conditions to promote tumor growth. The connection between senescence and aging was initially based on the observation that senescent cells accumulate in aged tissue. The use of transgenic models has enabled the detection of senescent cells systematically in many aging-related disorders. Strategies to selectively eliminate senescent cells have demonstrated that senescent cells play a causal role in aging-related disorders.

Cellular senescence is a series of progressive and phenotypically diverse cellular states that are acquired after initial growth arrest (van Deursen, *Nature* 509(7501):439-446, 2014) Thus, senescent cells are heterogeneous populations of cells with few shared core properties (Dou et al., *Nature* 550(7676):402-406, 2017). Identifying common senolytic drug targets, therefore, is difficult. This further precludes the achievement of a goal of developing senolytics that selectively, safety, and effectively eliminate senescent cells upon systemic administration. As described above, immune cells are the effector cells to remove senescent cells naturally after the fulfillment of senescent-cell physiological roles.(Brighton et al., *Elife* 6, 2017) The weakening of the immune system during the aging process allows the accumulation of senescent cells.(Karin et al., *Nat. Comm.* 10(1):5495, 2019; Chambers et al., *J. Allergy Clin. Immunol.* 145(5):1323-1331, 2020).

SUMMARY

The present ~~invention~~ disclosure is based on the discovery that subcutaneous administration of an agent that results in a decrease in the activation of a TGF-β receptor or a common gamma-chain family cytokine receptor activating agent (e.g., complexes of gamma-chain cytokines and their cognate receptors) to a mammal promotes and activates immune cells to regain their capabilities of reducing senescent cells in vivo effectively, selectively, and safely. In view of this discovery, provided herein are methods of killing or reducing the number of naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor. Also provided herein are methods of decreasing the accumulation of naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a

therapeutically effective amount of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor. Also provided herein are methods of decreasing a level of a marker of naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor. Also provided herein are methods of reducing the activity of naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor. Also provided herein are methods of decreasing levels and/or activity of one or more senescence associated secretory phenotype("SASP") factor(s) derived from naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor.

Also provided herein are methods of killing and reducing the number of naturally-occurring and/or treatment-induced senescent cells (and methods of decreasing the accumulation or reducing markers of senescent cells) in a subject that include administering to the subject a therapeutically effective amount of one or more common gamma-chain family cytokine receptor activating agent(s) (e.g., complexes of gamma-chain cytokines and their cognate receptors). Also provided herein are methods of reducing the activity of naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more common gamma-chain family cytokine receptor activating agent(s). Also provided herein are methods of decreasing levels and/or activity of one or more SASP factor(s) derived from naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more common gamma-chain family cytokine receptor activating agent(s).

The present ~~invention~~disclosure is also based on the discovery that administration of NK cell activating agents to a mammal having a cancer resulted in a tumor inhibition and administration of NK cell activating agents to a diabetic animal model demonstrated improved skin and hair appearance and texture, and decreased blood glucose levels. In

view of this discovery provided herein are methods of treating an aging-related disease or condition in a subject in need thereof that include administering to a subject identified as having an aging-related disease or condition a therapeutically effective amount of one or more natural killer (NK) cell activating agent (s) and/or a therapeutically effective number of activated NK cells. Also provided herein are methods of killing or reducing the number of senescent cells in a subject in need thereof that include administering to the subject a therapeutically effective amount of one or more NK cell activating agent(s) and/or or a therapeutically effective number of activated NK cells. Also provided herein are methods of improving the texture and/or appearance of skin and/or hair in a subject in need thereof over a period of time that include administering to the subject a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s) and/or a therapeutically effective number of activated NK cells. Also provided herein are methods of assisting in the treatment of obesity in a subject in need thereof over a period of time that include administering to the subject a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s) and/or a therapeutically effective number of activated NK cells.

Provided herein are methods of killing or reducing the number of naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor. In one aspect, the present disclosure refers to the use of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor in the manufacture of a medicament for killing or reducing the number of naturally-occurring and/or treatment-induced senescent cells in a subject.

Also provided herein are methods of decreasing the accumulation of naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor. In one aspect, the present disclosure refers to the use of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor in the manufacture of a medicament for decreasing the accumulation of naturally-occurring and/or treatment-induced senescent cells in a subject.

Also provided herein are methods of decreasing a level of a marker of naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor. In one aspect, the present disclosure refers to the use of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor in the manufacture of a medicament for decreasing a level of a marker of naturally-occurring and/or treatment-induced senescent cells in a subject.

