

adverse health outcomes, such as vascular leakage, through systemic immune activation. These findings illustrate the need for developing cytokine therapeutics that effectively target tumors without the side effects associated with systemic immune activation. Provided herein are masked cytokines, compositions thereof and methods of use thereof for addressing this need.

[0006] All references cited herein, including patent applications, patent publications, and scientific literature, are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY

[0007] The disclosed ~~invention~~disclosure relates to the engineering and use of masked cytokines or functional fragments thereof that, in some embodimentexamples, are engineered to be masked by a masking moiety at one or more receptor binding site(s) of the cytokine or functional fragment thereof. In some embodimentexamples, the cytokines are engineered to be activatable by a protease at a target site, such as in a tumor microenvironment, by including a proteolytically cleavable linker. In some embodimentexamples, the proteolytically cleavable linker links the cytokine to the masking moiety, links the cytokine to a half-life extension domain, and/or links the masking moiety to a half-life extension domain. The masking moiety blocks, occludes, inhibits (e.g., decreases) or otherwise prevents (e.g., masks) the activity or binding of the cytokine to its cognate receptor or protein. Upon proteolytic cleavage of the cleavable linker at the target site, the cytokine becomes activated, which renders it capable of binding to its cognate receptor or protein with increased affinity.

[0008] One embodimentexample comprises a masked cytokine comprising a masking moiety; and a cytokine or functional fragment thereof, wherein the masking moiety is linked to the cytokine or functional fragment thereof via a linker. In one aspectexample, the masked cytokine further comprises a half-life extension domain that is linked to either the masking moiety or the cytokine or functional fragment thereof.

[0009] In one embodimentexample, the masked cytokine comprises in an N to C-terminal or in a C to N-terminal direction: a) a masking moiety; b) a first linker; c) a cytokine or functional fragment thereof; and d) a half-life extension domain. In another embodimentexample, the masked cytokine comprises in an N to C-terminal or in a C to N-terminal direction: a) a masking moiety; b) a first linker; c) a cytokine or functional fragment thereof; d) a second linker and e) a half-life extension domain. In another embodimentexample, the masked cytokine comprises in an N to C-terminal or in a C to N-terminal direction: a) a cytokine or functional fragment thereof; b) a first linker; c) a masking moiety; and d) a half-life extension domain.

[0010] In another embodimentexample, the masked cytokine comprises in an N to C-terminal or in a C to N-terminal direction: a) a cytokine or functional fragment thereof; b) a first linker; c) a masking moiety; d) a second linker; and e) a half-life extension domain.

[0011] In one [embodimentexample](#), the cytokine or functional fragment thereof is an IL-2 polypeptide or functional fragment thereof or an IL-15 polypeptide or functional fragment thereof.

[0012] In one [embodimentexample](#), the masked cytokine one or more amino acid substitutions into the amino acid sequence of the IL-2 or IL-15 polypeptide or functional fragment thereof. In one [embodimentexample](#), the amino acid substitutions reduce the affinity of the IL-2 polypeptide or functional fragment thereof for CD25 (IL-2R α).

[0013] In one [embodimentexample](#), the IL-2 polypeptide or functional fragment thereof comprises an amino acid sequence produced by introducing one or more amino acid substitutions into the amino acid sequence of the IL-2 polypeptide or functional fragment thereof that increases the affinity of the IL-2 polypeptide or functional fragment thereof for IL-2R β or IL-2R γ .

[0014] In one [embodimentexample](#), the half-life extension domain is an antibody or fragment thereof, and in some [embodimentexamples](#), one or more amino acid substitutions altering effector function.

[0015] In one [embodimentexample](#), the antibody or fragment thereof is a Fragment crystallizable domain (Fc domain) or fragment thereof.

[0016] In one [embodimentexample](#), the half-life extension domain is a polyamino acid sequence, such as a PAS polypeptide or an XTEN polypeptide.

[0017] In one [embodimentexample](#) the masked cytokine comprises a second masking moiety, wherein the second masking moiety is linked to the cytokine or functional fragment thereof via a second linker.

[0018] In one [embodimentexample](#), the masked cytokine comprises in an N to C-terminal or in a C to N-terminal direction: a) a first masking moiety; b) a first linker; c) a cytokine or functional fragment thereof; d) a second linker; e) a second masking moiety; and f) a half-life extension domain.

