A, Q74P, K35E, and H16N or H16L. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions V69A, Q74P, K35E, and H16N. In an embodimentexample, the IL-2 agent comprises the amino acid substitution is V69A, Q74P, K35E, and H16L.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position V69, Q74, K35, H16, and R38, optionally wherein the amino acid substitution is V69A, Q74P, K35E, H16N, and R38N, respectively. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions V69A, Q74P, K35E, H16N, and R38N.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position V69, Q74, H16, and R38, optionally wherein the amino acid substitution is V69A, Q74P, H16N or H16L, and R38N or R38Q, respectively, optionally wherein the amino acid substitutions are V69A, Q74P, H16N or H16L, and R38Q. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions V69A, Q74P, H16L, and R38Q.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position I28, E68, S87, N88, Q126, or a combination thereof. In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position I28, optionally wherein the amino acid substitution is I28T or I28F. In an embodimentexample, the IL-2 agent comprises the amino acid substitution I28T. In an embodimentexample, the IL-2 agent comprises the amino acid substitution I28F.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position E68, optionally wherein the amino acid substitution is E68Q or E68N. In an embodimentexample, the

IL-2 agent comprises the amino acid substitution E68Q. In an embodimentexample, the IL-2 agent comprises the amino acid substitution E68N.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position S87, optionally wherein the amino acid substitution is S87R. In an embodimentexample, the IL-2 agent comprises the amino acid substitution S87R.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position N88, optionally wherein the amino acid substitution is N88R, N88S, N88L, or N88D. In an embodimentexample, the IL-2 agent comprises the amino acid substitution N88R. In an embodimentexample, the IL-2 agent comprises the amino acid substitution N88S. In an embodimentexample, the IL-2 agent comprises the amino acid substitution N88L. In an embodimentexample, the IL-2 agent comprises the amino acid substitution N88D.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position Q126, optionally wherein the amino acid substitution is Q126T, Q126K, or Q126R. In an embodimentexample, the IL-2 agent comprises the amino acid substitution Q126T. In an embodimentexample, the IL-2 agent comprises the amino acid substitution Q126K. In an embodimentexample, the IL-2 agent comprises the amino acid substitution Q126R.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position C125, optionally wherein the amino acid substitution is C125S. In an embodimentexample, the IL-2 agent comprises the amino acid substitution C125S.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position T3, optionally wherein the amino acid substitution is T3A. In an embodimentexample, the IL-2 agent comprises the amino acid substitution T3A.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position V69, Q74, and C125, optionally wherein the amino acid substitution is V69A, Q74P, and C125S, respectively. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions V69A, Q74P, and C125S.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position T3, H16, I92, or a combination thereof, optionally wherein the amino acid substitution is T3A, H16N, and I92S, respectively.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position H16, V69, Q74, and C125, optionally wherein the amino acid substitution is H16N, V69A, Q74P, and C125S, respectively. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions H16N, V69A, Q74P, and C125S.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position H16, V69, Q74, and C125, optionally wherein the amino acid substitution is H16L, V69A, Q74P, and C125S, respectively. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions H16L, V69A, Q74P, and C125S. Various technical effects are associated with an IL-2

agent comprising the aforesaid combination of amino acid alterations. Without wishing to be bound by theory, it is believed that in an embodimentexample, an IL-2 agent comprising the amino acid substitutions H16L, V69A, Q74P, and C125S can have at least one or more of the following advantageous properties: (i) has reduced binding affinity for CD122 and/or CD132, which increases the potency and selectivity of the IL-2 agent for regulatory T cells (Treg) compared to other T cell types; (ii) is significantly stable, *e.g.*, due to the presence of stabilizing V69A and Q74P mutations; (iii) has reduced or decreased (or has no more than a minimal effect on) binding capacity and/or binding affinity for CD25, which improves the lifetime of the IL-2 agent; (iv) does not substantially promote expansion, activation, survival, and/or proliferation of T effector cells and/or natural killer (NK) cells *in vitro* and/or *in vivo*; and/or (v) reduced incorrect disulfide pairing and improved stability, *e.g.*, due to the presence of the C125S mutation. In an embodimentexample, an IL-2 agent comprising the H16L mutation has reduced binding affinity for CD122 and/or CD132 and/or increased potency and selectivity for Treg over other T cell types, compared to an IL-2 agent comprising other H16 mutations. These properties make an IL-2 agent comprising the amino acid substitutions H16L, V69A, Q74P, and C125S particularly suitable for treating disorders and conditions arising from abnormal immune responses, such as autoimmune diseases.

Thus, in an embodimentexample, an IL-2 agent comprising the amino acid substitutions H16L, V69A, Q74P, and C125S, has *inter alia* one or more (*e.g.*, 2, 3, 4, 5, 6, 7, or all) of the following properties relative to a wild-type IL-2 or a reference IL-2 variant that does not comprise the amino acid substitutions: (i) enhanced or increased stability *in vitro* or *in vivo*; (ii) reduced or decreased binding capacity and/or binding affinity for human CD122 *in vitro* and/or *in vivo*; (iii) reduced or decreased binding capacity and/or binding affinity for human CD132 *in vitro* and/or *in vivo*; (iv) reduced or decreased affinity of the IL-2 variant for the heterodimeric IL-2 receptor composed of human CD122 and human CD132 (*i.e.* human CD122/CD132 heterodimer) *in vitro* and/or *in vivo*; (v) reduced or decreased (*e.g.*, moderately reduced or decreased) binding capacity and/or binding affinity for human CD25 *in vitro* and/or *in vivo*; (vi) selective binding to regulatory T cells (*e.g.* Foxp3⁺ T cells); (vii) selective activation of the IL-2 signaling pathway in T regulatory cells (Tregs) *in vitro* or *in vivo*; or (viii) enhanced or increased ability to induce or promote Treg expansion, activity, survival and/or proliferation.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position H16, V69, Q74, I92, and C125, optionally wherein the amino acid substitution is H16L, V69A, Q74P, I92S, and C125S, respectively. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions H16L, V69A, Q74P, I92S, and C125S.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position T3, V69, Q74, and C125, optionally wherein the amino acid substitution is T3A, V69A, Q74P, and C125S, respectively. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions T3A, V69A, Q74P, and C125S.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position T3, H16, V69, Q74, and C125, optionally wherein the amino acid substitution is T3A, H16N or H16L, V69A, Q74P, and C125S, respectively. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions T3A, H16N, V69A, Q74P, and C125S. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions T3A, H16L, V69A, Q74P, and C125S.

In an embodimentexample, the IL-2 agent comprises an amino acid alteration (*e.g.*, substitution) at position T3, V69, Q74, I92, and C125, optionally wherein the amino acid substitution is T3A, V69A, Q74P, I92S, and C125S, respectively. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions T3A, V69A, Q74P, I92S, and C125S. In an embodimentexample, the IL-2 agent comprises the amino acid substitutions T3A, V69A, Q74P, I92S, and C125S.

In an embodimentexample, the IL-2 agent comprises a human IL-2 variant comprising an amino acid sequence chosen from: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 1000, SEQ ID NO: 1001, SEQ ID NO: 1002, or a functional fragment thereof, or an amino acid sequence with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity thereof, or differing by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, or 30 amino acids thereto.