Also provided herein are methods of reducing the activity of naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the

subject a therapeutically effective amount of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor. In one aspect, the present disclosure refers to the use of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor in the manufacture of a medicament for reducing the activity of naturally-occurring and/or treatment-induced senescent cells in a subject.

Also provided herein are methods of decreasing levels and/or activity of one or more SASP factor(s) derived from naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor. In one aspect, the present disclosure refers to the use of one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor in the manufacture of a medicament for decreasing levels and/or activity of one or more SASP factor(s) derived from naturally-occurring and/or treatment-induced senescent cells in a subject.

In some ~~embodiment~~examples of any of the methods described herein, the subject has been previously diagnosed or identified as having an aging-related disease or an inflammatory disease. In some ~~embodiment~~examples of any of the methods described herein, the aging-related disease is inflamm-aging related. In some ~~embodiment~~examples of any of the methods described herein, the aging-related disease is selected from the group of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, inflammatory bowel disease, intervertebral disc degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, age-related macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, neurodegeneration, stroke, cancer, dementia, vascular disease, infection susceptibility, chronic inflammation, and renal dysfunction. In some ~~embodiment~~examples of any of the methods described herein, the aging-related disease is a cancer selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung

cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some ~~embodiment~~examples of any of the methods described herein, the inflammatory disease is selected from the group of: rheumatoid arthritis, inflammatory bowel disease, lupus erythematosus, lupus nephritis, diabetic nephropathy, CNS injury, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Crohn's disease, multiple sclerosis, Guillain-Barre syndrome, psoriasis, Grave's disease, ulcerative colitis, nonalcoholic steatohepatitis, mood disorders and cancer treatment-related cognitive impairment.

In some ~~embodiment~~examples of any of the methods described herein, the treatment-induced senescent cells are chemotherapy-induced senescent cells. In some ~~embodiment~~examples of any of the methods described herein, the administration of the one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor results in a decrease in the number or activity of naturally-occurring senescent cells and/or treatment-induced senescent cells in a target tissue in the subject. In some ~~embodiment~~examples of any of the methods described herein, the target tissue is selected from the group of: adipose tissue, pancreatic tissue, liver tissue, kidney tissue, lung tissue, heart tissue, vasculature, bone tissue, central nervous system (CNS) tissue, eye tissue, skin tissue, muscle tissue, and secondary lympho-organ tissue.

In some ~~embodiment~~examples of any of the methods described herein, the TGFβ receptor is a TGF-β receptor II (TGFβRII). In some ~~embodiment~~examples of any of the methods described herein, the TGFβ receptor is a TGFβRIII.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor is a soluble TGF-β receptor, an extracellular domain of TGF-β receptor, an antibody that binds specifically to TGF-β, an antagonistic antibody that binds to a TGF-β receptor, an agent that binds to a latency-associated peptide ("LAP"), or an agent that binds to a TGF-β/LAP complex. In some ~~embodiment~~examples of any of the methods described herein, the one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor

decrease(s) the activation of a TGF-β receptor through binding to a LAP, or to a TGF-β/LAP complex.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor is a multi-chain chimeric polypeptide including: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where one or both of the first target-binding domain and the second target-binding domain binds specifically to a ligand of a TGF-β receptor; or one or both of the first target-binding domain and the second target-binding domain is an antagonistic antigen-binding domain that binds specifically to a TGF-β receptor. In some ~~embodiment~~examples of any of the methods described herein, the TGF-β receptor is TGFβRII. In some ~~embodiment~~examples of any of the methods described herein, the TGF-β receptor is TGFβRIII.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some ~~embodiment~~examples of any of the methods described herein, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some ~~embodiment~~examples of any of the methods described herein, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the second chimeric polypeptide further includes a linker sequence

between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to different antigens. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide further comprises one or more additional target-binding domain(s). In some ~~embodiment~~examples of any of the methods described herein, the second chimeric polypeptide further comprises one or more additional target-binding domain(s).

In some ~~embodiment~~examples of any of the methods described herein, the soluble tissue factor domain is a soluble human tissue factor domain. In some ~~embodiment~~examples of any of the methods described herein, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 93.