[0019] In one [embodimentexample](#), the masked cytokine comprises in an N to C-terminal or in a C to N-terminal direction: a) a first masking moiety; b) a first linker; c) a cytokine or functional fragment thereof; d) a second linker; e) a second masking moiety; f) a third linker; and g) a half-life extension domain.

[0020] In one [embodimentexample](#), the masked cytokine comprises in an N to C-terminal or in a C to N-terminal direction: a) a second masking moiety; b) a second linker; c) a cytokine or functional fragment thereof; d) a first linker; e) a first masking moiety; and f) a half-life extension domain.

[0021] In one [embodimentexample](#), the masked cytokine comprises in an N to C-terminal or in a C to N-terminal direction: a) a second masking moiety; b) a second linker; c) a cytokine or functional fragment thereof; d) a first linker; e) a first masking moiety; f) a third linker; and g) a half-life extension domain.

[0022] In one [embodimentexample](#), the half-life extension domain is an albumin polypeptide or functional fragment thereof.

[0023] In one [embodimentexample](#), the cleavable peptide is cleaved by one or more enzyme selected from the group consisting of: ABHD12, ADAM12, ABHD12B, ABHD13, ABHD17A, ADAM19, ADAM20,

ADAM21, ADAM28, ADAM30, ADAM33, ADAM8, ABHD17A, ADAMDEC1, ADAMTS1, ADAMTS10, ADAMTS12, ADAMTS13, ADAMTS14, ADAMTS15, ADAMTS16, ADAMTS17, ADAMTS18, ADAMTS19, ADAMTS2, ADAMTS20, ADAMTS3, ADAMTS4, ABHD17B, ADAMTS5, ADAMTS6, ADAMTS7, ADAMTS8, ADAMTS9, ADAMTSL1, ADAMTSL2, ADAMTSL3, ABHD17C, ADAMTSL5, ASTL, BMP1, CELA1, CELA2A, CELA2B, CELA3A, CELA3B, ADAM10, ADAM15, ADAM17, ADAM9, ADAMTS4, CTSE, CTSF, ADAMTSL4, CMA1, CTRB1, CTRC, CTSO, CTRI, CTSA, CTSW, CTSB, CTSC, CTSD, ESP1, CTSG, CTSH, GZMA, GZMB, GZMH, CTSK, GZMM, CTSL, CTSS, CTSV, CTSZ, HTRA4, KLK10, KLK11, KLK13, KLK14, KLK2, KLK4, DPP4, KLK6, KLK7, KLKB1, ECE1, ECE2, ECEL1, MASP2, MEP1A, MEP1B, ELANE, FAP, GZMA, MMP11, GZMK, HGFAC, HPN, HTRA1, MMP11, MMP16, MMP17, MMP19, HTRA2, MMP20, MMP21, HTRA3, HTRA4, KEL, MMP23B, MMP24, MMP25, MMP26, MMP27, MMP28, KLK5, MMP3, MMP7, MMP8, MMP9, LGMN, LNPEP, MASP1, PAPP, PAPP2, PCSK1, NAPS, PCSK5, PCSK6, MME, MMP1, MMP10, PLAT, PLAU, PLG, PRSS1, PRSS12, PRSS2, PRSS21, PRSS3, PRSS33, PRSS4, PRSS55, PRSS57, MMP12, PRSS8, PRSS9, PRTN3, MMP13, MMP14, ST14, TMPRSS10, TMPRSS11A, TMPRSS11D, TMPRSS11E, TMPRSS11F, TMPRSS12, TMPRSS13, MMP15, TMPRSS15, MMP2, TMPRSS2, TMPRSS3, TMPRSS4, TMPRSS5, TMPRSS6, TMPRSS7, TMPRSS9, NRDC, OVCH1, PAMR1, PCSK3, PHEX, TINAG, TPSAB1, TPSD1, and TPSG1.

[0024] In one embodimentexample, the half-life extension domain is conjugated to an agent, such as an inhibitor of tubulin polymerization, a DNA damaging agent, or a DNA synthesis inhibitor, a maytansinoid, an auristatin, a pyrrolobenzodiazepine (PBD) dimer, a calicheamicin, a duocarmycin, a indo-linobenzodiazepine dimer or exatecan derivative Dxd.