In an embodimentexample, the amino acid alteration(s) (*e.g.*, substitution(s)) provides the IL-2 agent with at least one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or all) of the following properties relative to a reference IL-2 agent that does not comprise the amino acid alteration(s) (*e.g.*, substitution(s)):

- (i) enhanced or increased expression of the IL-2 agent;
- (ii) inhibited or decreased aggregation of the IL-2 agent;
- (iii) enhanced or increased stability of the IL-2 agent;
- (iv) enhanced or increased half-life of the IL-2 agent;
- (v) inhibited or decreased turnover and/or clearance of the IL-2 agent;
- (vi) inhibited or decreased (*e.g.*, moderately inhibited or decreased) or substantially unchanged binding of the IL-2 agent to human CD25;
- (vii) inhibited or decreased affinity of the IL-2 agent for human CD122;
- (viii) inhibited or decreased affinity of the IL-2 agent for human CD132; or
- (ix) inhibited or decreased affinity of the IL-2 agent for the dimeric IL-2 receptor composed of human CD122 and human CD132;
- (x) selective binding to regulatory T cells (*e.g.*, Foxp3+ T cells);
- (xi) selective activation of the IL-2 signaling pathway in Tregs; or

(xii) enhanced or increased, or reduced or decreased, ability to induce or promote Treg expansion, activity, survival and/or proliferation.

In an ~~embodiment~~ example, the IL-2 agent comprises a human IL-2 variant comprising one or more amino acid alteration(s) (*e.g.*, substitution(s)) chosen from H16D, H16N, H16L, I28T, K35E, R38Q, R38N, R38E, F42K, F42Q, V69A, Q74P, D84V, S87R, N88L, N88S, I92S, C125S; a polypeptide linker described herein; and a non-IL-2 moiety described herein; wherein the amino acid alteration(s) (*e.g.*, substitution(s)) provide(s) the IL-2 agent with at least one or more of the following properties relative to a reference IL-2 agent that does not comprise the amino acid alteration(s) (*e.g.*, substitution(s)):

- (i) enhanced or increased expression of the IL-2 agent;
- (ii) inhibited or decreased aggregation of the IL-2 agent;
- (iii) enhanced or increased stability of the IL-2 agent;
- (iv) enhanced or increased half-life of the IL-2 agent;
- (v) inhibited or decreased turnover and/or clearance of the IL-2 agent;
- (vi) inhibited or decreased (*e.g.*, moderately inhibited or decreased) or substantially unchanged binding of the IL-2 agent to human CD25;
- (vii) inhibited or decreased affinity of the IL-2 agent for human CD122;
- (viii) inhibited or decreased affinity of the IL-2 agent for human CD132;
- (ix) inhibited or decreased affinity of the IL-2 agent for the dimeric IL-2 receptor composed of human CD122 and human CD132;
- (x) selective binding to regulatory T cells (*e.g.*, Foxp3+ T cells);
- (xi) selective activation of the IL-2 signaling pathway in Tregs; and/or
- (xii) enhanced or increased, or reduced or decreased, ability to induce or promote Treg expansion, activity, survival, and/or proliferation.

In an ~~embodiment~~ example, the human IL-2 variant comprises the amino acid alteration(s) (*e.g.*, substitution(s)):

- (i) C125S;
- (ii) V69A, Q74P, and C125S;
- (iii) H16D, V69A, Q74P, and C125S;
- (iv) H16N, V69A, Q74P, and C125S;
- (v) H16L, V69A, Q74P, and C125S;
- (vi) I28T, V69A, Q74P, and C125S;
- (vii) V69A, Q74P, D84V, and C125S;
- (viii) V69A, Q74P, S87R, and C125S;
- (ix) V69A, Q74P, N88L, and C125S;
- (x) V69A, Q74P, N88S, and C125S;
- (xi) V69A, Q74P, I92S, and C125S;

- (xii) K35E, V69A, Q74P, and C125S;
- (xiii) K35E, H16N, V69A, Q74P, and C125S;
- (xiv) K35E, H16L, V69A, Q74P, and C125S;
- (xv) K35E, D84V, V69A, Q74P, and C125S;
- 5 (xvi) K35E, I92S, V69A, Q74P, and C125S;
- (xvii) R38Q, V69A, Q74P, and C125S;
- (xviii) R38Q, H16N, V69A, Q74P, and C125S;
- (xix) R38Q, H16L, V69A, Q74P, and C125S;
- (xx) R38Q, D84V, V69A, Q74P, and C125S;
- 10 (xxi) R38Q, I92S, Q74P, and C125S;
- (xxii) R38N, V69A, Q74P, and C125S;
- (xxiii) R38N, H16N, V69A, Q74P, and C125S;
- (xxiv) R38N, H16L, V69A, Q74P, and C125S;
- (xxv) R38N, D84V, V69A, Q74P, and C125S;
- 15 (xxvi) R38N, I92S, Q74P, and C125S;
- (xxvii) R38E, V69A, Q74P, and C125S;
- (xxviii) F42K, V69A, Q74P, and C125S;
- (xxix) F42Q, V69A, Q74P, and C125S;
- (xxx) F42A, Y45A, L72G, N88D, V69A, Q74P, and C125S;
- 20 (xxxi) R38N, S87R, V69A, Q74P, and C125S;
- (xxxii) R38E, H16N, V69A, Q74P, and C125S;
- (xxxiii) R38E, D84V, V69A, Q74P, and C125S;
- (xxxiv) R38E, S87R, V69A, Q74P, and C125S;
- (xxxv) R38E, I92S, V69A, Q74P, and C125S;
- 25 (xxxvi) F42Q, H16N, V69A, Q74P, and C125S;
- (xxxvii) F42Q, I92S, V69A, Q74P, and C125S; or
- (xxxviii) K35E, R38N, H16N, V69A, Q74P, and C125S.
- (xxxix) T3A, H16N, V69A, Q74P, and C125S;
- (xl) T3A, H16L, V69A, Q74P, and C125S; or
- (xli) T3A, V69A, Q74P, I92S, and C125S.

In an embodiment example, the IL-2 agent comprises a human IL-2 variant comprising an amino acid sequence chosen from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35,

SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 1000, SEQ ID NO: 1001, or SEQ ID NO: 1002, or a functional fragment thereof, or an amino acid sequence with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity thereof, or differing by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, or 30 amino acids thereto; a

5 polypeptide linker described herein; and a non-IL-2 moiety described herein; wherein the IL-2 agent exhibits at least one or more of the following properties relative to a reference IL-2 agent that does not comprise the human IL-2 polypeptide variant:

- (i) enhanced or increased expression of the IL-2 agent;
- (ii) inhibited or decreased aggregation of the IL-2 agent;
- 10 (iii) enhanced or increased stability of the IL-2 agent;
- (iv) enhanced or increased half-life of the IL-2 agent;
- (v) inhibited or decreased turnover and/or clearance of the IL-2 agent;
- (vi) inhibited or decreased (*e.g.*, moderately inhibited or decreased) or substantially unchanged binding of the IL-2 agent to human CD25;
- 15 (vii) inhibited or decreased affinity of the IL-2 agent for human CD122;
- (viii) inhibited or decreased affinity of the IL-2 agent for human CD132;
- (ix) inhibited or decreased affinity of the IL-2 agent for dimeric IL-2 receptor composed of human CD122 and human CD132;
- (x) selective binding to regulatory T cells (*e.g.*, Foxp3+ T cells);
- 20 (xi) selective activation of the IL-2 signaling pathway in Tregs; and/or
- (xii) enhanced or increased, or reduced or decreased, ability to induce or promote Treg expansion, activity and/or proliferation.

