

## Claims

What is claimed is:

1. A human interleukin-2 (IL-2) mutein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO:1, wherein said IL-2 mutein has one or more mutations of V91K,H16E; V91K,D20L; D84R,E61Q; V91K,D20A,E61Q,M104T; N88K,M104L; V91H,M104L; V91K,H16E,M104V; V91K,H16R,M104V; V91K,H16R,M104T; V91K,D20A,M104T; V91K,H16E,M104T; V91K,H16E,E61Q,M104T; V91K,H16R,E61Q,M104T; ~~V91K,H16E~~; V91H,D20A,M104T; H16E,V91H,M104V; V91H,D20A,E61Q,M104T; V91H,H16R,E16Q; V91K,D20A,M104V; H16E,V91H; V91H,D20A,M104V; H16E,V91H,M104T; H16E,V91H,E61Q,M104T; V91K,E61Q,H16E; V91K,H16R,M104L; H16E,V91H,E16Q; V91K,E61Q,H16R; D20W,V91K,E61Q; V91H,H16R; V91K,H16R; D20W,V91K,E61Q,M104T; V91K,D20A; V91H,D20A,E16Q; V91K,D20A,M104L; V91H,D20A; V91K,E61Q,D20A; V91H,M104T; V91H,M104V; V91K,E61Q; V91K,N88K,E61Q,M104T; V91K,N88K,E61Q; V91H,E61Q; V91K,N88K; D20A,H16E,M104T; D20A,M104T; H16E,N88K; D20A,M104V; D20A,M104L; H16E,M104T; H16E,M104V; N88K,M104V; N88K,E61Q; D20A,E61Q; H16R,D20A; D20W,E61Q; H16E,E61Q; H16E,M104L; N88K,M104T; D20A,H16E; D20A,H16E,E16Q; D20A,H16R,E16Q; or V91K,D20W; and preferentially stimulates T regulatory cells relative to other T cells.

2. The human IL-2 mutein of claim 1, wherein the IL-2 mutein further preferentially stimulates T regulatory cells relative to NK cells.

~~3. The human IL-2 mutein of claim 1, wherein said mutein is at least 95% identical to the amino acid sequence set forth in SEQ ID NO:1.~~

~~4. The human IL-2 mutein of claim 1, wherein said mutein is at least 99% identical to the amino acid sequence set forth in SEQ ID NO:1.~~

~~53.~~ The human IL-2 mutein of any one of claims 1-~~42~~, wherein the amino acid sequence of said mutein differs from the amino acid sequence set forth in SEQ ID NO:1 at C125A and at the mutation V91K,H16E; V91K,D20L; D84R,E61Q; V91K,D20A,E61Q,M104T; N88K,M104L; V91H,M104L; V91K,H16E,M104V; V91K,H16R,M104V; V91K,H16R,M104T; V91K,D20A,M104T; V91K,H16E,M104T; V91K,H16E,E61Q,M104T; V91K,H16R,E61Q,M104T; ~~V91K,H16E~~; V91H,D20A,M104T; H16E,V91H,M104V; V91H,D20A,E61Q,M104T; V91H,H16R,E16Q; V91K,D20A,M104V; H16E,V91H; V91H,D20A,M104V; H16E,V91H,M104T; H16E,V91H,E61Q,M104T; V91K,E61Q,H16E; V91K,H16R,M104L; H16E,V91H,E16Q;

V91K,E61Q,H16R; D20W,V91K,E61Q; V91H,H16R; V91K,H16R; D20W,V91K,E61Q,M104T; V91K,D20A; V91H,D20A,E16Q; V91K,D20A,M104L; V91H,D20A; V91K,E61Q,D20A; V91H,M104T; V91H,M104V; V91K,E61Q; V91K,N88K,E61Q,M104T; V91K,N88K,E61Q; V91H,E61Q; V91K,N88K; D20A,H16E,M104T; D20A,M104T; H16E,N88K; D20A,M104V; D20A,M104L; H16E,M104T; H16E,M104V; N88K,M104V; N88K,E61Q; D20A,E61Q; H16R,D20A; D20W,E61Q; H16E,E61Q; H16E,M104L; N88K,M104T; D20A,H16E; D20A,H16E,E16Q; D20A,H16R,E16Q; or V91K,D20W.

