

IMMUNOMODULATORY FUSION PROTEINS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of, and priority to, U.S. provisional patent application serial number 62/564,145, filed September 27, 2017, which is hereby incorporated by reference
5 herein in its entirety.

FIELD OF THE INVENTION

[0002] The field of the invention is molecular biology, specifically immunology and fusion proteins, *e.g.*, cytokine receptor fusion proteins.

BACKGROUND

10 [0003] Cytokines are small, secreted cell signaling proteins that have a wide range of activities including regulation of cell growth and differentiation and modulation of immune function. Cytokines, cytokine receptors, and certain other immunomodulatory proteins have been used as therapeutics to treat a variety of medical conditions. However, the administration of such proteins, for example, by subcutaneous or vascular routes, can result in inappropriate cellular
15 and extracellular localization thereby limiting therapeutic activity and/or increasing the risk of toxicity.

[0004] IL-10 is a homodimeric cytokine with immunoregulatory properties produced by cells including activated Th2 cells, B cells, keratinocytes, monocytes and macrophages (Moore *et al.* (1993) ANNU. REV. IMMUNOL. 11:165). IL-10 inhibits activation and effector functions of a
20 variety of cells including T cells, monocytes and macrophages. In particular, IL-10 inhibits cytokine synthesis, including that of IL-1, IFN- γ , and TNF, by cells such as Th1 cells, natural killer cells, monocytes, and macrophages (Fiorentino *et al.* (1989) J. EXP. MED. 170:2081-2095; Fiorentino *et al.* (1991) J. IMMUNOL. 146:3444; Hsu *et al.* (1992) INT. IMMUNOL. 4:563; Hsu *et al.* (1992) INT. IMMUNOL. 4:563; D'Andrea *et al.* (1993) J. EXP. MED. 178:1041; de Waal Malefyt *et al.*
25 (1991) J. EXP. MED. 174:915; Fiorentino *et al.* (1991) J. IMMUNOL. 147:3815). Multiple pathogens, including intracellular pathogens, elicit IL-10 production to slow or stall the effective removal of the pathogen by the immune system (Moore *et al.* (1993) *supra*).

[0005] Despite the advances that have been made to date in treating IL-10 mediated disorders, there is a need for improved therapies for treating such disorders.

SUMMARY OF THE INVENTION

30 [0006] The ~~invention~~disclosure is based, in part, upon the discovery of linker sequences that improve the function of fusion proteins, *e.g.*, cytokine receptor fusion proteins, *e.g.*, IL-10 receptor (IL-10R)

fusion proteins, *e.g.*, IL-10 receptor subunit alpha (IL-10RA) fusion proteins, *e.g.*, IL-10 traps. The linker sequences may permit a ligand binding portion of a fusion protein (*e.g.*, a cytokine receptor) to bind optimally to a ligand (*e.g.*, a cytokine), provide temporal and spatial colocalization of two or more components of a fusion protein (*e.g.*, two subunits of a dimeric cytokine), optimize expression from an expression vector (*e.g.*, a viral vector), reduce immunogenicity, or provide a cleavage site to allow for release of a component of the fusion protein. For example, the linker sequences may provide sufficient flexibility to allow a ligand binding domain of a cytokine receptor to adopt a native conformation in the context of a fusion protein, and minimize the potential immunogenicity of the fusion protein for use as a therapeutic agent.

[0007] In one aspect, the present disclosure provides an isolated fusion protein comprising, in an N- to C- terminal orientation: (i) a soluble portion of an extracellular domain of a human IL-10 receptor subunit alpha, wherein the soluble portion of an extracellular domain of a human IL-10 receptor has greater than 95% sequence identity to amino acid residues 22-229 of SEQ ID NO: 12; (ii) an amino acid linker; (iii) an immunoglobulin (Ig) hinge region; and (iv) an immunoglobulin (Ig) Fc domain; wherein the linker consists of 10 to 40 amino acid residues, wherein the linker is a sequence derived from an endogenous human protein, wherein the Ig hinge region and the Ig Fc domain together comprise an amino acid sequence having greater than 95% identity to SEQ ID NOs: 13-21, and wherein the linker comprises a sequence derived from a human protein selected from albumin and casein, or comprises a C-terminal portion of an immunoglobulin (Ig) CH1 domain having greater than 95% sequence identity to an amino acid sequence selected from SEQ ID NO: 1, 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: 53, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63. In an ~~one aspect~~ example, the ~~invention~~ disclosure provides an isolated fusion protein that comprises, for example, in an N- to C- terminal orientation: a first portion of an extracellular domain, transmembrane domain, or intracellular domain of a cytokine, cytokine receptor, or immunomodulatory protein; an amino acid linker; and at least one of, a second portion of an extracellular domain, transmembrane domain, or intracellular domain of a cytokine, cytokine receptor, or immunomodulatory protein; an immunoglobulin (Ig) hinge region; and an immunoglobulin (Ig) Fc domain. In certain ~~embodiment~~ examples, the linker comprises from about 5 to about 40 amino acid residues. In certain ~~embodiment~~ examples, the fusion protein comprises a portion of an IL-10 receptor, *e.g.*, a human IL-10 receptor, *e.g.*, IL-10RA.