Various technical effects are associated with an IL-2 agent comprising the amino acid sequence of SEQ ID NO: 5. Without wishing to be bound by theory, it is believed that in an
25 embodiment example, an IL-2 agent comprising the amino acid sequence of SEQ ID NO: 5 can have at least one or more of the following advantageous properties: (i) has reduced binding affinity for CD122 and/or CD132, which increases the potency and selectivity of the IL-2 agent for regulatory T cells (Treg) compared to other T cell types; (ii) is significantly stable, *e.g.*, due to the presence of stabilizing V69A and Q74P mutations; (iii) has reduced or decreased (or has no more than a minimal effect on) binding capacity and/or binding affinity for CD25, which improves the lifetime of the IL-2
30 agent; (iv) does not substantially promote expansion, activation, survival, and/or proliferation of T effector cells and/or natural killer (NK) cells *in vitro* and/or *in vivo*; and/or (v) has reduced incorrect disulfide pairing and improved stability, *e.g.*, due to the presence of the C125S mutation. In an
| embodiment example, an IL-2 agent comprising the H16L mutation has reduced binding affinity for
35 CD122 and/or CD132 and/or increased potency and selectivity for Treg over other T cell types, compared to an IL-2 agent comprising other H16 mutations. These properties make an IL-2 agent comprising the

amino acid sequence of SEQ ID NO: 5 particularly suitable for treating disorders and conditions arising from abnormal immune responses, such as autoimmune diseases.

Thus, in an embodimentexample, an IL-2 agent comprising the amino acid sequence of SEQ ID NO: 5, has *inter alia* one or more (*e.g.*, 2, 3, 4, 5, 6, 7, or all) of the following properties relative to a wild-type IL-2 or a reference IL-2 variant that does not comprise the amino acid substitutions: (i) enhanced or increased stability *in vitro* or *in vivo*; (ii) reduced or decreased binding capacity and/or binding affinity for human CD122 *in vitro* and/or *in vivo*; (iii) reduced or decreased binding capacity and/or binding affinity for human CD132 *in vitro* and/or *in vivo*; (iv) reduced or decreased affinity of the IL-2 variant for the heterodimeric IL-2 receptor composed of human CD122 and human CD132 (*i.e.* human CD122/CD132 heterodimer) *in vitro* and/or *in vivo*; (v) reduced or decreased (*e.g.*, moderately reduced or decreased) binding capacity and/or binding affinity for human CD25 *in vitro* and/or *in vivo*; (vi) selective binding to regulatory T cells (*e.g.* Foxp3⁺ T cells); (vii) selective activation of the IL-2 signaling pathway in T regulatory cells (Tregs) *in vitro* or *in vivo*; or (viii) enhanced or increased ability to induce or promote Treg expansion, activity, survival and/or proliferation.

In an embodimentexample, the reference IL-2 agent comprises the amino acid sequence of SEQ ID NO: 1031, SEQ ID NO: 1, or SEQ ID NO: 2, or a functional fragment thereof. In an embodimentexample, the reference IL-2 agent comprises the amino acid sequence of SEQ ID NO: 1031. In an embodimentexample, the reference IL-2 agent comprises the amino acid sequence of SEQ ID NO: 1. In an embodimentexample, the reference IL-2 agent comprises the amino acid sequence of SEQ ID NO: 2.

In an embodimentexample, the IL-2 agent comprises a human IL-2 variant described herein fused to a non-IL-2 moiety described herein by a linker, wherein the linker is a polypeptide linker, optionally wherein the polypeptide linker is a flexible linker, a rigid linker, or a cleavable linker. In an embodimentexample, the polypeptide linker is a Gly-Ser linker (*e.g.*, a (G4S)_n linker, wherein n = 1, 2, 3, 4, 5, 6 or more (SEQ ID NO: 1020)), a proline-rich extended linker (*e.g.*, V1 GPc, V2, GPGc, V3 GcGcP, cellulase linker 4, cellulase linker 4), a rigid linker (*e.g.*, A(EAAAK)_nA, wherein n = 2, 3, 4, 5, or more (SEQ ID NO: 1021); REPR_12), a non-GS linker (*e.g.*, (GGGSA)_n, wherein n = 1, 2, 3, 4, 5, or more (SEQ ID NO: 1022)), or an immunoglobulin hinge region or portion thereof. In an embodimentexample, the polypeptide linker is a Gly-Ser linker comprising (G4S)₁ (SEQ ID NO: 1023), (G4S)₂ (SEQ ID NO: 1024), (G4S)₃ (SEQ ID NO: 1025), (G4S)₄ (SEQ ID NO: 48), (G4S)₅ (SEQ ID NO: 1026), or (G4S)₆ (SEQ ID NO: 1027). In an embodimentexample, the polypeptide linker is a Gly-Ser linker comprising (G4S)₄ (SEQ ID NO: 48). In an embodimentexample, the polypeptide linker comprises an amino acid sequence chosen from SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, or SEQ ID NO: 55. In an embodimentexample, the polypeptide linker comprises the amino acid sequence of SEQ ID NO: 48.

In an embodimentexample, the non-IL-2 moiety is an immunoglobulin Fc region, or a fragment or portion thereof (*e.g.*, a functional fragment). In an embodimentexample, the immunoglobulin Fc region comprises an IgG Fc region, an IgD Fc region, an IgA Fc region, an IgM Fc region, or an IgE Fc

region, or fragment or portion thereof. In an ~~embodiment~~example, the IgG Fc region comprises a wild type human IgG1 Fc region (*e.g.*, IgG1 m3 allotype), a wild type IgG2 Fc region, or a wild type human IgG4 Fc region, or a fragment or portion thereof.

In an ~~embodiment~~example, the IgG Fc region comprises a mutant IgG1 or mutant IgG4 Fc region, or a fragment or portion thereof. In an ~~embodiment~~example, the IgG Fc region comprises one or more (*e.g.*, two, three, four, or five) mutations, *e.g.*, one or more (*e.g.*, two, three, four, or five) mutations described herein.

In an ~~embodiment~~example, the IgG Fc region comprises a mutant IgG4 Fc region, or a fragment or portion thereof, wherein the mutant IgG4 Fc region is human.

In an ~~embodiment~~example, the mutant IgG4 Fc region, or fragment or portion thereof, comprises an amino acid alteration (*e.g.*, substitution) at Ser228, numbering according to EU numbering, optionally wherein the amino acid alteration (*e.g.*, substitution) at Ser228 is S228P. In an ~~embodiment~~example, the mutant IgG4 Fc region comprises the amino acid substitution S228P.

In an ~~embodiment~~example, the mutant IgG4 Fc region, or fragment or portion thereof, comprises an amino acid alteration (*e.g.*, substitution) at Arg409, numbering according to EU numbering, optionally wherein the amino acid alteration (*e.g.*, substitution) at Arg409 is R409K. In an ~~embodiment~~example, the mutant IgG4 Fc region comprises the amino acid substitution R409K.

