In one aspect, the present disclosure refers to a human interleukin-2 (IL-2) chimeric molecule comprising a human IL-2 polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence set forth in SEQ ID NO:1, and a tumor necrosis factor receptor (TNFR) agonist selected from the group consisting of anti-OX40, anti-DR3, and TNF.

In another aspect, the present disclosure refers to an isolated nucleic acid encoding the human IL-2 chimeric molecule as disclosed herein.

In another aspect, the present disclosure refers to an isolated nucleic acid encoding the Fc fusion protein as disclosed herein.

In another aspect, the present disclosure refers to an expression vector comprising the isolated nucleic acid as disclosed herein operably linked to a promoter.

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

In yet another aspect, the present disclosure refers to a method of making a human IL-2 chimeric molecule, comprising culturing a host cell as disclosed herein under conditions in which said promoter is expressed and harvesting the human IL-2 chimeric molecule from said culture.

In yet another aspect, the present disclosure refers to a method of making a Fc-fusion protein, comprising culturing a host cell as disclosed herein under conditions in which said promoter is expressed and harvesting the Fc-fusion protein from said culture.

In yet another aspect, the present disclosure refers to an *in vitro* method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells or within peripheral blood of a subject, comprising contacting the population of T cells with an effective amount of a human IL-2 chimeric molecule as disclosed herein or an Fc-fusion protein as disclosed herein.

In yet another aspect, the present disclosure refers to an *in vitro* method of increasing the ratio of regulatory T cells (Tregs) to natural killer (NK) cells within the peripheral blood of a subject, comprising contacting the population of T cells with an effective amount of a human IL-2 chimeric molecule as disclosed herein or an Fc-fusion protein as disclosed herein.

In yet another aspect, the present disclosure refers to the use of a human IL-2 chimeric molecule of as disclosed herein or an Fc-fusion protein as disclosed herein in the manufacture of a medicament for treating a subject with an inflammatory or autoimmune disease.

In some examples, the disclosure is an Fc-fusion protein comprising an Fc, a human IL-2 polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence set forth in SEQ ID NO:1, and a tumor necrosis factor receptor (TNFR) agonist selected from the group consisting of anti-OX40, anti-DR3, and TNF.

In some examples, the disclosure is a method of treating a subject with an inflammatory or autoimmune disease, said method comprising administering to said subject a therapeutically effective amount of a human interleukin-2 (IL-2) chimeric molecule comprising a human IL-2 polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence set forth in SEQ ID NO:1, and a tumor necrosis factor receptor (TNFR) agonist selected from the group consisting of anti-OX40, anti-DR3, and TNF complexes, or an Fc-fusion protein comprising an Fc, a human IL-2 polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence set forth in SEQ ID NO:1, and a tumor necrosis factor receptor (TNFR) agonist selected from the group consisting of anti-OX40, anti-DR3, and TNF.

**Brief Description of the Figures**

Figure 1 shows proliferation of Tregs upon stimulation with IL-2 in combination with a TNFR agonist (anti-OX40, recombinant TNF, or anti-DR3). (A) Cell Trace Violet (CTV) labeled human PBMCs were stimulated with indicated reagents for 4 days followed by flow cytometry analysis. Histograms are arranged in the following order (from bottom to top): Unstimulated, stimulated with 1µg/ml anti-CD3, 20U/ml IL-2, IgG, TNFR agonist (anti-OX40, recombinant TNF, anti-DR3), TNFR agonist plus IL-2. Histograms were gated on Treg cells (CD4+Foxp3+). Only the positive control anti-CD3 plots and the combination of TNFR agonist (anti-OX40, recombinant TNF, and anti-DR3) showed proliferation of Tregs.

Figure 2 shows histogram plots of anti-OX40 and IL-2 on PBMCs. (A) CTV labeled human PBMCs were stimulated with indicated titrating doses of anti-OX40 (clone 15A9) for 4 days followed by flow cytometry analysis. Histograms were gated on Treg cells (CD4+Foxp3+). Cells stimulated with CD3 show robust proliferation and serve as a positive control for the assay. Stimulation with IL2 or control IgG does not result in any CTV dilution. No proliferation was observed with any of the indicated doses of anti-OX40 alone. However, combined stimulation using anti-OX40 and IL2 lead to Treg cell proliferation as seen by CTV dilution. (B) Summary of the data from (A). Graphs show three replicates from one donor for each condition. Response of T cells to these stimulations was also assessed and only very low levels of T cell proliferation were observed at higher doses of anti-OX40/IL2 treatment.