In some ~~embodiment~~examples of any of the methods described herein, the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL15Rα) and a soluble IL-15. In some ~~embodiment~~examples of any of the methods described herein, the soluble IL-15 has a D8N or D8A amino acid substitution. In some ~~embodiment~~examples of any of the methods described herein, the soluble IL-15 comprises a mutation to reduce or eliminate IL-15 activity.

In some ~~embodiment~~examples of any of the methods described herein, the pair of affinity domains is selected from the group of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25. In some ~~embodiment~~examples of any of the methods described herein, the first domain or the second domain of a pair of affinity domains is a soluble common gamma-chain family cytokine or an antigen-binding domain that binds specifically to a common gamma-chain family cytokine receptor.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and/or the second target-binding domain include a soluble TGF-β

receptor. In some ~~embodiment~~examples of any of the methods described herein, the soluble TGF-β receptor is a soluble TGFβRII. In some ~~embodiment~~examples of any of the methods described herein, the soluble TGFβRII includes a first sequence that is at least 80% identical to SEQ ID NO: 183, and a second sequence that is at least 80% identical to SEQ ID NO: 183, wherein the first and second sequence are separated by a linker. In some ~~embodiment~~examples of any of the methods described herein, the soluble TGFβRII comprises a first sequence that is at least 90% identical to SEQ ID NO: 183, and a second sequence that is at least 90% identical to SEQ ID NO: 183. In some ~~embodiment~~examples of any of the methods described herein, the soluble TGFβRII includes a first sequence of SEQ ID NO: 183, and a second sequence of SEQ ID NO: 183. In some ~~embodiment~~examples of any of the methods described herein, the linker includes a sequence of SEQ ID NO: 102. In some ~~embodiment~~examples of any of the methods described herein, the soluble TGFβRII includes a sequence that is at least 80% identical to SEQ ID NO: 188. In some ~~embodiment~~examples of any of the methods described herein, the soluble TGFβRII includes a sequence that is at least 90% identical to SEQ ID NO: 188. In some ~~embodiment~~examples of any of the methods described herein, the soluble TGF-βRII includes a sequence of SEQ ID NO: 188.

In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide includes a sequence that is at least 80% identical to SEQ ID NO: 236. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide includes a sequence that is at least 90% identical to SEQ ID NO: 236. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide includes a sequence of SEQ ID NO: 236. In some ~~embodiment~~examples of any of the methods described herein, the second chimeric polypeptide includes a sequence that is at least 80% identical to SEQ ID NO: 193. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide includes a sequence that is at least 80% identical to SEQ ID NO: 236. In some ~~embodiment~~examples of any of the methods described herein, the second chimeric polypeptide includes a sequence that is at least 90% identical to SEQ ID NO: 193. In some ~~embodiment~~examples of any of the methods described herein, the second chimeric polypeptide includes a sequence of SEQ ID NO: 193. In some

~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide comprises a sequence of SEQ ID NO: 236.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor is a single-chain chimeric polypeptide including: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a second target-binding domain, wherein one or both of the first target-binding domain and the second target-binding domain binds specifically to a ligand of a TGF-β receptor; or one or both of the first target-binding domain and the second target-binding domain is an antagonistic antigen-binding domain that binds specifically to a TGF-β receptor. In some ~~embodiment~~examples of any of the methods described herein, the TGF-β receptor is TGF-βRII. In some ~~embodiment~~examples of any of the methods described herein, the TGF-β receptor is TGFβRIII.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the soluble tissue factor domain directly abut each other. In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between the first target-binding domain and the soluble tissue factor domain. In some ~~embodiment~~examples of any of the methods described herein, the soluble tissue factor domain and the second target-binding domain directly abut each other. In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between the soluble tissue factor domain and the second target-binding domain.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to different antigens.

In some ~~embodiment~~examples of any of the methods described herein, the soluble tissue factor domain is a soluble human tissue factor domain. In some ~~embodiment~~examples of any of the methods described herein, the soluble human tissue factor domain includes a sequence that is at least 80% identical to SEQ ID NO: 93.

In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide further includes one or more additional target-binding domains at its N- and/or C-terminus. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and/or the second target-binding domain comprise a soluble TGF-β receptor. In some ~~embodiment~~examples of any of the methods described herein, the soluble TGF-β receptor is a soluble TGF-βRII.