In an ~~embodiment~~example, the mutant IgG4 Fc region, or a fragment or portion thereof, comprises amino acid alterations (*e.g.*, substitutions) at Thr307, Gln311, and Ala378, numbering according to EU numbering, optionally wherein the amino acid alterations (*e.g.*, substitutions) are T307Q, Q311V, and A378V, respectively. In an ~~embodiment~~example, the mutant IgG4 Fc region comprises the amino acid substitutions T307Q, Q311V, and A378V.

In an ~~embodiment~~example, the mutant IgG4 Fc region comprises an amino acid sequence chosen from SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, or SEQ ID NO: 47, or an amino acid sequence with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity thereof, or differing by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, or 30 amino acids thereto.

In an ~~embodiment~~example, the IgG Fc region comprises a mutant IgG1 Fc region, or a fragment or portion thereof, wherein the mutant IgG1 Fc region is human. In an ~~embodiment~~example, the mutant IgG1 Fc region (*e.g.*, comprising an N297G substitution) has an IgG1 m3 allotype.

In an ~~embodiment~~example, the mutant IgG1 Fc region, or a fragment or portion thereof, comprises an amino acid alteration (*e.g.*, substitution) at Asn297, numbering according to EU numbering, optionally wherein the amino acid alteration (*e.g.*, substitution) at Asn297 is N297G. In an ~~embodiment~~example, the mutant IgG1 Fc region comprises the amino acid substitution N297G.

In an ~~embodiment~~example, the mutant IgG1 Fc region, or a fragment or portion thereof, comprises amino acid alterations (*e.g.*, substitutions) at Leu234, Leu235, and Pro329, numbering according to EU numbering, optionally wherein the amino acid alterations (*e.g.*, substitutions) are L234A, L235A,

and P329G, respectively. In an embodimentexample, the mutant IgG1 Fc region comprises the amino acid substitutions L234A, L235A, and P329G.

In an embodimentexample, the mutant IgG1 Fc region, or a fragment or portion thereof, comprises amino acid alterations (*e.g.*, substitutions) at Thr307, Gln311, and Ala378, numbering according to EU numbering, optionally wherein the amino acid alterations (*e.g.*, substitutions) are T307Q, Q311V, and A378V, respectively. In an embodimentexample, the mutant IgG1 Fc region comprises the amino acid substitutions T307Q, Q311V, and A378V.

In an embodimentexample, the mutant IgG1 Fc region comprises an amino acid sequence chosen from SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, or SEQ ID NO: 1003, or an amino acid sequence with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity thereof, or differing by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, or 30 amino acids thereto. In an embodimentexample, the mutant IgG1 Fc region comprises an amino acid sequence of SEQ ID NO: 1003, or an amino acid sequence with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity thereof, or differing by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, or 30 amino acids thereto. In an embodimentexample, the mutant IgG1 Fc region comprises an amino acid sequence of SEQ ID NO: 1003.

In an embodimentexample, the non-IL-2 moiety inhibits or decreases the ability of the IL-2 agent to elicit Fc-receptor-mediated immune effector functions.

In an embodimentexample, the IL-2 agent comprises an IL-2 variant comprising an amino acid sequence chosen from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, or SEQ ID NO: 38, SEQ ID NO: 1000, SEQ ID NO: 1001, or SEQ ID NO: 1002, or a functional fragment thereof; wherein the IL-2 agent comprises a Gly-Ser linker, optionally wherein the Gly-Ser linker comprises (G₄S)₄ (SEQ ID NO: 48), and wherein the IL-2 variant is fused by the Gly-Ser linker to an IgG Fc region comprising an amino acid sequence chosen from SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, or SEQ ID NO: 1003.

In an embodimentexample, the IL-2 agent comprises an amino acid sequence chosen from SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, or SEQ ID NO: 100, or a functional fragment thereof; wherein the IL-2 agent comprises a Gly-Ser linker, optionally wherein the Gly-Ser linker comprises (G₄S)₄ (SEQ ID NO: 48), and wherein the IL-2 variant is fused by the Gly-Ser linker to an IgG Fc region comprising an amino acid sequence chosen from SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, or SEQ ID NO: 1003.

NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 1004, SEQ ID NO: 1005, SEQ ID NO: 1006, SEQ ID NO: 1007, SEQ ID NO: 1008, or SEQ ID NO: 1009, or a functional fragment thereof.

In an ~~embodiment~~example, the IL-2 agent comprises an amino acid sequence chosen from SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, or SEQ ID NO: 131, or a functional fragment thereof.

In an ~~embodiment~~example, the IL-2 agent comprises an amino acid sequence chosen from SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, or SEQ ID NO: 169, or a functional fragment thereof.

In an ~~embodiment~~example, the IL-2 agent comprises an amino acid sequence chosen from SEQ ID NO: 170, SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, SEQ ID NO: 190, SEQ ID NO: 191, SEQ ID NO: 192, SEQ ID NO: 193, SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197, SEQ ID NO: 198, SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, or SEQ ID NO: 207, or a functional fragment thereof.

In an ~~embodiment~~example, the IL-2 agent comprises an amino acid sequence chosen from SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224, SEQ ID NO: 225, SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID

NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 236, SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, SEQ ID NO: 244, or SEQ ID NO: 245, or a functional fragment thereof.

5 In an embodimentexample, the IL-2 agent comprises an amino acid sequence chosen from SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252, SEQ ID NO: 253, SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265, SEQ ID NO: 266, SEQ ID NO: 267, 10 SEQ ID NO: 268, SEQ ID NO: 269, SEQ ID NO: 270, SEQ ID NO: 271, SEQ ID NO: 272, SEQ ID NO: 273, SEQ ID NO: 274, SEQ ID NO: 275, SEQ ID NO: 276, SEQ ID NO: 277, SEQ ID NO: 278, SEQ ID NO: 279, SEQ ID NO: 280, SEQ ID NO: 281, SEQ ID NO: 282, or SEQ ID NO: 283, or a functional fragment thereof.

15 In an embodimentexample, the IL-2 agent comprises an amino acid sequence chosen from SEQ ID NO: 284, SEQ ID NO: 285, SEQ ID NO: 286, SEQ ID NO: 287, SEQ ID NO: 288, SEQ ID NO: 289, SEQ ID NO: 290, SEQ ID NO: 291, SEQ ID NO: 292, SEQ ID NO: 293, SEQ ID NO: 294, SEQ ID NO: 295, SEQ ID NO: 296, SEQ ID NO: 297, SEQ ID NO: 298, SEQ ID NO: 299, SEQ ID NO: 300, SEQ ID NO: 301, SEQ ID NO: 302, SEQ ID NO: 303, SEQ ID NO: 304, SEQ ID NO: 305, SEQ ID NO: 306, SEQ ID NO: 307, SEQ ID NO: 308, SEQ ID NO: 309, SEQ ID NO: 310, SEQ ID NO: 311, SEQ ID NO: 312, SEQ ID NO: 313, SEQ ID NO: 314, SEQ ID NO: 315, SEQ ID NO: 316, 20 SEQ ID NO: 317, SEQ ID NO: 318, SEQ ID NO: 319, SEQ ID NO: 320, or SEQ ID NO: 321, or a functional fragment thereof.