## Claims

What is claimed is:

1. A human interleukin-2 (IL-2) chimeric molecule comprising a human IL-2 polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence set forth in SEQ ID NO:1, and a tumor necrosis factor receptor (TNFR) agonist selected from the group consisting of anti-OX40, anti-DR3, and TNF.

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

3. The human IL-2 chimeric molecule of any of the above claims, wherein the human IL-2 polypeptide is a human IL-2 polypeptide mutein, wherein said IL-2 mutein has at least one mutation selected from V91K, N30S, N30D, Y31H, Y31S, K35R, V69A, Q74P, 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, V91K/D20W, V91A/H16A, V91A/H16D, V91A/H16E, V91A/H16S, V91E/H16A, V91E/H16D, V91E/H16E, V91E/H16S, V91K/H16A, V91K/H16D, V91K/H16S, V91S/H16E, L12G, L12K, L12Q, L12S, Q13G, E15A, E15G, E15S, H16A, H16D, H16G, H16K, H16M, H16N, H16R, H16S, H16T, H16V, H16Y, L19A, L19D, L19E, L19G, L19N, L19R, L19S, L19T, L19V, D20A, D20E, D20F, D20G, D20T, D20W, M23R, N30S, Y31H, K35R, V69A, Q74P, R81A, R81G, R81S, R81T, D84A, D84E, D84G, D84I, D84M, D84Q, D84R, D84S, D84T, S87R, N88A, N88D, N88E, N88F, N88G, N88M, N88R, N88S, N88V, N88W, V91D, V91E, V91G, V91S, I92K, I92R, and/or E95G and preferentially stimulates T regulatory cells.

4. The human IL-2 chimeric molecule of claim 3, further comprising a substitution at C125A.

5. An Fc-fusion protein comprising an Fc, a human IL-2 polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence set forth in SEQ ID NO:1, and a tumor necrosis factor receptor (TNFR) agonist selected from the group consisting of anti-OX40, anti-DR3, and TNF.

6. The Fc-fusion protein of claim 5, wherein the anti-OX40 is an anti-OX40 antibody, wherein the anti-OX40 antibody has a heavy chain amino acid sequence of SEQ ID NO:9, a light chain amino acid sequence of SEQ ID NO:10, or both a heavy chain antibody sequence of SEQ ID NO:9 and a light chain amino acid sequence of SEQ ID NO:10.

7. The Fc-fusion protein of claim 5 or 6, wherein the Fc is a human IgG1 Fc.

8. The Fc-fusion protein of claim 7, wherein the human IgG1 Fc comprises one or more mutations altering effector function of said Fc, wherein the human IgG1 comprises an N297G substitution.

9. The Fc-fusion protein of any of claims 5-8, comprising a substitution or deletion of the C-terminal lysine of said human IgG Fc.

10. The Fc-fusion protein of claim 9, wherein the C-terminal lysine of said human IgG Fc is deleted.

11. The Fc-fusion protein of any of claims 5-10, wherein a linker connects the Fc and human IL-2 polypeptide portions of said protein.

12. The Fc-fusion protein of any of claims 5-10, wherein a linker connects the Fc and tumor necrosis factor receptor (TNFR) agonist portions of said protein.

13. The Fc-fusion protein of claim 11 or 12, wherein the linker is GGGGS (SEQ ID NO: 5), GGNGT, or (SEQ ID NO: 6), and YGNGT (SEQ ID NO: 7).

14. The Fc-fusion protein of any of claims 5-13, wherein a first linker connects the Fc and human IL-2 polypeptide portions of said protein and a second linker connects the Fc and tumor necrosis factor receptor (TNFR) agonist portions of said protein.

15. The Fc-fusion protein of claim 14, wherein the first linker is GGGGS (SEQ ID NO: 5), GGNGT, or (SEQ ID NO: 6), and YGNGT (SEQ ID NO: 7) and the second linker is GGGGS (SEQ ID NO: 5), GGNGT, or (SEQ ID NO: 6), and YGNGT (SEQ ID NO: 7).

16. The Fc-fusion protein of any of claims 5-15, wherein the IL-2 chimeric molecule further comprises an amino acid addition, substitution, or deletion altering glycosylation of said Fc-fusion protein when expressed in mammalian cells, wherein the addition, substitution, or deletion altering glycosylation is a T3N, T3A, or S5T substitution.