[0008] In another aspectexample, the inventiondisclosure provides an isolated fusion protein that comprises, in an N- to C-terminal orientation: a soluble portion of an extracellular domain of a cytokine receptor; an amino acid linker; an immunoglobulin (Ig) hinge region; and an immunoglobulin (Ig) Fc domain; wherein the linker comprises from about 5 to about 40 amino acid residues. In certain embodimentexamples, the cytokine receptor is an IL-10 receptor, *e.g.*, a human IL-10 receptor, *e.g.*, IL-10RA.

[0009] In certain embodimentexamples of any of the foregoing fusion proteins, the amino acid linker may comprise, *e.g.*, from about 5 to about 15, from about 5 to about 20, from about 5 to about 30, from about 10 to about 15, from about 10 to about 20, from about 10 to about 30, from about 10 to about 40, from about 15 to about 20, from about 15 to about 30, or from about 15 to about 40 amino acid residues.

[0010] In certain embodimentexamples of any of the foregoing fusion proteins, the amino acid linker sequence is derived from an endogenous human protein, *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, IgM, albumin, or casein. In certain embodimentexamples, the amino acid linker comprises a C-terminal portion of an immunoglobulin (Ig) CH1 domain, *e.g.*, an IgG1, IgG2,

IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM CH1 domain. In certain [embodimentexamples](#), the amino acid linker comprises an amino acid sequence selected from SEQ ID NO: 1, 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: 53, SEQ ID NO: 54, and SEQ ID NO: 57. In certain [embodimentexamples](#), the amino acid linker comprises a C-terminal portion of an IgG1 CH1 domain, *e.g.*, the amino acid linker comprises an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 53, SEQ ID NO: 54, and SEQ ID NO: 57, *e.g.*, the amino acid sequence of SEQ ID NO: 57.

[0011] In certain [embodimentexamples](#) of any of the foregoing fusion proteins, the amino acid linker comprises a sequence derived from a cytokine, signaling molecule, immunomodulatory protein or peptide, or a biologically active peptide.

[0012] In certain [embodimentexamples](#) of any of the foregoing fusion proteins, the amino acid linker comprises a cleavage site, *e.g.*, a proteolytic cleavage site, *e.g.*, a proteolytic cleavage site that is cleaved by a protease present in the endoplasmic reticulum or golgi of a eukaryotic cell.

In certain [embodimentexamples](#), the proteolytic cleavage site is a furin cleavage site, *e.g.*, a furin cleavage site comprising the sequence RX_1X_2R (SEQ ID NO: 50), wherein X_1 is any amino acid, and X_2 is Lys or Arg, *e.g.*, a furin cleavage site comprising the sequence RAKR (SEQ ID NO: 51). In certain [embodimentexamples](#) of any of the foregoing fusion proteins, the amino acid linker is proteolytically stable in a mammal or plant.

[0013] In certain [embodimentexamples](#) of any of the foregoing fusion proteins, the soluble portion of an extracellular domain of a cytokine receptor is a soluble portion of an extracellular domain of the human IL-10R, *e.g.*, IL-10RA. For example, in certain [embodimentexamples](#), the soluble portion of an extracellular domain of a cytokine receptor comprises the amino acid sequence of SEQ ID NO: 12 or amino acid residues 22-229 of SEQ ID NO: 12.