In an embodimentexample, the IL-2 agent comprises an amino acid sequence chosen from SEQ ID NO: 322, SEQ ID NO: 323, SEQ ID NO: 324, SEQ ID NO: 325, SEQ ID NO: 326, SEQ ID NO: 327, SEQ ID NO: 328, SEQ ID NO: 329, SEQ ID NO: 330, SEQ ID NO: 331, SEQ ID NO: 332, 25 SEQ ID NO: 333, SEQ ID NO: 334, SEQ ID NO: 335, SEQ ID NO: 336, SEQ ID NO: 337, SEQ ID NO: 338, SEQ ID NO: 339, SEQ ID NO: 340, SEQ ID NO: 341, SEQ ID NO: 342, SEQ ID NO: 343, SEQ ID NO: 344, SEQ ID NO: 345, SEQ ID NO: 346, SEQ ID NO: 347, SEQ ID NO: 348, SEQ ID NO: 349, SEQ ID NO: 350, SEQ ID NO: 351, SEQ ID NO: 352, SEQ ID NO: 353, SEQ ID NO: 354, 30 SEQ ID NO: 355, SEQ ID NO: 356, SEQ ID NO: 357, SEQ ID NO: 358, or SEQ ID NO: 359, or a functional fragment thereof.

In an embodimentexample, the IL-2 agent comprises the amino acid sequence of SEQ ID NO: 59, or a functional fragment thereof. In an embodimentexample, the IL-2 agent comprises the amino acid sequence of SEQ ID NO: 97, or a functional fragment thereof. In an embodimentexample, the 35 IL-2 agent comprises the amino acid sequence of SEQ ID NO: 135, or a functional fragment thereof. In an embodimentexample, the IL-2 agent comprises the amino acid sequence of SEQ ID NO: 173, or a functional fragment thereof. In an embodimentexample, the IL-2 agent comprises the amino acid sequence of SEQ ID NO: 211, or a

[illegible]

acid sequence of SEQ ID NO: 1006, or a functional fragment thereof. In an embodimentexample, the IL-2 agent comprises the amino acid sequence of SEQ ID NO: 1007, or a functional fragment thereof. In an embodimentexample, the IL-2 agent comprises the amino acid sequence of SEQ ID NO: 1008, or a functional fragment thereof. In an embodimentexample, the IL-2 agent comprises the amino acid sequence of SEQ ID NO: 1009, or a functional fragment thereof.

Various technical effects are associated with an IL-2 agent comprising the amino acid sequence of SEQ ID NO: 1008. Without wishing to be bound by theory, it is believed that in an embodimentexample, an IL-2 agent comprising the amino acid sequence of SEQ ID NO: 1008 can have at least one or more of the following advantageous properties: (i) has reduced binding affinity for CD122 and/or CD132, which increases the potency and selectivity of the IL-2 agent for regulatory T cells (Treg) compared to other T cell types; (ii) is significantly stable, *e.g.*, due to the presence of stabilizing V69A and Q74P mutations; (iii) has reduced or decreased (or has no more than a minimal effect on) binding capacity and/or binding affinity for CD25, which improves the lifetime of the IL-2 agent; (iv) does not substantially promote expansion, activation, survival, and/or proliferation of T effector cells and/or natural killer (NK) cells *in vitro* and/or *in vivo*; and/or (v) has reduced incorrect disulfide pairing and improved stability, *e.g.*, due to the presence of the C125S mutation. In an embodimentexample, an IL-2 agent comprising the H16L mutation has reduced binding affinity for CD122 and/or CD132 and/or increased potency and selectivity for Treg over other T cell types, compared to an IL-2 agent comprising other H16 mutations. These properties make an IL-2 variant an IL-2 agent comprising the amino acid sequence of SEQ ID NO: 1008 particularly suitable for treating disorders and conditions arising from abnormal immune responses, such as autoimmune diseases.

Thus, in an embodimentexample, an IL-2 agent comprising the amino acid sequence of SEQ ID NO: 1008, has *inter alia* one or more (*e.g.*, 2, 3, 4, 5, 6, 7, or all) of the following properties relative to a wild-type IL-2 or a reference IL-2 variant that does not comprise the amino acid substitutions: (i) enhanced or increased stability *in vitro* or *in vivo*; (ii) reduced or decreased binding capacity and/or binding affinity for human CD122 *in vitro* and/or *in vivo*; (iii) reduced or decreased binding capacity and/or binding affinity for human CD132 *in vitro* and/or *in vivo*; (iv) reduced or decreased affinity of the IL-2 variant for the heterodimeric IL-2 receptor composed of human CD122 and human CD132 (*i.e.* human CD122/CD132 heterodimer) *in vitro* and/or *in vivo*; (v) reduced or decreased (*e.g.*, moderately reduced or decreased) binding capacity and/or binding affinity for human CD25 *in vitro* and/or *in vivo*; (vi) selective binding to regulatory T cells (*e.g.* Foxp3⁺ T cells); (vii) selective activation of the IL-2 signaling pathway in T regulatory cells (Tregs) *in vitro* or *in vivo*; or (viii) enhanced or increased ability to induce or promote Treg expansion, activity, survival and/or proliferation.

In an embodimentexample, the IL-2 agent forms a dimer (*e.g.*, a homodimer or heterodimer).

In an embodimentexample, the IL-2 agent comprises an IL-2 fusion protein. In an embodimentexample, the IL-2 agent comprises an IL-2 agent/anti-IL-2 antibody complex. In an embodimentexample, the IL-2 agent comprises a conjugate.

In some aspectexamples, the disclosure provides a pharmaceutical composition comprising an IL-2 agent described, and a pharmaceutically acceptable carrier. In some aspectexamples, the disclosure provides a nucleic acid encoding an IL-2 agent described herein. In some aspectexamples, the disclosure provides a vector (*e.g.*, expression vector) comprising a nucleic acid encoding an IL-2 agent described herein. In some aspectexamples, the disclosure provides a cell (*e.g.*, isolated cell) comprising a nucleic acid encoding an IL-2 agent described herein or a vector (*e.g.*, expression vector) comprising a nucleic acid encoding an IL-2 agent described herein.

In some aspectexamples, the disclosure provides a method of producing an IL-2 agent, comprising culturing (*e.g.*, maintaining) a cell comprising a nucleic acid encoding an IL-2 agent described herein or a vector (*e.g.*, expression vector) comprising a nucleic acid encoding an IL-2 agent described herein under conditions permitting expression of the IL-2 agent. In an embodimentexample, the method further comprising obtaining the IL-2 agent. In an embodimentexample, the method further comprising purifying the IL-2 agent.

In some aspectexamples, the disclosure provides a method of enhancing regulatory T cell (Treg) expansion, activity, survival, and/or proliferation, comprising contacting a Treg cell or a population of Treg cells (*e.g.*, *in vitro*, *ex vivo*, or *in vivo*) or administering to a subject in need thereof an effective amount of an IL-2 agent described herein, or a pharmaceutical composition comprising the IL-2 agent. The IL-2 agent may, for example, comprise the amino acid substitutions H16L, V69A, Q74P and C125S, or the amino acid substitutions H16N, V69A, Q74P and C125S. In an embodimentexample, the IL-2 agent comprises amino acid substitutions H16L, V69A, Q74P and C125S.