~~64.~~ The human IL-2 mutein of any one of claims 1-~~53~~, wherein the IL-2 mutein has: (a) less than 30% pSTAT activation of T effector cells at 1nM IL-2 treatment; (b) less than 30% pSTAT activation of T effector cells and greater than 40% pSTAT activation of T regulatory cells at 1nM IL - 2 treatment; or (c) greater than 40% pSTAT activation of T regulatory cells at 1nM IL - 2 treatment; using the protocol of Example 1.

~~7. The human IL-2 mutein of any one of claims 1-5, wherein the IL-2 mutein has less than 30% pSTAT activation of T effector cells and greater than 40% pSTAT activation of T regulatory cells at 1nM IL-2 treatment, using the protocol of Example 1.~~

~~8. The human IL-2 mutein of any one of claims 1-5, wherein the IL-2 mutein has greater than 40% pSTAT activation of T regulatory cells at 1nM IL-2 treatment, using the protocol of Example 1.~~

~~95.~~ The human IL-2 mutein of any one of claims 1-~~84~~, further wherein the IL-2 mutein has greater than 30% stability, using the protocol of Example 2.

~~106.~~ An Fc-fusion protein comprising an Fc and the human IL-2 mutein of any of claims 1-~~95~~.

~~117.~~ The Fc-fusion protein of claim ~~106~~, wherein: (a) the Fc is a human IgG1 Fc, wherein the human IgG1 Fc comprises one or more mutations altering effector function of said Fc, wherein the human IgG1 comprises a substitution N297G; (b) the Fc-fusion protein comprises a substitution or deletion of the C-terminal lysine of said human IgG Fc; (c) a linker connects the Fc and human IL-2 mutein portions of said protein, wherein the linker is GGGGS (SEQ ID NO: 5), GGNGT, (SEQ ID NO: 6), or YGNGT (SEQ ID NO: 7); (d) the IL-2 mutein further comprises an amino acid addition, substitution, or deletion altering glycosylation of said Fc-fusion protein when expressed in mammalian cells; (e) the IL-2 mutein comprises a T3 substitution, wherein the IL-2 mutein comprises a T3N or T3A substitution (f) the IL-2 mutein further comprises an S5 mutation, wherein the IL-2 mutein further comprises an S5T mutation;

(g) said Fc-fusion protein comprises an Fc dimer; (h) said Fc-fusion protein comprises two IL-2 muteins; or (i) said Fc-fusion protein comprises a single IL-2 mutein.

~~12. The Fc-fusion protein of claim 11, wherein the human IgG1 Fc comprises one or more mutations altering effector function of said Fc.~~

~~13. The Fc-fusion protein of claim 12, wherein the human IgG1 comprises a substitution at N297.~~

~~14. The Fc-fusion protein of claim 13, wherein the substitution at N297 is N297G.~~

~~15. The Fc-fusion protein of any of claims 11-14, comprising a substitution or deletion of the C-terminal lysine of said human IgG Fc.~~

~~16. The Fc-fusion protein of claim 15, wherein the C-terminal lysine of said human IgG Fc is deleted.~~

~~17. The Fc-fusion protein of any of claims 11-16, wherein a linker connects the Fc and human IL-2 mutein portions of said protein.~~

~~18. The Fc-fusion protein of claim 17, wherein the linker is GGGGS (SEQ ID NO: 5), GGNGT, or (SEQ ID NO: 6), and YGNGT (SEQ ID NO: 7).~~

~~19. The Fc-fusion protein of claim 18, wherein the linker is GGGGS (SEQ ID NO: 5).~~

~~20. The Fc-fusion protein of any of claims 11-19, wherein the IL-2 mutein further comprises an amino acid addition, substitution, or deletion altering glycosylation of said Fc-fusion protein when expressed in mammalian cells.~~

~~21. The Fc-fusion protein of claim 20, wherein the IL-2 mutein comprises a T3 substitution.~~

~~22. The Fc-fusion protein of claim 21, wherein the IL-2 mutein comprises a T3N or T3A substitution.~~

~~23. The Fc-fusion protein of claim 22, wherein the IL-2 mutein comprises a T3N substitution.~~

~~24. The Fc-fusion protein of claim 23, wherein the IL-2 mutein further comprises an S5 mutation.~~