[0014] In certain [embodimentexamples](#) of any of the foregoing fusion proteins, the fusion protein comprises one or more of IL-10, TGF- β , a TGF β receptor, *e.g.*, the TGF β type II receptor (T β RII), CD80, CD19, CD20, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-12B/p40, IL-23A/p19, IL27A/p28, IL-27B/EBI3, IL-15, CD154, CD70, TNF-alpha, CD86, CD137, CD137L, BORIS/CTCFL, FGF, ICAM, IL-24, GM-CSF, MAGE, NY-ESO-1, angiostatin, endostatin, acetylcholine, interferon-gamma, DKK1/Wnt, p53, Ox40L, GM-CSF, an IL-15 receptor fusion protein, GITRL, CD40L, CD70, secreted flagellin, IL-12, thymidine kinase, an anti-PD-1 antibody heavy chain or light chain, an anti-PD-L1 antibody heavy chain or light chain, and an anti-CTLA-4 antibody heavy chain or light chain, or a functional fragment thereof.

[0015] In certain embodimentexamples of any of the foregoing fusion proteins, the Ig hinge region is selected from an IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, and IgM hinge region, and the Ig Fc domain, is selected from IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, and IgM Fc domain. In certain embodimentexamples, the Ig hinge region and Fc domain together comprise an amino acid sequence selected from 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 and SEQ ID NO: 21. In certain embodimentexamples, the Ig Fc, Ig hinge region, and Ig CH1 domain are derived from a single immunoglobulin.

[0016] In certain embodimentexamples of any of the foregoing fusion proteins, the fusion protein comprises an amino acid sequence selected from 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: 55, SEQ ID NO: 56, and SEQ ID NO: 58. In certain embodimentexamples, the fusion protein comprises an amino acid sequence selected from SEQ ID NO: 22, SEQ ID NO: 55, SEQ ID NO: 56, and SEQ ID NO: 58. In certain embodimentexamples, the fusion protein comprises the amino acid sequence of SEQ ID NO: 58.

[0017] In certain embodimentexamples of any of the foregoing fusion proteins, the fusion protein comprises an amino acid sequence having greater than 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to a sequence selected from 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: 55, SEQ ID NO: 56, and SEQ ID NO: 58.

[0018] In another aspectexample, the inventiondisclosure provides a dimeric cytokine binding protein comprising two of any of the foregoing fusion proteins covalently linked together, wherein each fusion protein comprises an extracellular domain of a cytokine receptor, and wherein the two extracellular domains together define a binding site for a cytokine.

[0019] In another aspectexample, the inventiondisclosure provides an isolated nucleic acid comprising a nucleotide sequence that encodes for any of the foregoing fusion proteins.

[0020] In another aspect, the inventiondisclosure provides an expression vector comprising any of the foregoing nucleic acids.

[0021] In another aspect, the inventiondisclosure provides a host cell comprising any of the foregoing the expression vectors. In another aspect, the inventiondisclosure provides a method of producing a fusion protein comprising growing a host cell under conditions to express the fusion protein and

purifying the fusion protein. In yet another aspect, the present disclosure provides a pharmaceutical composition comprising: (i) the fusion protein as described herein; and (ii) at least one pharmaceutically acceptable carrier or diluent. In another aspectexample, the inventiondisclosure provides a method of expressing a fusion protein in a target cell comprising exposing the cell to an effective amount of any of the foregoing expression vectors. In certain embodimentexamples, the fusion protein is cleaved posttranslationally into two polypeptide chains.

[0022] In another aspectexample, any of foregoing fusion proteins or expression vectors can be used, *e.g.*, to reduce cytokine activity in a subject, thereby treating various medical indications that are mediated by a cytokine, for example, IL-10. In another aspectexample, any of the foregoing fusion proteins or expression vectors can be used to inhibit proliferation of tumor cells *in vitro* and/or *in vivo*, inhibit tumor growth in a subject in need thereof, or treat cancer in a subject in need thereof. The subject may be, *e.g.*, an animal, *e.g.*, a mammal, *e.g.*, a human, *e.g.*, a pediatric human. For example, when administered to a human subject with cancer, the fusion proteins or expression vectors inhibit or reduce tumor growth, or, reduce the tumor load, in the subject.