In some aspectexamples, the disclosure provides a method of selectively activating the IL-2 signaling pathway in regulatory T cells (Tregs), comprising contacting a Treg cell or a population of Treg cells (*e.g.*, *in vitro*, *ex vivo*, or *in vivo*) or administering to a subject in need thereof an effective amount of an IL-2 agent described herein, or a pharmaceutical composition of comprising the IL-2 agent. The IL-2 agent may, for example, comprise the amino acid substitutions H16L, V69A, Q74P and C125S, or the amino acid substitutions H16N, V69A, Q74P and C125S. In an embodimentexample, the IL-2 agent comprises amino acid substitutions H16L, V69A, Q74P and C125S.

In some aspectexamples, the disclosure provides a method of inducing immune tolerance in a subject in need thereof, comprising administering an effective amount of an IL-2 agent described herein, or a pharmaceutical composition comprising the IL-2 agent. The IL-2 agent may, for example, comprise the amino acid substitutions H16L, V69A, Q74P and C125S, or the amino acid substitutions H16N, V69A, Q74P and C125S. In an embodimentexample, the IL-2 agent comprises amino acid substitutions H16L, V69A, Q74P and C125S.

In some aspectexamples, the disclosure provides a method of treating a subject having a disorder (*e.g.*, a disorder described herein, *e.g.*, an autoimmune disease, lupus nephritis, autoimmune hepatitis, nephrotic syndrome, or a cancer) comprising administering to the subject an effective amount of an IL-2 agent described herein, or a pharmaceutical composition comprising the IL-2 agent. The IL-2 agent may, for example, comprise the amino acid substitutions H16L, V69A, Q74P and C125S, or the amino acid substitutions H16N, V69A, Q74P and C125S. In an embodimentexample, the IL-2 agent comprises amino acid substitutions H16L, V69A, Q74P and C125S.

In some aspectexamples, the disclosure provides an IL-2 agent or a composition for use in a method for the treatment of a subject having a disorder (*e.g.*, a disorder described herein, *e.g.*, an autoimmune disease, lupus nephritis, autoimmune hepatitis, nephrotic syndrome, or a cancer), the method comprising administering an IL-2 agent described herein, or a pharmaceutical composition comprising the IL-2 agent, to said subject. The IL-2 agent may, for example, comprise the amino acid substitutions H16L, V69A, Q74P and C125S, or the amino acid substitutions H16N, V69A, Q74P and C125S. In an embodimentexample, the IL-2 agent comprises amino acid substitutions H16L, V69A, Q74P and C125S.

In some aspectexamples, the disclosure provides use of an IL-2 agent or a composition in the manufacture of a medicament in a method for the treatment of a subject having a disorder (*e.g.*, a disorder described herein, *e.g.*, an autoimmune disease, lupus nephritis, autoimmune hepatitis, nephrotic syndrome, or a cancer), the method comprising administering an IL-2 agent described herein, or a pharmaceutical composition comprising the IL-2 agent, to said subject. The IL-2 agent may, for example, comprise the amino acid substitutions H16L, V69A, Q74P and C125S, or the amino acid substitutions H16N, V69A, Q74P and C125S. In an embodimentexample, the IL-2 agent comprises amino acid substitutions H16L, V69A, Q74P and C125S.

In some aspectexamples, the disclosure provides a kit comprising an IL-2 agent described herein, or a pharmaceutical composition comprising the IL-2 agent, and instructions for use. The IL-2 agent may, for example, comprise the amino acid substitutions H16L, V69A, Q74P and C125S, or the amino acid substitutions H16N, V69A, Q74P and C125S. In an embodimentexample, the IL-2 agent comprises amino acid substitutions H16L, V69A, Q74P and C125S.

In some aspectexamples, the disclosure provides a container comprising an IL-2 agent described herein, or a pharmaceutical composition comprising the IL-2 agent. The IL-2 agent may, for example, comprise the amino acid substitutions H16L, V69A, Q74P and C125S, or the amino acid substitutions H16N, V69A, Q74P and C125S. In an embodimentexample, the IL-2 agent comprises amino acid substitutions H16L, V69A, Q74P and C125S.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A provides a schematic illustrating the domain structure of an exemplary, non-limiting embodiment of an IL-2 agent provided for herein. The IL-2 agent comprises an IL-2 moiety or variant

CLAIMS

1. An interleukin-2 (IL-2) variant, comprising:
 - (i) the amino acid substitution H16L or H16N, and/or the amino acid substitution I92S, and
 - (ii) the amino acid substitutions V69A, Q74P, and C125S, corresponding to human IL-2 (SEQ ID NO: 1031).
2. The IL-2 variant of claim 1, wherein:
 - (i) the IL-2 variant further comprises~~ing~~ the amino acid substitution T3A;
 - (ii) the IL-2 variant comprises the amino acid sequence of any of SEQ ID NOs: 4, 5, 11, 1000, 1001, or 1002, an amino acid sequence that is at least 95% identical thereto or differs by no more than 1, 2, 3, 4, or 5 amino acids therefrom, or a functional fragment thereof; and/or
 - (iii) the IL-2 variant selectively stimulates regulatory T cells (Tregs).
- ~~3. The IL-2 variant of claim 1 or 2, comprising the amino acid sequence of any of SEQ ID NOs: 4, 5, 11, 1000, 1001, or 1002, an amino acid sequence that is at least 95% identical thereto or differs by no more than 1, 2, 3, 4, or 5 amino acids therefrom, or a functional fragment thereof.~~
- ~~4. The IL-2 variant of any of claims 1-3, which selectively stimulates regulatory T cells (Tregs).~~
- ~~53.~~ An IL-2 fusion protein comprising the IL-2 variant of ~~any of claims 1-4~~ claim 1 or 2.
- ~~64.~~ The IL-2 fusion protein of claim ~~53~~, wherein:
 - (i) the IL-2 fusion further comprising~~comprises~~ an Fc region, optionally wherein the Fc region comprises an Fc region of IgG1 allotype m3 comprising an N297G substitution according to EU numbering;
 - (ii) the Fc region comprises the amino acid sequence of SEQ ID NO: 1003, or an amino acid sequence that is at least 95% identical thereto or differs by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids therefrom, or a functional fragment thereof;
 - (iii) the Fc region is fused to the C-terminus of the IL-2 variant;
 - (iv) the IL-2 fusion protein further comprises a linker, optionally wherein the linker comprises (G₄S)₄ (SEQ ID NO: 48);

(v) the IL-2 fusion protein comprises an amino acid sequence of any of SEQ ID NOs: 1004, 1005, 1006, 1007, 1008, or 1009, an amino acid sequence that is at least 95% identical thereto or differs by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids therefrom, or a functional fragment thereof; and/or

(vi) the IL-2 fusion protein forms a dimer.