In some ~~embodiment~~examples of any of the methods described herein, the soluble TGF-βRII includes a first sequence that is at least 80% identical to SEQ ID NO: 183, and a second sequence that is at least 80% identical to SEQ ID NO: 183, wherein the first and second sequence are separated by a linker. In some ~~embodiment~~examples of any of the methods described herein, the soluble TGFβRII includes a first sequence that is at least 90% identical to SEQ ID NO: 183, and a second sequence that is at least 90% identical to SEQ ID NO: 183. In some ~~embodiment~~examples of any of the methods described herein, the soluble TGFβRII includes a first sequence of SEQ ID NO: 183, and a second sequence of SEQ ID NO: 183. In some ~~embodiment~~examples of any of the methods described herein, the linker includes a sequence of SEQ ID NO: 102. In some ~~embodiment~~examples of any of the methods described herein, the soluble TGFβRII includes a sequence that is at least 80% identical to SEQ ID NO: 188. In some ~~embodiment~~examples of any of the methods described herein, the soluble TGFβRII includes a sequence that is at least 90% identical to SEQ ID NO: 188. In some ~~embodiment~~examples of any of the methods described herein, the soluble TGFβRII includes a sequence of SEQ ID NO: 188.

In some ~~embodiment~~examples of any of the methods described herein, the method includes administering two or more doses of the one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor to the subject. In some ~~embodiment~~examples of any of the methods described herein, any two consecutive doses of the two or more doses are administered about 1 week to about one year apart. In some ~~embodiment~~examples of any of the methods described herein, any two consecutive doses of the two or more doses are administered about 1 week to about 6 months apart. In some ~~embodiment~~examples of any of the methods described herein, any two consecutive doses of the two or more doses are administered about 1 week to about 2 months apart. In some ~~embodiment~~examples of any of the

methods described herein, any two consecutive doses of the two or more doses are administered about 1 week to about 1 month apart.

In some ~~embodiment~~examples of any of the methods described herein, the two or more doses are administered by subcutaneous administration. In some ~~embodiment~~examples of any of the methods described herein, the two or more doses are administered by intramuscular administration.

In some ~~embodiment~~examples of any of the methods described herein, the two or more doses are administered over a period of time of about 1 year to about 60 years. In some ~~embodiment~~examples of any of the methods described herein, the two or more doses are administered over a period of time of about 1 year to about 50 years. In some ~~embodiment~~examples of any of the methods described herein, the two or more doses are administered over a period of time of about 1 year to about 40 years. In some ~~embodiment~~examples of any of the methods described herein, the two or more doses are administered over a period of time of about 1 year to about 30 years. In some ~~embodiment~~examples of any of the methods described herein, the two or more doses are administered over a period of time of about 1 year to about 20 years. In some ~~embodiment~~examples of any of the methods described herein, the two or more doses are administered over a period of time of about 1 year to about 10 years.

In some ~~embodiment~~examples of any of the methods described herein, a first dose of the one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor begins when the subject reaches an age of at least 30 years. In some ~~embodiment~~examples of any of the methods described herein, a first dose of the one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor begins when the subject reaches an age of at least 40 years. In some ~~embodiment~~examples of any of the methods described herein, a first dose of the one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor begins when the subject reaches an age of at least 50 years. In some ~~embodiment~~examples of any of the methods described herein, a first dose of the one or more agent(s) that result(s) in a decrease in the activation of a TGF-β receptor begins when the subject reaches an age of at least 60 years.

In some ~~embodiment~~examples of any of the methods described herein, each of the two or more doses are administered at a dosage of about 0.01 mg of each agent that results in a decrease in the activation of a TGF-β receptor/kg to about 10 mg of each agent that results in a decrease in the activation of a TGF-β receptor/kg. In some ~~embodiment~~examples of any of the methods described herein, each of the two or more doses are administered at a dosage of about 0.02 mg of each agent that results in a decrease in the activation of a TGF-β receptor/kg to about 5 mg of each agent that results in a decrease in the activation of a TGF-β receptor/kg.

In some ~~embodiment~~examples of any of the methods described herein, the subject is not diagnosed or identified as having an aging-related disease or an inflammatory disease. In some ~~embodiment~~examples of any of the methods described herein, the subject has not been previously treated with a chemotherapeutic agent. In some ~~embodiment~~examples of any of the methods described herein, the subject has not been previously treated with a therapeutic agent that induces cellular senescence.

Provided herein are methods of treating an aging-related disease or condition in a subject in need thereof that include administering to a subject identified as having an aging-related disease or condition a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s).