[0023] In certain embodimentexamples, the cancer may be selected from melanoma, squamous cell carcinoma of the skin, basal cell carcinoma, head and neck cancer, breast cancer, anal cancer, cervical cancer, non-small cell lung cancer, mesothelioma, small cell lung cancer, renal cell carcinoma, prostate cancer, gastroesophageal cancer, colorectal cancer, testicular cancer, bladder cancer, ovarian cancer, liver cancer, hepatocellular carcinoma, cholangiocarcinoma, brain and central nervous system cancer, thyroid cancer, parathyroid cancer (*e.g.*, parathyroid carcinoma), endometrial cancer, neuroendocrine cancer, lymphoma (*e.g.*, Hodgkin and non-Hodgkin), leukemia, merkel cell carcinoma, gastrointestinal stromal tumors, multiple myeloma, uterine cancer, a sarcoma, kidney cancer, ocular cancer, pancreatic cancer, and a germ cell cancer (*e.g.*, ovarian germ cell cancer). In certain embodimentexamples, the cancer may be selected from leukemia, breast cancer, lung cancer, pancreatic cancer, endometrial cancer, ovarian cancer, prostate cancer, cervical cancer, brain cancer, skin cancer, colorectal cancer, gastric cancer, head and neck cancer, and leukemia.

[0024] In certain embodimentexamples, the fusion protein or expression vector is administered in combination with one or more therapies selected from surgery, radiation, chemotherapy, immunotherapy, hormone therapy, and virotherapy. In certain embodimentexamples, the fusion protein or expression vector is administered in combination with a lymphocyte, *e.g.*, a T-cell, *e.g.*, a CAR T-cell.

[0025] Any of the foregoing fusion proteins or expression vectors can also be used to treat an

inflammatory condition or infection in a subject in need thereof.

[0026] These and other aspectexamples and advantages of the inventiondisclosure are illustrated by the following figures, detailed description and claims.

DESCRIPTION OF THE DRAWINGS

[0027] The invention can be more completely understood with reference to the following
5 drawings.

[0028] **FIGURE 1A** depicts a schematic of a dimeric cytokine receptor on the cell surface (left), an antibody (middle), and a receptor-Fc fusion that optimally binds a target cytokine (right).

FIGURE 1B depicts a receptor-Fc fusion, *e.g.*, a cytokine trap, that is sterically constrained from optimal binding to a target cytokine (left), or that adopts an optimal binding configuration
10 (right).

[0029] **FIGURE 2** depicts a sequence alignment of the amino acid sequences of the human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, and IgM CH1 domains (top) and CH2 domains (bottom).

[0030] **FIGURE 3** depicts a Western blot for phosphorylated Stat3 following treatment of
15 reporter cells with IL-10 and/or the IL-10RA fusion proteins IL-10R-IgG and IL-10R-Fc as indicated. Total Stat3 was used as a loading control. IL-10 activity was markedly reduced by IL-10R-IgG compared to IL-10R-Fc.

DETAILED DESCRIPTION

[0031] The invention provides a recombinant fusion protein for use in the treatment of a variety
20 of medical conditions, for example, in inhibiting proliferation of a tumor cell, inhibiting tumor growth, treating cancer, treating an inflammatory condition, or treating an infection, in a subject. Exemplary fusion proteins comprise: a first portion of an extracellular domain, transmembrane domain, or intracellular domain of a cytokine, cytokine receptor, or immunomodulatory protein; an amino acid linker; and at least one of, a second portion of an extracellular domain,
25 transmembrane domain, or intracellular domain of a cytokine, cytokine receptor, or immunomodulatory protein; an immunoglobulin (Ig) hinge region; or an immunoglobulin (Ig) Fc domain. It is contemplated that the first and second portions can be portions of the same protein or portions of different proteins, and, even if the same protein, the first and second portions can be different portions of the same protein. In certain embodiments, the linker comprises from
30 about 5 to about 40 amino acid residues. Exemplary fusion proteins of the invention include cytokine traps.

WHAT IS CLAIMED IS:

~~1. An isolated fusion protein comprising:~~

- ~~(i) a portion of an IL-10 receptor; and~~
- ~~(ii) an amino acid linker; and at least one of~~
- ~~(iii) a portion of an extracellular domain, transmembrane domain, or intracellular domain of a cytokine, cytokine receptor, or immunomodulatory protein;~~
- ~~(iv) an immunoglobulin (Ig) hinge region; or~~
- ~~(v) an immunoglobulin (Ig) Fc domain.~~

~~2. The isolated fusion protein of claim 1, wherein the linker comprises from about 5 to about 40 amino acid residues.~~