~~7. The IL-2 fusion protein of claim 6, wherein the Fc region comprises an Fc region of IgG1 allotype m3 comprising an N297G substitution according to EU numbering.~~

~~8. The IL-2 fusion protein of claim 6 or 7, wherein the Fc region comprises the amino acid sequence of SEQ ID NO: 1003, or an amino acid sequence that is at least 95% identical thereto or differs by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids therefrom, or a functional fragment thereof.~~

~~9. The IL-2 fusion protein of any of claims 6-8, wherein the Fc region is fused to the C-terminus of the IL-2 variant.~~

~~10. The IL-2 fusion protein of any of claims 6-9, further comprising a linker.~~

~~11. The IL-2 fusion protein of claim 10, wherein the linker comprises (G₄S)₄ (SEQ ID NO: 48).~~

~~12. The IL-2 fusion protein of any of claims 6-11, comprising an amino acid sequence of any of SEQ ID NOs: 1004, 1005, 1006, 1007, 1008, or 1009, an amino acid sequence that is at least 95% identical thereto or differs by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids therefrom, or a functional fragment thereof.~~

~~13. The IL-2 fusion protein of any of claims 6-12, which forms a dimer.~~

~~145.~~ An IL-2 complex comprising the IL-2 variant of ~~any of claims 1-4~~ claim 1 or 2 and an anti-IL-2 antibody molecule.

~~156.~~ An IL-2 conjugate comprising the IL-2 variant of ~~any of claims 1-4~~ claim 1 or 2 and a non-IL-2 moiety.

~~167.~~ A pharmaceutical composition comprising any of:

(i) the IL-2 variant of claim 1 or 2 ~~any of claims 1-4.~~

(ii) the IL-2 fusion protein of claim 3 or 4,
(iii) the IL-2 complex of claim 5, or
(iv) the IL-2 conjugate of claim 6; and
a pharmaceutically acceptable carrier.

~~17. — A pharmaceutical composition comprising the IL-2 fusion protein of any of claims 5-13 and a pharmaceutically acceptable carrier.~~

~~18. — A pharmaceutical composition comprising the IL-2 complex of claim 14 and a pharmaceutically acceptable carrier.~~

~~19. — A pharmaceutical composition comprising the IL-2 conjugate of claim 15 and a pharmaceutically acceptable carrier.~~

~~208.~~ A nucleic acid encoding any of:

- (i) the IL-2 variant of claim 1 or 2,
- (ii) the IL-2 fusion protein of claim 3 or 4,
- (iii) the IL-2 complex of claim 5, or
- (iv) the IL-2 conjugate of claim 6~~the IL-2 variant of any of claims 1-4.~~

~~21. — A nucleic acid encoding the IL-2 fusion protein any of claims 5-13.~~

~~22. — A nucleic acid encoding the IL-2 complex of claim 14.~~

~~23. — A nucleic acid encoding the IL-2 conjugate of claim 15.~~

~~249.~~ A vector comprising the nucleic acid of claim 8~~claim 20~~.

~~25. — A vector comprising the nucleic acid of claim 21.~~

~~26. — A vector comprising the nucleic acid of claim 22.~~

~~27. — A vector comprising the nucleic acid of claim 23.~~

~~28~~10. A cell comprising the nucleic acid of claim 8~~claim 20~~.

~~29~~. — A cell comprising the nucleic acid of claim 21.

~~30~~. — A cell comprising the nucleic acid of claim 22.

~~31~~. — A cell comprising the nucleic acid of claim 23.

~~32~~11. A method of producing an IL-2 variant, comprising culturing the cell of claim 10 ~~claim 28~~ under conditions that allow expression of the IL-2 variant.

~~33~~. — A method of producing an IL-2 fusion protein, comprising culturing the cell of claim 29 under conditions that allow expression of the IL-2 fusion protein.

~~34~~. — A method of producing an IL-2 complex, comprising culturing the cell of claim 30 under conditions that allow expression of the IL-2 complex.

~~35~~. — A method of producing an IL-2 conjugate, comprising culturing the cell of claim 31 under conditions that allow expression of the IL-2 conjugate.

~~36~~12. A method of enhancing regulatory T cell (Treg) expansion, activity, survival, and/or proliferation, comprising contacting a Treg cell or a population of Treg cells *in vitro*, *ex vivo*, or *in vivo*, or administering to a subject in need thereof an effective amount of any of:

(i) the IL-2 variant of claim 1 or 2,

(ii) the IL-2 fusion protein of claim 3 or 4,

(iii) the IL-2 complex of claim 5, or

(iv) the IL-2 conjugate of claim 6 ~~the IL-2 variant of any of claims 1-4.~~

~~37~~. — A method of enhancing regulatory T cell (Treg) expansion, activity, survival, and/or proliferation, comprising contacting a Treg cell or a population of Treg cells *in vitro*, *ex vivo*, or *in vivo*, or administering to a subject in need thereof an effective amount of the IL-2 fusion protein of any of claims ~~5-13~~.

~~38. — A method of enhancing regulatory T cell (Treg) expansion, activity, survival, and/or proliferation, comprising contacting a Treg cell or a population of Treg cells *in vitro*, *ex vivo*, or *in vivo*, or administering to a subject in need thereof an effective amount of the IL-2 complex of claim 14.~~

~~39. — A method of enhancing regulatory T cell (Treg) expansion, activity, survival, and/or proliferation, comprising contacting a Treg cell or a population of Treg cells *in vitro*, *ex vivo*, or *in vivo*, or administering to a subject in need thereof an effective amount of the IL-2 conjugate of claim 15.~~

4013. A method of selectively activating the IL-2 signaling pathway in regulatory T cells (Tregs), comprising contacting a Treg cell or a population of Treg cells *in vitro*, *ex vivo*, or *in vivo*, or administering to a subject in need thereof an effective amount of any of:

- (i) the IL-2 variant of claim 1 or 2,
- (ii) the IL-2 fusion protein of claim 3 or 4,
- (iii) the IL-2 complex of claim 5, or
- (iv) the IL-2 conjugate of claim 6~~the IL-2 variant of any of claims 1-4.~~

~~41. — A method of selectively activating the IL-2 signaling pathway in regulatory T cells (Tregs), comprising contacting a Treg cell or a population of Treg cells *in vitro*, *ex vivo*, or *in vivo*, or administering to a subject in need thereof an effective amount of the IL-2 fusion protein of any of claims 5-13.~~

~~42. — A method of selectively activating the IL-2 signaling pathway in regulatory T cells (Tregs), comprising contacting a Treg cell or a population of Treg cells *in vitro*, *ex vivo*, or *in vivo*, or administering to a subject in need thereof an effective amount of the IL-2 complex of claim 14.~~

~~43. — A method of selectively activating the IL-2 signaling pathway in regulatory T cells (Tregs), comprising contacting a Treg cell or a population of Treg cells *in vitro*, *ex vivo*, or *in vivo*, or administering to a subject in need thereof an effective amount of the IL-2 conjugate of claim 15.~~

4414. A method of inducing immune tolerance, comprising administering to a subject in need thereof an Use of effective amount of any of:

- (i) the IL-2 variant of claim 1 or 2,
- (ii) the IL-2 fusion protein of claim 3 or 4,
- (iii) the IL-2 complex of claim 5, or

(iv) the IL-2 conjugate of claim 6~~the IL-2 variant of any of claims 1-4;~~
in the manufacture of a medicament for inducing immune tolerance.