[0025] In one embodimentexample, the half-life extension domain is conjugated to an immune stimulant, such as a stimulator of interferon genes (STING) agonist, such as a cyclic dinucleotide (CDN), such as cGAMP, c-di-AMP, c-di-GMP, cAIMP, c-di-IMP, or 4-(2-chloro-6-fluorobenzyl)-N-(furan-2-ylmethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazine-6-carboxamide or a toll-like receptor (TLR) agonist, such as TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, or TLR10.

[0026] In one embodimentexample, the masked cytokine comprises a first half-life extension domain and a second half-life extension domain, wherein the masking moiety is linked to the first half-life extension domain, and wherein the cytokine or functional fragment thereof is linked to the second half-life extension domain,

[0027] In one embodimentexample, the first half-life extension domain and the second half-life extension domain contain modifications promoting the association of the first and the second half-life extension domain.

[0027a] In one aspect, the present disclosure refers to a masked cytokine comprising:

a) a first half-life extension domain and a second half-life extension domain, wherein the first half-life extension domain is a first Fc domain or fragment thereof, and the second half-life extension domain is a second Fc domain or fragment thereof;

b) a masking moiety comprising an amino acid sequence having at least about 98% sequence identity to the amino acid sequence of SEQ ID NO: 10; and

c) a cytokine, wherein the cytokine is an IL-2 polypeptide, wherein the IL-2 polypeptide comprises the amino acid sequence of SEQ ID NO: 3;

wherein the masking moiety is linked to the first half-life extension domain via a first linker, wherein the first linker comprises a cleavable peptide;

wherein the cytokine is linked to the second half-life extension domain via a second linker; and wherein the first half-life extension domain and the second half-life extension domain contain modifications promoting the association of the first and the second half-life extension domain.

[0028] One embodimentexample comprises a nucleic acid encoding the masked cytokines described herein. Another embodimentexample encompasses a vector comprising the nucleic acid. In another embodimentexample a host

cell comprising the nucleic acid. Another [embodimentexample](#) comprises one or more nucleic acids encoding the masked cytokines described herein. Another [embodimentexample](#) encompasses a vector comprising the one or more nucleic acids. Another [embodimentexample](#) encompasses one or more vectors comprising the one or more nucleic acids.

[0029] In another [embodimentexample](#) is provided a method of producing a masked cytokine comprising culturing the host cell under a condition that produces the masked cytokine. In one [embodimentexample](#), the masked cytokine produced by the host cell is recovered. One [embodimentexample](#) encompasses a masked cytokine produced by the disclosed methods.