Also provided herein are methods of killing or reducing the number of senescent cells in a subject in need thereof that include administering to the subject a therapeutically effective amount of one or more NK cell activating agent(s). In some ~~embodiment~~examples of any of the methods described herein, the senescent cells are senescent cancer cells, senescent monocytes, senescent lymphocytes, senescent astrocytes, senescent microglia, senescent neurons, senescent tissue fibroblasts, senescent dermal fibroblasts, senescent keratinocytes, or other differentiated tissue-specific dividing functional cells. In some ~~embodiment~~examples of any of the methods described herein, the senescent cancer cells are chemotherapy-induced senescent cells or radiation-induced senescent cells. In some ~~embodiment~~examples of any of the methods described herein, the subject has been identified or diagnosed as having an aging-related disease or condition.

In some ~~embodiment~~examples of any of the methods described herein, the aging-related disease or condition is selected from the group of: a cancer, an autoimmune disease, a metabolic disease, a neurodegenerative disease, a cardiovascular disease, a skin disease, a progeria disease, and a fragility disease. In some ~~embodiment~~examples of any of the methods described herein, the cancer is selected from the group of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some ~~embodiment~~examples of any of the methods described herein, the autoimmune disease is type-1 diabetes.

In some ~~embodiment~~examples of any of the methods described herein, the metabolic disease is selected from the group of: obesity, a lipodystrophy, and type-2 diabetes mellitus.

In some ~~embodiment~~examples of any of the methods described herein, the neurodegenerative disease is selected from the group of: Alzheimer's disease, Parkinson's disease, and dementia.

In some ~~embodiment~~examples of any of the methods described herein, the cardiovascular disease is selected from the group of: coronary artery disease, atherosclerosis, and pulmonary arterial hypertension.

In some ~~embodiment~~examples of any of the methods described herein, the skin disease is selected from the group of: wound healing, alopecia, wrinkles, senile lentigo, skin thinning, xeroderma pigmentosum, and dyskeratosis congenita.

In some ~~embodiment~~examples of any of the methods described herein, the progeria disease is selected from the group of: progeria and Hutchinson-Gilford Progeria Syndrome.

In some ~~embodiment~~examples of any of the methods described herein, the fragility disease is selected from the group of: frailty, responsiveness to vaccination, osteoporosis, and sarcopenia.

In some ~~embodiment~~examples of any of the methods described herein, the aging-related disease or condition is selected from the group of: osteoarthritis, adipose atrophy, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, sarcopenia, age-associated loss of lung tissue elasticity, osteoporosis, age-associated renal dysfunction, and chemical-induced renal dysfunction.

In some ~~embodiment~~examples of any of the methods described herein, the aging-related disease or condition is type-2 diabetes or atherosclerosis.

In some ~~embodiment~~examples of any of the methods described herein, the administering results in a decrease in the number of senescent cells in a target tissue in the subject. In some ~~embodiment~~examples of any of the methods described herein, the target tissue is selected from the group of: adipose tissue, pancreatic tissue, liver tissue, lung tissue, vasculature, bone tissue, central nervous system (CNS) tissue, eye tissue, skin tissue, muscle tissue, and secondary lympho-organ tissue.

In some ~~embodiment~~examples of any of the methods described herein, the administering results in an increase in the expression levels of CD25, CD69, mTORC1, SREBP1, IFN-γ, and granzyme B in activated NK cells.

Also provided herein are methods of treating an aging-related disease or condition in a subject in need thereof that include administering to a subject identified as having an aging-related disease or condition a therapeutically effective number of activated NK cells.

Also provided herein are methods of killing or reducing the number of senescent cells in a subject in need thereof that include administering to the subject a therapeutically effective number of activated NK cells. In some ~~embodiment~~examples of any of the methods described herein, the senescent cells are senescent cancer cells, senescent

monocytes, senescent lymphocytes, senescent astrocytes, senescent microglia, senescent neurons, senescent tissue fibroblasts, senescent dermal fibroblasts, senescent keratinocytes, or other differentiated tissue-specific dividing functional cells. In some ~~embodiment~~examples of any of the methods described herein, the senescent cancer cells are chemotherapy-induced senescent cells or radiation-induced senescent cells. In some ~~embodiment~~examples of any of the methods described herein, the subject has been identified or diagnosed as having an aging-related disease or condition.