~~25. The Fc-fusion protein of claim 24, wherein the IL-2 mutein further comprises an S5T mutation.~~

~~26. The Fc-fusion protein of any of claim 11-25, wherein said Fc-fusion protein comprises an Fc dimer.~~

- ~~27. The Fc fusion protein of claim 26, wherein said Fc fusion protein comprises two IL-2 muteins.~~
- ~~28. The Fc fusion protein of claim 26, wherein said Fc fusion protein comprises a single IL-2 mutein.~~
- ~~298.~~ An isolated nucleic acid encoding the human IL-2 mutein of any of claims 1-~~95~~.
- ~~309.~~ An isolated nucleic acid encoding an Fc portion of an antibody and the human IL-2 muteins of ~~any of claims 1-9~~claim 6 or 7.
- ~~3110.~~ The isolated nucleic acid of claim 30, wherein: (a) said Fc portion of an antibody and the human IL-2 mutein are encoded within a single open-reading frame; (b) the Fc is a human IgG1 Fc; (c) the human IgG1 Fc comprises one or more mutations altering effector function of said Fc, wherein the human IgG1 comprises an N297G substitution; (d) the isolated nucleic acid encodes a substitution or deletion of the C-terminal lysine of said human IgG Fc; (e) , further encoding a linker connecting the Fc portion of an antibody and the human IL-2 mutein, wherein the linker is GGGGS (SEQ ID NO: 5), GGNGT, (SEQ ID NO: 6), or YGNGT (SEQ ID NO: 7); or (f) the nucleic acid further encodes an amino acid addition, substitution, or deletion altering glycosylation of said Fc-fusion protein when expressed in mammalian cells, wherein the substitution is a T3N, T3A, or S5T mutation.
- ~~32. The isolated nucleic acid of claim 30 or 31, wherein the Fc is a human IgG1 Fc.~~
- ~~33. The isolated nucleic acid of claim 32, wherein the human IgG1 Fc comprises one or more mutations altering effector function of said Fc.~~
- ~~34. The isolated nucleic acid of claim 33, wherein the human IgG1 comprises a substitution at N297.~~
- ~~35. The isolated nucleic acid of claim 32, wherein the substitution at N297 is N297G.~~
- ~~36. The isolated nucleic acid of any of claims 32-35 encoding a substitution or deletion of the C-terminal lysine of said human IgG Fc.~~
- ~~37. The isolated nucleic acid of claim 36, wherein the C-terminal lysine of said human IgG Fc is deleted.~~
- ~~38. The isolated nucleic acid of any of claims 30-37, further encoding a linker connecting the Fc portion of an antibody and the human IL-2 mutein.~~

~~39. — The isolated nucleic acid of claim 38, wherein the linker is GGGGS (SEQ ID NO: 5), GGNGT, or (SEQ ID NO: 6), and YGNGT (SEQ ID NO: 7).~~

~~40. — The isolated nucleic acid of claim 39, wherein the linker is GGGGS (SEQ ID NO: 5).~~

~~41. — The isolated nucleic acid of any of claims 30-40, wherein the IL-2 mutein further comprises an amino acid addition, substitution, or deletion altering glycosylation of a protein comprising said IL-2 mutein when expressed in mammalian cells.~~

~~42. — The isolated nucleic acid of claim 41, wherein the IL-2 mutein comprises a T3 substitution.~~

~~43. — The isolated nucleic acid of claim 42, wherein the IL-2 mutein comprises a T3N or T3A substitution.~~

~~44. — The isolated nucleic acid of claim 43, wherein the IL-2 mutein comprises a T3N substitution.~~

~~45. — The isolated nucleic acid of claim 44, wherein the IL-2 mutein further comprises an S5 mutation.~~

~~46. — The isolated nucleic acid of claim 45, wherein the IL-2 mutein further comprises an S5T mutation.~~

~~47~~11. An expression vector comprising the isolated nucleic acid of any of claims ~~29-46~~7-10 operably linked to a promoter.

~~48~~12. A host cell comprising the isolated nucleic acid of any of claims ~~29-46~~7-10, wherein: (a) the isolated nucleic acid is operably linked to a promoter; (b) said host cell is a prokaryotic cell; (c) the host cell is E. coli; (d) said host cell is a eukaryotic cell; (e) the host cell is a mammalian cell; or (f) the host cell is a Chinese hamster ovary (CHO) cell line.