[0030] In one [embodimentexample](#), a composition comprising a disclosed masked cytokine is provided. In another [embodimentexample](#), the composition further comprises an anti-inflammatory agent or an anti-cancer agent, such as a PD-1 inhibitor, an EGFR inhibitor, a HER2 inhibitor, a VEGFR inhibitor, a CTLA-4 inhibitor, a BTLA inhibitor, a B7H4 inhibitor, a B7H3 inhibitor, a CSFIR inhibitor, an HVEM inhibitor, a CD27 inhibitor, a KIR inhibitor, an NKG2A inhibitor, an NKG2D agonist, a TWEAK inhibitor, an ALK inhibitor, a CD52 targeting antibody, a CCR4 targeting antibody, a PD-L1 inhibitor, a KIT inhibitor, a PDGFR inhibitor, a BAFF inhibitor, an HDAC inhibitor, a VEGF ligand inhibitor, a CD19 targeting molecule, a FOLR1 targeting molecule, a DLL3 targeting molecule, a DKK1 targeting molecule, a MUC1 targeting molecule, a MUC16 targeting molecule, a PSMA targeting molecule, an MSLN targeting molecule, an NY-ES0-1 targeting molecule, a B7H3 targeting molecule, a B7H4 targeting molecule, a BCMA targeting molecule, a CD29 targeting molecule, a CD151 targeting molecule, a CD123 targeting molecule, a CD33 targeting molecule, a CD37 targeting molecule, a CDH19 targeting molecule, a CEA targeting molecule, a Claudin 18.2 targeting molecule, a CLEC12A targeting molecule, an EGFRVIII targeting molecule, an EPCAM targeting molecule, an EPHA2 targeting molecule, an FCRH5 targeting molecule, an FLT3 targeting molecule, a GD2 targeting molecule, a glypican 3 targeting molecule, a gpA33 targeting molecule, a GPRC5D targeting molecule, an IL-23R targeting molecule, an IL-1RAP targeting molecule, a MCSP targeting molecule, a RON targeting molecule, a ROR1 targeting molecule, a STEAP2 targeting molecule, a TfR targeting molecule, a CD166 targeting molecule, a TPBG targeting molecule, a TROP2 targeting molecule, a proteasome inhibitor, an ABL inhibitor, a CD30 inhibitor, a FLT3 inhibitor, a MET inhibitor, a RET inhibitor, an IL-1 β inhibitor, a MEK inhibitor, a ROS1 inhibitor, a BRAF inhibitor, a CD38 inhibitor, a RANKL inhibitor, a B4GALNT1 inhibitor, a SLAMF7 inhibitor, an IDH2 inhibitor, an mTOR inhibitor, a CD20 targeting antibody, a BTK inhibitor, a PI3K inhibitor, a FLT3 inhibitor, a PARP inhibitor, a CDK4 inhibitor, a CDK6 inhibitor, an FGFR inhibitor, a RAF inhibitor, a JAK1 inhibitor, a JAK2 inhibitor, a JAK3 inhibitor, an IL-6 inhibitor, a IL-17 inhibitor, a Smoothened inhibitor, an IL-6R inhibitor, a BCL2 inhibitor, a PTCH inhibitor, a PIGF inhibitor, a TGFB inhibitor, a CD28 agonist, a CD3 agonist, CD40 agonist, a GITR agonist, a OX40 agonist, a VISTA agonist, a CD137 agonist, a LAG3 inhibitor, a TIM3 inhibitor, a TIGIT inhibitor, or an IL-2R inhibitor.

[0031] In one ~~embodiment~~example, the anti-inflammatory agent is a cyclooxygenase (COX) inhibitor, such as a COX-1 and/or COX-2 inhibitor, such as SC-560, FR122047, P6, mofezolac, TFAP, flurbiprofen, ketoprofen, celecoxib, rofecoxib, meloxicam, piroxicam, deracoxib, parecoxib, valdecoxib, etoricoxib, a chromene derivative, a chroman derivative, N-(2-cyclohexyloxynitrophenyl) methane sulfonamide, parecoxib, lumiracoxib, RS 57067, T-614, BMS-347070, JTE-522, S-2474, SVT- 2016, CT-3, ABT-963, SC-58125, nimesulide, flosulide, NS-398, L- 745337, RWJ-63556, L-784512, darbufelone, CS-502, LAS-34475, LAS- 34555, S-33516, diclofenac, mefenamic acid, SD-8381, ibuprofen, naproxen, ketorolac, indomethacin, aspirin, naproxen, tolmetin, piroxicam, or meclofenamate.

[0032] In one ~~embodiment~~example, the anti-inflammatory agent is an NF- κ B inhibitor, such as an IKK complex inhibitor, an I κ B degradation inhibitor, an NF- κ B nuclear translocation inhibitor, a p65 acetylation inhibitor, an NF- κ B DNA binding inhibitor, an NF- κ B transactivation inhibitor, or a p53 induction inhibitor.

[0033] In one ~~embodiment~~example, the NF- κ B inhibitor is TPCA-1, NF- κ B Activation Inhibitor VI (BOT-64), BMS-345541, amlexanox, SC-514 (GK-01140), IMD-0354, IKK-16, BAY-11-7082, MG-115, MG-132, lactacystin, epoxomicin, parthenolide, carfilzomib, MLN-4924 (pevonedistat), JSH-23 rolipram, gallic acid, anacardic acid, GYY-4137, p-XSC, CV-3988, prostaglandin E2 (PGE2), LY-294002, wortmannin, mesalamine, quinacrine, or flavopiridol.

[0034] ~~One embodiment encompasses~~In one aspect, the present disclosure refers to a pharmaceutical composition comprising a disclosed masked cytokine and a pharmaceutically acceptable carrier.

[0035] ~~Another embodiment encompasses~~In another aspect, the present disclosure refers to a kit comprising the disclosed masked cytokine.