31. An isolated fusion protein comprising, in an N- to C- terminal orientation:

- (i) a soluble portion of an extracellular domain of an human IL-10 receptor subunit alpha, wherein the soluble portion of an extracellular domain of a human IL-10 receptor has greater than 95% sequence identity to amino acid residues 22-229 of SEQ ID NO: 12;
- (ii) an amino acid linker;
- (iii) an immunoglobulin (Ig) hinge region; and
- (iv) an immunoglobulin (Ig) Fc domain;

wherein the linker consists of 10 to 40 amino acid residues, wherein the linker is a sequence derived from an endogenous human protein, wherein the Ig hinge region and the Ig Fc domain together comprise an amino acid sequence having greater than 95% identity to SEQ ID NOs: 13-21, and wherein the linker comprises a sequence derived from a human protein selected from albumin and casein, or comprises a C-terminal portion of an immunoglobulin (Ig) CH1 domain having greater than 95% sequence identity to an amino acid sequence selected from SEQ ID NO: 1, 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: 53, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63~~comprises from about 5 to about 40 amino acid residues.~~

~~4. The isolated fusion protein of any one of claims 1-3, wherein the linker comprises from about 5 to about 30 amino acid residues.~~

5. ~~The isolated fusion protein of any one of claims 1-3, wherein the linker comprises from about 5 to about 20 amino acid residues.~~
6. ~~The isolated fusion protein of any one of claims 1-3, wherein the linker comprises from about 5 to about 15 amino acid residues.~~
7. ~~The isolated fusion protein of any one of claims 1-3, wherein the linker comprises from about 10 to about 40 amino acid residues.~~
8. ~~The isolated fusion protein of any one of claims 1-3, wherein the linker comprises from about 10 to about 30 amino acid residues.~~
9. ~~The isolated fusion protein of any one of claims 1-3, wherein the linker comprises from about 10 to about 20 amino acid residues.~~
10. ~~The isolated fusion protein of any one of claims 1-3, wherein the linker comprises from about 10 to about 15 amino acid residues.~~
11. ~~The isolated fusion protein of any one of claims 1-10, wherein the linker comprises a sequence derived from an endogenous human protein.~~
12. ~~The isolated fusion protein of any one of claims 1-11, wherein the linker comprises a C-terminal portion of an immunoglobulin (Ig) CH1 domain.~~
13. ~~The isolated fusion protein of claim 12, wherein the Ig CH1 domain is selected from an IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, and IgM CH1 domain.~~
14. ~~The isolated fusion protein of claim 13, wherein the linker comprises an amino acid sequence selected from SEQ ID NO: 1, 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: 53, SEQ ID NO: 54, and SEQ ID NO: 57.~~
15. ~~The isolated fusion protein of claim 14, wherein the Ig CH1 domain is an IgG1 CH1 domain.~~
16. ~~The isolated fusion protein of claim 15, wherein the linker comprises an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 53, SEQ ID NO: 54, and SEQ ID NO: 57.~~
17. ~~The isolated fusion protein of claim 16, wherein the linker comprises the amino acid sequence of SEQ ID NO: 57.~~
18. ~~The isolated fusion protein of any one of claims 1-11, wherein the linker comprises a sequence derived from a human protein selected from albumin and casein.~~

~~19. The isolated fusion protein of claim 18, wherein the linker comprises an amino acid sequence selected from SEQ ID NO: 10 and SEQ ID NO: 11.~~

~~20. The isolated fusion protein of any one of claims 1-11, wherein the linker comprises a sequence derived from a cytokine, signaling molecule, immunomodulatory protein or a peptide.~~

2. The isolated fusion protein of claim 1, wherein the linker comprises a sequence derived from a human protein selected from albumin and casein, or comprises a C-terminal portion of an immunoglobulin (Ig) CH1 domain having greater than 98% sequence identity to an amino acid sequence selected from SEQ ID NO: 1, 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: 53, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63.

243. The isolated fusion protein of ~~any one of~~ claims ~~1-20~~ or claim 2, wherein the linker comprises an amino acid sequence which is proteolytically stable in a mammal or plant or comprises a cleavage site.