~~49. — The host cell of claim 48, wherein the isolated nucleic acid is operably linked to a promoter.~~

~~50. — The host cell of claim 48 or 49, wherein said host cell is a prokaryotic cell.~~

~~51. — The host cell of claim 50, wherein the host cell is *E. coli*.~~

~~52. — The host cell of claim 48 or 49, wherein said host cell is a eukaryotic cell.~~

~~53. — The host cell of claim 52, wherein the host cell is a mammalian cell.~~

~~54. — The host cell of claim 53, wherein the host cell is a Chinese hamster ovary (CHO) cell line.~~

~~5513.~~ A method of making a human IL-2 mutein, comprising culturing a host cell of ~~any of claims 49 to 54~~claim 12 under conditions in which said promoter is expressed and harvesting the human IL-2 mutein from said culture.

~~5614.~~ A method of making a Fc-fusion protein, comprising culturing a host cell of ~~any of claims 49 to 54~~claim 12 under conditions in which said promoter is expressed and harvesting the Fc-fusion protein from said culture.

~~57. A method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells, comprising contacting the population of T cells with an effective amount of a human IL-2 mutein of any of claims 1-9.~~

~~58. The method of claim 57, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases.~~

~~59. The method of claim 58, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.~~

~~60. A method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells, comprising contacting the population of T cells with an effective amount of an Fc-fusion protein of any of claims 10-28.~~

~~61. The method of claim 60, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases.~~

~~62. The method of claim 61, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.~~

~~63. A method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within peripheral blood of a subject, comprising administering an effective amount of a human IL-2 mutein of any of claims 1-9.~~

~~64. The method of claim 63, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases.~~

~~65. The method of claim 64, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.~~

~~66. A method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within the peripheral blood of a subject, comprising administering an effective amount of an Fc-fusion protein of any of claims 10-28.~~

~~67. — The method of claim 66, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases.~~

~~68. — The method of claim 67, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.~~

~~69. — A method of increasing the ratio of regulatory T cells (Tregs) to natural killer (NK) cells within the peripheral blood of a subject, comprising administering an effective amount of a human IL-2 mutein of any of claims 1-9.~~

~~70. — The method of claim 69, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases.~~

~~71. — The method of claim 70, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases at least 50%.~~

~~72. — A method of increasing the ratio of regulatory T cells (Tregs) to natural killer (NK) cells within the peripheral blood of a subject, comprising administering an effective amount of an Fc-fusion protein of any of claims 10-28.~~

~~73. — The method of claim 72, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases.~~

~~74. — The method of claim 73, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases at least 50%.~~

~~7515. Use of an IL-2 mutein of any of claims 1-5 in the manufacture of a medicament A method of for~~  
treating a subject with an inflammatory or autoimmune disease, ~~said method comprising administering~~  
to said subject a therapeutically effective amount of an IL-2 mutein of any of claims 1-9.

~~7616. Use of an Fc-fusion protein of claim 6 or 7 in the manufacture of a medicament for~~  
treating a subject with an inflammatory or autoimmune disease, ~~said method comprising administering~~  
to said subject a therapeutically effective amount of an Fc-fusion protein of any of claims 10-25.

~~7717. The method of treating a subject with an inflammatory or autoimmune disease~~  
use of claim 75  
15 or 7616, wherein administration causes reduction of at least one symptom of the disease.

~~78. — The method of claim 77, wherein the ratio of regulatory T cells (Tregs) to non-regulatory T cells within the peripheral blood of a subject increases after the administration.~~

~~79. —The method of claim 77, wherein the ratio of regulatory T cells (Tregs) to non-regulatory T cells within the peripheral blood of a subject remains essentially the same after the administration.~~

~~80~~18. The ~~method~~use of any of claims ~~75~~15-~~79~~17, wherein the inflammatory or autoimmune disease is lupus, graft-versus-host disease, hepatitis C-induced vasculitis, type I diabetes, type II diabetes, multiple sclerosis, rheumatoid arthritis, alopecia areata, atherosclerosis, psoriasis, organ transplant rejection, Sjögren's Syndrome, Behcet's disease, spontaneous loss of pregnancy, atopic diseases, asthma, or inflammatory bowel diseases.