[0036] Another ~~embodiment~~example comprises a method of treating or preventing a neoplastic disease in a subject, the method comprising administering to the subject an effective amount of a disclosed masked cytokine composition for the treatment of a disease or condition, such as a neoplastic disease, for example, cancer. In one ~~embodiment~~example the cancer is leukemia, lymphoma, head and neck cancer, colorectal cancer, prostate cancer, pancreatic cancer, melanoma, breast cancer, neuroblastoma, lung cancer, ovarian cancer, bone cancer (e.g., osteosarcoma, chondrosarcoma, Ewing sarcoma), bladder cancer, cervical cancer, liver cancer, kidney cancer, skin cancer, testicular cancer, adrenal cancer, adenoid cystic carcinoma, anal cancer, brain cancer, ductal carcinoma, endometrial cancer, esophageal cancer, gastric cancer, oral cancer, thyroid cancer, retinoblastoma, parathyroid cancer, pituitary cancer, bile duct cancer, or uterine cancer. In some ~~embodiment~~examples, the cancer is selected from the group consisting of lymphoma, sarcoma, bladder cancer, bone cancer, brain tumor, cervical cancer, colon cancer, esophageal cancer, gastric cancer, head and neck cancer, kidney cancer, myeloma, thyroid cancer, leukemia, prostate cancer, breast cancer (e.g. triple negative, ER positive, ER negative, chemotherapy resistant, Herceptin resistant, HER2 positive, doxorubicin resistant, tamoxifen resistant, ductal carcinoma, lobular carcinoma, primary, metastatic), ovarian cancer, pancreatic cancer, liver cancer (e.g. hepatocellular carcinoma), lung cancer (e.g. non-small cell lung carcinoma, squamous cell lung

carcinoma, adenocarcinoma, large cell lung carcinoma, small cell lung carcinoma, carcinoid, sarcoma), glioblastoma multiforme, glioma, melanoma, prostate cancer, castration-resistant prostate cancer, breast cancer, triple negative breast cancer, glioblastoma, ovarian cancer, lung cancer, squamous cell carcinoma (e.g., head, neck, or esophagus), colorectal cancer, leukemia, acute myeloid leukemia, lymphoma, B cell lymphoma, or multiple myeloma. Additional examples include, cancer of the thyroid, endocrine system, brain, breast, cervix, colon, head & neck, esophagus, liver, kidney, lung, non-small cell lung, melanoma, mesothelioma, ovary, sarcoma, stomach, uterus or Medulloblastoma, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, glioma, glioblastoma multiforme, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine or exocrine pancreas, medullary thyroid cancer, medullary thyroid carcinoma, melanoma, colorectal cancer, papillary thyroid cancer, hepatocellular carcinoma, Paget's Disease of the Nipple, Phyllodes Tumors, Lobular Carcinoma, Ductal Carcinoma, cancer of the pancreatic stellate cells, cancer of the hepatic stellate cells, or prostate cancer.

[0037] Another ~~embodiment~~example comprises a method of treating or preventing an inflammatory or autoimmune disease in a subject, the method comprising administering to the subject an effective amount of a disclosed masked cytokine composition, wherein the inflammatory or autoimmune disease is selected from the group consisting of atherosclerosis, obesity, inflammatory bowel disease (IBD), rheumatoid arthritis, allergic encephalitis, psoriasis, atopic skin disease, osteoporosis, peritonitis, hepatitis, lupus, celiac disease, Sjogren's syndrome, polymyalgia rheumatica, multiple sclerosis (MS), ankylosing spondylitis, type 1 diabetes mellitus, alopecia areata, vasculitis, and temporal arteritis, graft versus host disease (GVHD), asthma, COPD, a paraneoplastic autoimmune disease, cartilage inflammation, 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, systemic onset rheumatoid arthritis, enteropathic arthritis, reactive arthritis, Reiter's Syndrome, dermatomyositis, psoriatic arthritis, Scleroderma, vasculitis, myolitis, polymyolitis, dermatomyolitis, polyarteritis nodosa, Wegener's granulomatosis, arteritis, polymyalgia rheumatica, sarcoidosis, Sclerosis, primary biliary Sclerosis, Sclerosing cholangitis, psoriasis, plaque psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, erythrodermic psoriasis, dermatitis, atopic dermatitis, atherosclerosis, Still's disease, Systemic Lupus Erythematosus (SLE), myasthenia gravis, Crohn's disease, ulcerative colitis, celiac disease,