17. An isolated nucleic acid encoding the human IL-2 chimeric molecule of any of claims 1-4.

18. An isolated nucleic acid encoding the Fc fusion protein of any of claims 5-16.

19. An expression vector comprising the isolated nucleic acid of claim 17 or 18 operably linked to a promoter.

20. A host cell comprising the isolated nucleic acid of any of claims 17-19.

21. A method of making a human IL-2 chimeric molecule, comprising culturing a host cell of claim 20 under conditions in which said promoter is expressed and harvesting the human IL-2 chimeric molecule from said culture.

22. A method of making a Fc-fusion protein, comprising culturing a host cell of claim 20 under conditions in which said promoter is expressed and harvesting the Fc-fusion protein from said culture.

23. An *in vitro* method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells or within peripheral blood of a subject, comprising contacting the population of T cells with an effective amount of a human IL-2 chimeric molecule of any of claims 1-4 or an Fc-fusion protein of any one of claims 5-15.

24. The method of claim 23, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases.

25. The method of claim 24, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

26. An *in vitro* method of increasing the ratio of regulatory T cells (Tregs) to natural killer (NK) cells within the peripheral blood of a subject, comprising contacting the population of T cells with an effective amount of a human IL-2 chimeric molecule of any of claims 1-4 or an Fc-fusion protein of any one of claims 5-15.

27. The method of claim 26, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases.

28. The method of claim 27, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases at least 50%.

29. Use of a human IL-2 chimeric molecule of any of claims 1-4 or an Fc-fusion protein of any one of claims 5-15 in the manufacture of a medicament for treating a subject with an inflammatory or autoimmune disease.

30. The use of claim 29, wherein administration of the medicament causes reduction of at least one symptom of the disease.

31. The use of claim 30, wherein the ratio of regulatory T cells (Tregs) to non-regulatory T cells within the peripheral blood of a subject increases after administration.

32. The use of claim 30, 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 administration.

33. The use of any of claims 29-32, wherein the inflammatory or autoimmune disease is inflammation, autoimmune disease, atopic diseases, paraneoplastic autoimmune diseases, cartilage inflammation, arthritis, rheumatoid arthritis, juvenile arthritis, juvenile rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, polyarticular juvenile rheumatoid arthritis, systemic onset juvenile rheumatoid arthritis, juvenile ankylosing spondylitis, juvenile enteropathic arthritis, juvenile reactive arthritis, juvenile Reiter's Syndrome, SEA Syndrome (Seronegativity, Enthesopathy, Arthropathy Syndrome), juvenile dermatomyositis, juvenile psoriatic arthritis, juvenile scleroderma, juvenile systemic lupus erythematosus, juvenile vasculitis, pauciarticular rheumatoid arthritis, polyarticular rheumatoid arthritis, systemic onset rheumatoid arthritis, ankylosing spondylitis, enteropathic arthritis, reactive arthritis, Reiter's Syndrome, dermatomyositis, psoriatic arthritis, scleroderma, vasculitis, myolitis, polymyolitis, dermatomyolitis, polyarteritis nodossa, Wegener's granulomatosis, arteritis, ploymyalgia rheumatica, sarcoidosis, sclerosis, primary biliary sclerosis, sclerosing cholangitis, Sjogren's syndrome, psoriasis, plaque psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, erythrodermic psoriasis, dermatitis, atopic dermatitis, atherosclerosis, lupus, Still's disease, Systemic Lupus Erythematosus (SLE), myasthenia gravis, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, celiac disease, multiple sclerosis (MS), asthma, COPD, , rhinosinusitis, rhinosinusitis with polyps, eosinophilic esophogitis, eosinophilic bronchitis, Guillain-Barre disease, Type I diabetes mellitus, thyroiditis(e.g., Graves' disease), Addison's disease, Raynaud's phenomenon, autoimmune hepatitis, GVHD, transplantation rejection, kidney damage, hepatitis C-induced vasculitis, or spontaneous loss of pregnancy.

34. The use of any of claims 29-32, wherein the inflammatory or autoimmune disease is Systemic Lupus Erythematosus (SLE), graft-versus-host disease, hepatitis C-induced vasculitis, Type I diabetes, rheumatoid arthritis, multiple sclerosis, spontaneous loss of pregnancy, atopic diseases, and inflammatory bowel diseases, including ulcerative colitis, celiac disease.

35. The use of any of claims 29-32, 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.