~~45. — A method of inducing immune tolerance, comprising administering to a subject in need thereof an effective amount of the IL-2 fusion protein of claim 5-13.~~

~~46. — A method of inducing immune tolerance, comprising administering to a subject in need thereof an effective amount of the IL-2 complex of claim 14.~~

~~47. — A method of inducing immune tolerance, comprising administering to a subject in need thereof an effective amount of the IL-2 conjugate of claim 15.~~

~~48~~15. ~~A method of treating an autoimmune disease, comprising administering to a subject in need thereof~~Use of an effective amount of any of:

(i) the IL-2 variant of claim 1 or 2,

(ii) the IL-2 fusion protein of claim 3 or 4,

(iii) the IL-2 complex of claim 5, or

(iv) the IL-2 conjugate of claim 6~~the IL-2 variant of any of claims 1-4;~~
in the manufacture of a medicament for treating an autoimmune disease.

~~49. — A method of treating an autoimmune disease, comprising administering to a subject in need thereof an effective amount of the IL-2 fusion protein of any of claims 5-13.~~

~~50. — A method of treating an autoimmune disease, comprising administering to a subject in need thereof an effective amount of the IL-2 complex of claim 14.~~

~~51. — A method of treating an autoimmune disease, comprising administering to a subject in need thereof an effective amount of the IL-2 conjugate of claim 15.~~

~~52~~16. ~~A method of treating lupus nephritis, comprising administering to a subject in need thereof~~Use of an effective amount of any of:

(i) the IL-2 variant of claim 1 or 2,

(ii) the IL-2 fusion protein of claim 3 or 4,

(iii) the IL-2 complex of claim 5, or

(iv) the IL-2 conjugate of claim 6~~the IL-2 variant of any of claims 1-4;~~
in the manufacture of a medicament for treating lupus nephritis in a subject.

~~53. — A method of treating lupus nephritis, comprising administering to a subject in need thereof an effective amount of the IL-2 fusion protein of any of claims 5-13.~~

~~54. — A method of treating lupus nephritis, comprising administering to a subject in need thereof an effective amount of the IL-2 complex of claim 14.~~

~~55. — A method of treating lupus nephritis, comprising administering to a subject in need thereof an effective amount of the IL-2 conjugate of claim 15.~~

~~56~~17. ~~A method of treating autoimmune hepatitis, comprising administering to a subject in need thereof~~
Use of an effective amount of any of:

(i) the IL-2 variant of claim 1 or 2,

(ii) the IL-2 fusion protein of claim 3 or 4,

(iii) the IL-2 complex of claim 5, or

(iv) the IL-2 conjugate of claim 6~~the IL-2 variant of any of claims 1-4;~~

in the manufacture of a medicament for treating autoimmune hepatitis in a subject.

~~57. — A method of treating autoimmune hepatitis, comprising administering to a subject in need thereof an effective amount of the IL-2 fusion protein of any of claims 5-13.~~

~~58. — A method of treating autoimmune hepatitis, comprising administering to a subject in need thereof an effective amount of the IL-2 complex of claim 14.~~

~~59. — A method of treating autoimmune hepatitis, comprising administering to a subject in need thereof an effective amount of the IL-2 conjugate of claim 15.~~

~~60~~18. ~~A method of treating nephrotic syndrome, comprising administering to a subject in need thereof~~
Use of an effective amount of any of:

(i) the IL-2 variant of claim 1 or 2,

(ii) the IL-2 fusion protein of claim 3 or 4,

(iii) the IL-2 complex of claim 5, or

(iv) the IL-2 conjugate of claim 6~~the IL-2 variant of any of claims 1-4;~~
in the manufacture of a medicament for treating nephrotic syndrome in a subject.

~~61. — A method of treating nephrotic syndrome, comprising administering to a subject in need thereof an effective amount of the IL-2 fusion protein of any of claims 5-13.~~

~~62. — A method of treating nephrotic syndrome, comprising administering to a subject in need thereof an effective amount of the IL-2 complex of claim 14.~~

~~63. — A method of treating nephrotic syndrome, comprising administering to a subject in need thereof an effective amount of the IL-2 conjugate of claim 15.~~

~~64~~19. A kit comprising any of:

(i) the IL-2 variant of claim 1 or 2,

(ii) the IL-2 fusion protein of claim 3 or 4,

(iii) the IL-2 complex of claim 5, or

(iv) the IL-2 conjugate of claim 6; and

~~the IL-2 variant of any of claims 1-4 and instructions for use.~~

20. The IL-2 variant of claim 1 or 2, the IL-2 fusion protein of claim 3 or 4, the IL-2 complex of claim 5, or the IL-2 conjugate of claim 6 for use as a medicament.

~~65. — A kit comprising the IL-2 fusion protein of any of claims 5-13 and instructions for use.~~

~~66. — A kit comprising the IL-2 complex of claim 14 and instructions for use.~~

~~67. — A kit comprising the IL-2 conjugate of claim 15 and instructions for use.~~

~~68. — The IL-2 variant of any of claims 1-4 for use in a method of inducing immune tolerance in a subject.~~

~~69. — The IL-2 fusion protein of any of claims 5-13 for use in a method of inducing immune tolerance in a subject.~~

70. — ~~The IL 2 complex of claim 14 for use in a method of inducing immune tolerance in a subject.~~
71. — ~~The IL 2 conjugate of claim 15 for use in a method of inducing immune tolerance in a subject.~~
72. — ~~The IL 2 variant of any of claims 1-4 for use in a method of treating an autoimmune disease in a subject.~~
73. — ~~The IL 2 fusion protein of any of claims 5-13 for use in a method of autoimmune hepatitis an autoimmune disease in a subject.~~
74. — ~~The IL 2 complex of claim 14 for use in a method of autoimmune hepatitis an autoimmune disease in a subject.~~
75. — ~~The IL 2 conjugate of claim 15 for use in a method of autoimmune hepatitis an autoimmune disease in a subject.~~
76. — ~~The IL 2 variant of any of claims 1-4 for use in a method of treating lupus nephritis in a subject.~~
77. — ~~The IL 2 fusion protein of any of claims 5-13 for use in a method of lupus nephritis in a subject.~~
78. — ~~The IL 2 complex of claim 14 for use in a method of treating lupus nephritis in a subject.~~
79. — ~~The IL 2 conjugate of claim 15 for use in a method of treating lupus nephritis in a subject.~~
80. — ~~The IL 2 variant of any of claims 1-4 for use in a method of treating autoimmune hepatitis in a subject.~~
81. — ~~The IL 2 fusion protein of any of claims 5-13 for use in a method of treating autoimmune hepatitis in a subject.~~
82. — ~~The IL 2 complex of claim 14 for use in a method of treating autoimmune hepatitis in a subject.~~
83. — ~~The IL 2 conjugate of claim 15 for use in a method of treating autoimmune hepatitis in a subject.~~

84. — ~~The IL 2 variant of any of claims 1-4 for use in a method of treating nephrotic syndrome in a subject.~~

85. — ~~The IL 2 fusion protein of any of claims 5-13 for use in a method of treating nephrotic syndrome in a subject.~~

86. — ~~The IL 2 complex of claim 14 for use in a method of treating nephrotic syndrome in a subject.~~

87. — ~~The IL 2 conjugate of claim 15 for use in a method of treating nephrotic syndrome in a subject.~~