CLAIMS

What is claimed is:

1. ~~—— A masked cytokine comprising:~~

~~a) a masking moiety; and~~

~~b) a cytokine or functional fragment thereof,~~

~~wherein the masking moiety is linked to the cytokine or functional fragment thereof via a first linker.~~

2. ~~—— A masked cytokine comprising:~~

~~a) a first masking moiety;~~

~~b) a cytokine or functional fragment thereof,~~

~~wherein the first masking moiety is linked to the cytokine or functional fragment thereof via a first linker; and~~

~~e) a second masking moiety;~~

~~wherein the second masking moiety is linked to the cytokine or functional fragment thereof via a second linker.~~

3. ~~—— A masked cytokine comprising:~~

~~a) a first half life extension domain and a second half life extension domain;~~

~~b) a masking moiety; and~~

~~c) a cytokine or functional fragment thereof,~~

~~wherein the masking moiety is linked to the first half life extension domain;~~

~~wherein the cytokine or functional fragment thereof is linked to the second half life extension domain; and~~

~~wherein the first half life extension domain and the second half life extension domain contain modifications promoting the association of the first and the second half life extension domain.~~

4. ~~—— A masked cytokine comprising:~~

~~a) a first half life extension domain and a second half life extension domain;~~

~~b) a first masking moiety and a second masking moiety; and~~

~~c) a cytokine or functional fragment thereof,~~

~~wherein the first masking moiety is linked to the first half life extension domain;~~

~~wherein the second masking moiety is linked to the cytokine or functional fragment thereof;~~

~~wherein either the second masking moiety or the cytokine or functional fragment thereof is linked to the second half life extension domain, and~~

~~wherein the first half life extension domain and the second half life extension domain contain modifications promoting the association of the first and the second half life extension domain.~~

~~5. The masked cytokine of claim 1, further comprising a half life extension domain that is linked to either the masking moiety or the cytokine or functional fragment thereof.~~

~~6. The masked cytokine of claim 2, further comprising a half life extension domain that is linked to either the first masking moiety or the second masking moiety.~~

~~7. The masked cytokine of claim 3, wherein the masking moiety is linked to the first half life extension domain via a first linker; and/or wherein the cytokine or functional fragment thereof is linked to the second half life extension domain via a second linker.~~

~~8. The masked cytokine of claim 4, wherein:
a) the first masking moiety is linked to the first half life extension domain via a first linker; and/or
b) either the second masking moiety or the cytokine or functional fragment thereof is linked to the second half life extension domain via a second linker.~~

~~9. The masked cytokine of any one of claims 1-8, wherein the cytokine or functional fragment thereof is an IL-2 polypeptide or functional fragment thereof.~~

~~10. The masked cytokine of claim 9, wherein the IL-2 polypeptide or functional fragment thereof comprises:~~

~~a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-8, 160, 230, 243-251, 260, 775-792, and 813-822; or~~

~~b) an amino acid sequence produced by introducing one or more of the following amino acid substitutions into any one of SEQ ID NOs: 1-8, 160, 243-251, 260, 775-792, and 813-822: H16I, L18C, D20A, D20L, D20F, N29L, R38A, F42A, F42K, F42E, F43A, Y45A, Y45N, Y45R, E62A, E62R, E62S, L72G, L80F, R81D, L85V, I86V, I92F, and C125S.~~

~~11. —The masked cytokine of any one of claims 1, 3, 5, 7, 9, and 10, wherein the masking moiety comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 9, 10, 161-165, 187-218, 221-229, 231, and 261.~~

~~12. —The masked cytokine of any one of claims 2, 4, 6, and 8-10, wherein the first masking moiety comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 9, 10, 161-165, 187-218, 221-229, 231, and 261, and the second masking moiety comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 9, 10, 161-165, 187-218, 221-229, 231, and 261, and wherein the amino acid sequence of the first masking moiety and the second masking moiety are different.~~

~~13. —The masked cytokine of any one of claims 1-8, wherein the cytokine or functional fragment thereof is an IL-15 polypeptide or functional fragment thereof.~~