~~22. The isolated fusion protein of claim 21, wherein the cleavage site is a proteolytic cleavage site.~~

~~23. The isolated fusion protein of claim 22, wherein the proteolytic cleavage site is cleaved by a protease that is present in the endoplasmic reticulum or golgi of a eukaryotic cell.~~

~~24. The isolated fusion protein of claims 22 or 23, wherein the proteolytic cleavage site is a furin cleavage site.~~

~~25. The isolated fusion protein of claim 24, wherein the furin cleavage site comprises RX_1X_2R (SEQ ID NO: 50), wherein X_1 is any amino acid, and X_2 is Lys or Arg.~~

~~26. The isolated fusion protein of claim 22, wherein the furin cleavage site comprises RAKR (SEQ ID NO: 51).~~

~~27. The isolated fusion protein of any one of claims 1-20, wherein the linker comprises an amino acid sequence which is proteolytically stable in a mammal or plant.~~

~~28. The isolated fusion protein of any one of claims 1-27, wherein the IL-10 receptor is a human IL-10 receptor.~~

294. The isolated fusion protein of any one of claims ~~28~~1-3, wherein the soluble portion of an extracellular domain of an IL-10 receptor comprises the amino acid sequence of SEQ ID NO: 12 or amino acid residues 22-229 of SEQ ID NO: 12.

~~30. The isolated fusion protein of claim 28, wherein the soluble portion of an extracellular domain of an IL-10 receptor comprises amino acid residues 22-229 of SEQ ID NO: 12.~~

~~31. The isolated fusion protein of any one of claims 1-30, wherein the Ig Fc domain and hinge region are selected from a human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, and IgM Fc domain and hinge region.~~

~~325.~~ The isolated fusion protein of any one of claims ~~341-4~~, wherein the Ig Fc domain and Ig hinge region together comprise an amino acid sequence selected from 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 and SEQ ID NO: 21.

~~33. The isolated fusion protein of claim 32, wherein the Ig Fc domain and hinge region are a human IgG1 Fc domain and hinge region.~~

~~34. The isolated fusion protein of claim 33, wherein the Ig Fc domain and hinge region comprise the amino acid sequence of SEQ ID NO: 13.~~

~~35. The isolated fusion protein of any one of claims 1-34, wherein the Ig Fc, Ig hinge region, and Ig CH1 domain are derived from a single immunoglobulin.~~

~~366.~~ The isolated fusion protein of ~~any one of~~ claims ~~1-352~~, wherein the fusion protein has greater than 95% sequence identity to ~~comprises~~ an amino acid sequence selected from 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: 55, ~~SEQ ID NO: 56~~, and SEQ ID NO: 58.

~~37. The isolated fusion protein of claim 36, wherein the fusion protein comprises an amino acid sequence selected from SEQ ID NO: 22, SEQ ID NO: 55, SEQ ID NO: 56, and SEQ ID NO: 58.~~

~~38. The isolated fusion protein of claim 37, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 58.~~

~~39. A cytokine binding protein comprising two fusion proteins of any one of claims 1-38, wherein each fusion protein comprises an extracellular domain of a cytokine receptor, wherein the two fusion proteins are covalently linked together, and wherein the two extracellular domains together define a binding site for binding a cytokine.~~

~~407.~~ An isolated nucleic acid comprising a nucleotide sequence encoding the fusion protein of any one of claims 1-~~396~~.

418. An expression vector comprising the nucleic acid of claim 407.
429. A host cell comprising the expression vector of ~~any one of~~ claim 418.
4310. A method of producing a fusion protein comprising:
- (a) growing the host cell of claim 429 under conditions to express the fusion protein; and
 - (b) purifying the fusion protein.
4411. A pharmaceutical composition comprising: (i) the fusion protein of any one of claims 1-639 ~~or the expression vector of claim 41~~; and (ii) at least one pharmaceutically acceptable carrier or diluent.
- ~~45.—A method of expressing a fusion protein in a target cell comprising exposing the cell to an effective amount of the expression vector of claim 41 to express the fusion protein.~~
- ~~46.—The method of claim 45, wherein the fusion protein is cleaved posttranslationally into two polypeptide chains.~~
- ~~47.—A method of inhibiting proliferation of a tumor cell comprising exposing the cell to an effective amount of the dimeric fusion protein of claim 39 to inhibit proliferation of the tumor cell.~~
- ~~48.—A method of inhibiting proliferation of a tumor cell comprising exposing the cell to an effective amount of the fusion protein of any one of claims 1-38 to inhibit proliferation of the tumor cell.~~
- ~~49.—A method of inhibiting tumor growth in a subject in need thereof, the method comprising administering to the subject to an effective amount of the dimeric fusion protein of claim 39 to inhibit growth of the tumor.~~
- ~~50.—A method of inhibiting tumor growth in a subject in need thereof, the method comprising administering to the subject to an effective amount of the fusion protein of any one of claims 1-38 to inhibit growth of the tumor.~~
- ~~51.—A method of treating cancer in a subject in need thereof, the method comprising administering an effective amount of the dimeric fusion protein of claim 39 to the subject.~~
- ~~52.—A method of treating cancer in a subject in need thereof, the method comprising administering an effective amount of the fusion protein of any one of claims 1-38 to the subject.~~