In some ~~embodiment~~examples of any of the methods described herein, the aging-related disease or condition is selected from the group of: a cancer, an autoimmune disease, a metabolic disease, a neurodegenerative disease, a cardiovascular disease, a skin disease, a progeria disease, and a fragility disease. In some ~~embodiment~~examples of any of the methods described herein, the cancer is selected from the group of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some ~~embodiment~~examples of any of the methods described herein, the autoimmune disease is type-1 diabetes.

In some ~~embodiment~~examples of any of the methods described herein, the metabolic disease is selected from the group of: obesity, a lipodystrophy, and type-2 diabetes mellitus.

In some ~~embodiment~~examples of any of the methods described herein, the neurodegenerative disease is selected from the group of: Alzheimer's disease, Parkinson's disease, and dementia.

In some ~~embodiment~~examples of any of the methods described herein, the cardiovascular disease is selected from the group of: coronary artery disease, atherosclerosis, and pulmonary arterial hypertension.

In some ~~embodiment~~examples of any of the methods described herein, the skin disease is selected from the group of: wound healing, alopecia, wrinkles, senile lentigo, skin thinning, xeroderma pigmentosum, and dyskeratosis congenita.

In some ~~embodiment~~examples of any of the methods described herein, the progeria disease is selected from the group of: progeria and Hutchinson-Gilford Progeria Syndrome.

In some ~~embodiment~~examples of any of the methods described herein, the fragility disease is selected from the group of: frailty, responsiveness to vaccination, osteoporosis, and sarcopenia.

In some ~~embodiment~~examples of any of the methods described herein, the aging-related disease or condition is selected from the group of: age-related macular degeneration, osteoarthritis, adipose atrophy, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, sarcopenia, age-associated loss of lung tissue elasticity, osteoporosis, age-associated renal dysfunction, and chemical-induced renal dysfunction.

Some ~~embodiment~~examples of any of the methods described herein further include: obtaining a resting NK cell; and contacting the resting NK cell *in vitro* in a liquid culture medium including one or more NK cell activating agent(s), where the contacting results in the generation of the activated NK cells that are subsequently administered to the subject. In some ~~embodiment~~examples of any of the methods described herein, the resting NK cell is an autologous NK cell obtained from the subject. In some ~~embodiment~~examples of any of the methods described herein, the resting NK cell is an allogeneic resting NK cell. In some ~~embodiment~~examples of any of the methods described herein, the resting NK cell is an artificial NK cell. In some ~~embodiment~~examples of any of the methods described herein, the resting NK cell is a haploidentical resting NK cell. In some ~~embodiment~~examples of any of the methods described herein, the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor. Some ~~embodiment~~examples of any of the methods described herein further include isolating the activated NK cells

before the activated NK cells are administered to the subject. Some ~~embodiment~~examples of any of the methods described herein further include introducing a nucleic acid that encodes a chimeric antigen receptor or a recombinant T cell receptor into the resting NK cell or the activated NK cell prior to administration to the subject.

Also provided herein are methods of improving the texture and/or appearance of skin and/or hair in a subject in need thereof over a period of time that include administering to the subject a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s).

Also provided herein are methods of improving the texture and/or appearance of skin and/or hair in a subject in need thereof over a period of time that include administering to the subject a therapeutically effective number of activated NK cells. Some ~~embodiment~~examples of any of the methods described herein further include: obtaining a resting NK cell; and contacting the resting NK cell *in vitro* in a liquid culture medium including one or more NK cell activating agent(s), where the contacting results in the generation of the activated NK cells that are subsequently administered to the subject. In some ~~embodiment~~examples of any of the methods described herein, the resting NK cell is an autologous NK cell obtained from the subject. In some ~~embodiment~~examples of any of the methods described herein, the resting NK cell is an allogeneic resting NK cell. In some ~~embodiment~~examples of any of the methods described herein, the resting NK cell is an artificial NK cell. In some ~~embodiment~~examples of any of the methods described herein, the resting NK cell is a haploidentical resting NK cell. In some ~~embodiment~~examples of any of the methods described herein, the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor. Some ~~embodiment~~examples of any of the methods described herein further include isolating the activated NK cells before the activated NK cells are administered to the subject.