~~14. —The masked cytokine of claim 13, wherein the IL-15 polypeptide or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 167.~~

~~15. —The masked cytokine of any one of claims 1, 3, 5, 7, 13, and 14, wherein the masking moiety comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 161-165, 219-229, 232-234, 261, and 823-825.~~

~~16. —The masked cytokine of any one of claims 2, 4, 6, 8, 13, and 14, wherein:~~

~~a) the first masking moiety comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 232-234, and 823-825, and the second masking moiety comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 161-165, 219-229, and 261;~~

~~b) the first masking moiety comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 161-165, 219-229, and 261, and the second masking moiety comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 232-234, and 823-825;~~

~~c) the first masking moiety comprises an amino acid sequence produced by introducing one or more of the following amino acid substitutions into the amino acid sequence of any one of SEQ ID NOs: 232-234, and 823-825: R24A, R26A, K34A, S40A, L42A, and P67A, and the second masking moiety comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 161-165, 219-229, and 261; or~~

~~———d) the first masking moiety comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 161-165, 219-229, and 261, and the second masking moiety comprises an amino acid~~

sequence produced by introducing one or more of the following amino acid substitutions into the amino acid sequence of any one of SEQ ID NOs: 232-234, and 823-825: R24A, R26A, K34A, S40A, L42A, and P67A.

17. — The masked cytokine of any one of claims 5, 6, and 9-16, wherein the half-life extension domain is an antibody or fragment thereof.

18. — The masked cytokine of claim 17, wherein the antibody or fragment thereof is a Fragment crystallizable domain (Fc domain) or fragment thereof.

19. — The masked cytokine of claim 18, wherein the Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 154.

20. — The masked cytokine of any one of claims 3, 4, and 7-19, wherein the first half-life extension domain is a first antibody or fragment thereof, and the second half-life extension domain is a second antibody or fragment thereof.

21. — The masked cytokine of claim 20, wherein the first antibody or fragment thereof is a first Fc domain or fragment thereof, and second antibody or fragment thereof is a second Fc domain or fragment thereof.

22. — The masked cytokine of claim 21, wherein:

a) the first Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 155, and the second Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 156;

b) the first Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 156, and the second Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 155;

c) the first Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 154, and the second Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 154;

d) the first Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 265, and the second Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 156;

e) the first Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 156, and the second Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 265;

f) the first Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 155, and the second Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 616;

g) the first Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 616; and the second Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 155;

~~h) the first Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 157, and the second Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 158;~~

i) the first Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 158, and the second Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 157;

j) the first Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 796, and the second Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 774;

k) the first Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 774, and the second Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 796;

1) the first Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 721, and the second Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 619;

m) the first Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 619, and the second Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 721;

n) the first Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 721, and the second Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 772;

~~e) the first Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 772, and the second Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 721;~~

p) the first Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 793, and the second Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 622;

~~g) the first Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 622, and the second Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 793;~~

r) the first Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 793, and the second Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 773;

s) the first Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 773, and the second Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 793;

t) the first Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 796, and the second Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 625;

u) the first Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 625, and the second Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 796;

~~v) the first Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 156, and the second Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 156;~~

~~w) the first Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 796, and the second Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 625; or~~

~~x) the first Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 625, and the second Fe domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 796.~~

~~23. — The masked cytokine of any one of claims 1, 5, and 9-22, wherein the first linker comprises a first cleavable peptide.~~

~~24. — The masked cytokine of claim 23, wherein the first cleavable peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 96-153, 236-242, 264, 270-302, 306-317, 342-347, 356-415, 420-491, 494-501, 504-535, and 538-555.~~

~~25. — The masked cytokine of any one of claims 1, 5, and 9-24, wherein the first linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 11-153, 235-242, 262-264, 268-320, 323-338, 340-354, 356-555, 668, 691, 724, 725, 727, 762-771, 794, and 797-812.~~

~~26. — The masked cytokine of any one of claims 2 and 7-22, wherein the first linker comprises a first cleavable peptide, and/or the second linker comprises a second cleavable peptide.~~

~~27. — The masked cytokine of claim 26, wherein the first cleavable peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 96-153, 236-242, 264, 270-302, 306-317, 342-347, 356-415, 420-491, 494-501, 504-535, and 538-555, and/or the second cleavable peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 96-153, 236-242, 264, 270-302, 306-317, 342-347, 356-415, 420-491, 494-501, 504-535, and 538-555.~~