53. — ~~A method of reducing IL-10 activity in a cell comprising exposing the cell to an effective amount of the dimeric fusion protein of claim 39 to reduce IL-10 activity.~~

54. — ~~A method of reducing IL-10 activity in a cell comprising exposing the cell to an effective amount of the fusion protein of any one of claims 1-38 to reduce IL-10 activity.~~

55. — ~~A method of treating an inflammatory condition in a subject in need thereof, the method comprising administering an effective amount of the dimeric fusion protein of claim 39 to the subject.~~

56. — ~~A method of treating an inflammatory condition in a subject in need thereof, the method comprising administering an effective amount of the fusion protein of any one of claims 1-38 to the subject.~~

57. — ~~A method of inhibiting proliferation of a tumor cell comprising exposing the cell to an effective amount of the expression vector of claim 41 to inhibit proliferation of the tumor cell.~~

58. — ~~A method of inhibiting tumor growth in a subject in need thereof, the method comprising administering to the subject an effective amount of the expression vector of claim 41 to inhibit growth of the tumor.~~

59. — ~~A method of treating cancer in a subject in need thereof, the method comprising administering an effective amount of the expression vector of claim 41 to the subject.~~

60. — ~~A method of reducing IL-10 activity in a cell comprising exposing the cell to an effective amount of the expression vector of claim 41 to reduce IL-10 activity.~~

61. — ~~A method of treating an inflammatory condition in a subject in need thereof, the method comprising administering an effective amount of the expression vector of claim 41 to the subject.~~

62. — ~~A method of treating an infection in a subject in need thereof, the method comprising administering an effective amount of the expression vector of claim 41 to the subject.~~

63. — ~~The method of any one of claims 51, 52, or 59, wherein the cancer is selected from melanoma, squamous cell carcinoma of the skin, basal cell carcinoma, head and neck cancer, breast cancer, anal cancer, cervical cancer, non-small cell lung cancer, mesothelioma, small cell lung cancer, renal cell carcinoma, prostate cancer, gastroesophageal cancer, colorectal cancer, testicular cancer, bladder cancer, ovarian cancer, liver cancer, hepatocellular carcinoma, cholangiocarcinoma, brain and central nervous system cancer, thyroid cancer, parathyroid cancer (e.g., parathyroid carcinoma), endometrial cancer, neuroendocrine cancer, lymphoma (e.g., Hodgkin and non-Hodgkin), leukemia, merkel cell carcinoma, gastrointestinal stromal tumors,~~

~~multiple myeloma, uterine cancer, a sarcoma, kidney cancer, ocular cancer, pancreatic cancer, and a germ cell cancer (e.g., ovarian germ cell cancer).~~

~~64. —The method of any one of claims 51, 52, or 59, wherein the cancer is selected from leukemia, breast cancer, lung cancer, pancreatic cancer, endometrial cancer, ovarian cancer, prostate cancer, cervical cancer, brain cancer, skin cancer, colorectal cancer, gastric cancer, head and neck cancer, and leukemia.~~

~~65. —The method of claim 64, wherein the cancer is selected from skin cancer, head and neck cancer, and lung cancer.~~

~~66. —The method of any one of claims 49-52, 55, 56, 58, 59, or 61-65, wherein the fusion protein or expression vector is administered to the subject in combination with one or more therapies selected from surgery, radiation, chemotherapy, immunotherapy, hormone therapy, and virotherapy.~~

~~67. —The method of any one of claims 49-52, 55, 56, 58, 59, or 61-65, wherein the fusion protein or expression vector is administered to the subject in combination with a lymphocyte.~~

~~68. —The method of claim 67, wherein the lymphocyte is a T-cell.~~

~~69. —The method of claim 68, wherein the T-cell is a CAR T-cell.~~

~~70. —The method of any one of claims 49-52, 55, 56, 58, 59, or 61-69, wherein the subject is a human or animal.~~

~~71. —The method of claim 70, wherein the subject is a pediatric human.~~