~~28. — The masked cytokine of any one of claims 2, 7-22, 26, and 27, wherein the first linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 11-153, 235-242, 262-264, 268-320, 323-338, 340-354, 356-555, 668, 691, 724, 725, 727, 762-771, 794, and 797-812, and/or the second linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 11-153, 235-242, 262-264, 268-320, 323-338, 340-354, 356-555, 668, 691, 724, 725, 727, 762-771, 794, and 797-812.~~

~~29. — A nucleic acid encoding the masked cytokine of any one of claims 1-28.~~

- ~~30. — A vector comprising the nucleic acid of claim 29.~~
- ~~31. — A host cell comprising the nucleic acid of claim 29.~~
- ~~32. — A method of producing a masked cytokine comprising culturing the host cell of claim 31 under a condition that produces the masked cytokine.~~
- ~~33. — A composition comprising the masked cytokine of any one of claims 1-28.~~
- ~~34. — A pharmaceutical composition comprising the masked cytokine of any one of claims 1-28, and a pharmaceutically acceptable carrier.~~
- ~~35. — A kit comprising the masked cytokine of any one of claims 1-28, or the composition of claim 33, or the pharmaceutical composition of claim 34.~~
- ~~36. — A method of treating or preventing a neoplastic disease in a subject, the method comprising administering to the subject an effective amount of the masked cytokine of any one of claims 1-28, or the composition of claim 33, or the pharmaceutical composition of claim 34.~~
- ~~37. — A method of treating or preventing an inflammatory or autoimmune disease in a subject, the method comprising administering to the subject an effective amount of the masked cytokine of any one of claims 1-28, or the composition of claim 33, or the pharmaceutical composition of claim 34.~~

1. A masked cytokine comprising:

a) a first half-life extension domain and a second half-life extension domain, wherein the first half-life extension domain is a first Fc domain or fragment thereof, and the second half-life extension domain is a second Fc domain or fragment thereof;

b) a masking moiety comprising an amino acid sequence having at least about 98% sequence identity to the amino acid sequence of SEQ ID NO: 10; and

c) a cytokine, wherein the cytokine is an IL-2 polypeptide, wherein the IL-2 polypeptide comprises the amino acid sequence of SEQ ID NO: 3;

wherein the masking moiety is linked to the first half-life extension domain via a first linker, wherein the first linker comprises a cleavable peptide;

wherein the cytokine is linked to the second half-life extension domain via a second linker;
and

wherein the first half-life extension domain and the second half-life extension domain contain modifications promoting the association of the first and the second half-life extension domain.

2. The masked cytokine of claim 1, wherein the masking moiety comprises the amino acid sequence of SEQ ID NO: 826.

3. The masked cytokine of claim 1, wherein the first Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 155, and the second Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 156.

4. The masked cytokine of claim 1, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 96.

5. The masked cytokine of claim 1, wherein the first linker comprises an amino acid sequence of SEQ ID NO: 15, and the second linker comprises an amino acid sequence of SEQ ID NO: 339.

6. A pharmaceutical composition comprising the masked cytokine of claim 1, and a pharmaceutically acceptable carrier.

7. A kit comprising the masked cytokine of claim 1.

8. The masked cytokine of claim 2, wherein the first Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 155, and the second Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 156.

9. The masked cytokine of claim 2, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 96.

10. The masked cytokine of claim 2, wherein the first linker comprises an amino acid sequence of SEQ ID NO: 15, and the second linker comprises an amino acid sequence of SEQ ID NO: 339.

11. The masked cytokine of claim 1, wherein the masking moiety comprises an amino acid sequence of SEQ ID NO: 826; wherein the first Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 155, and the second Fc domain or fragment thereof comprises the amino acid sequence of SEQ ID NO: 156; and wherein the first linker comprises an amino acid sequence of SEQ ID NO: 15, and the second linker comprises an amino acid sequence of SEQ ID NO: 339.

12. A pharmaceutical composition comprising the masked cytokine of claim 2, and a pharmaceutically acceptable carrier.

13. A kit comprising the masked cytokine of claim 2.

14. A pharmaceutical composition comprising the masked cytokine of claim 11, and a pharmaceutically acceptable carrier.

15. A kit comprising the masked cytokine of claim 11.