In some ~~embodiment~~examples of any of the methods described herein, the method provides for an improvement in the texture and/or appearance of skin of the subject over the period of time. In some ~~embodiment~~examples of any of the methods described herein, the method results in a decrease in the rate of formation of wrinkles in the skin of the subject over the period of time. In some ~~embodiment~~examples of any of the methods described herein,

the method results in an improvement in the coloration of skin of the subject over the period of time. In some ~~embodiment~~examples of any of the methods described herein, the method results in a reduction of age spots on skin of the subject over the period of time. In some ~~embodiment~~examples of any of the methods described herein, the method results in an improvement in the texture of skin of the subject over the period of time. In some ~~embodiment~~examples of any of the methods described herein, the method provides for an improvement in the texture and/or appearance of hair of the subject over the period of time. In some ~~embodiment~~examples of any of the methods described herein, the method results in a decrease in the rate of formation of gray hair in the subject over the period of time. In some ~~embodiment~~examples of any of the methods described herein, the method results in a decrease in the number of gray hairs of the subject over the period of time. In some ~~embodiment~~examples of any of the methods described herein, the method results in a decrease in the rate of hair loss in the subject over time. In some ~~embodiment~~examples of any of the methods described herein, the method results in an improvement in the texture of hair of the subject over the period of time.

In some ~~embodiment~~examples of any of the methods described herein, the period of time is between about one month and about 10 years. In some ~~embodiment~~examples of any of the methods described herein, the method results in a decrease in the number of senescent dermal fibroblasts in the skin of the subject over the period of time.

Also provided herein are methods of assisting in the treatment of obesity in a subject in need thereof over a period of time that include administering to the subject a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s).

Also provided herein are methods of assisting in the treatment of obesity in a subject in need thereof over a period of time that include administering to the subject a therapeutically effective number of activated NK cells. Some ~~embodiment~~examples of any of the methods described herein further include: obtaining a resting NK cell; and contacting the resting NK cell *in vitro* in a liquid culture medium including one or more NK cell activating agent(s), where the contacting results in the generation of the activated NK cells that are subsequently administered to the subject. In some ~~embodiment~~examples of any of

the methods described herein, the resting NK cell is an autologous NK cell obtained from the subject. In some ~~embodiment~~examples of any of the methods described herein, the resting NK cell is an allogeneic resting NK cell. In some ~~embodiment~~examples of any of the methods described herein, the resting NK cell is an artificial NK cell. In some ~~embodiment~~examples of any of the methods described herein, the resting NK cell is a haploidentical resting NK cell. In some ~~embodiment~~examples of any of the methods described herein, the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor. Some ~~embodiment~~examples of any of the methods described herein further include isolating the activated NK cells before the activated NK cells are administered to the subject.

In some ~~embodiment~~examples of any of the methods described herein, the method results in a decrease in the mass of the subject over the period of time. In some ~~embodiment~~examples of any of the methods described herein, the method results in a decrease in the body mass index (BMI) of the subject over the period of time. In some ~~embodiment~~examples of any of the methods described herein, the method results in a decrease in the rate of progression from pre-diabetes to type-2 diabetes in the subject. In some ~~embodiment~~examples of any of the methods described herein, the method results in a decrease in fasting serum glucose level in the subject. In some ~~embodiment~~examples of any of the methods described herein, the method results in an increase in insulin sensitivity in the subject. In some ~~embodiment~~examples of any of the methods described herein, the method results in a decrease in the severity of atherosclerosis in the subject. In some ~~embodiment~~examples of any of the methods described herein, the period of time is between about two weeks and about 10 years.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) results in activation of one or more of: a receptor for IL-2, a receptor for IL-7, a receptor for IL-12, a receptor for IL-15, a receptor for IL-18, a receptor for IL-21, a receptor for IL-33, CD16, CD69, CD25, CD59, CD352, NKp80, DNAM-1, 2B4, NKp30, NKp44, NKp46, NKG2D, KIR2DS1, KIR2Ds2/3, KIR2DL4, KIR2DS4, KIR2DS5, and KIR3DS1.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-2 is a soluble IL-2 or an agonistic antibody that binds specifically to an IL-2 receptor.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-7 is a soluble IL-7 or an agonistic antibody that binds specifically to an IL-7 receptor.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-12 is a soluble IL-12 or an agonistic antibody that binds specifically to an IL-12 receptor.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-15 is a soluble IL-15 or an agonistic antibody that binds specifically to an IL-15 receptor.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-21 is a soluble IL-21 or an agonistic antibody that binds specifically to an IL-21 receptor.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-33 is a soluble IL-33 or an agonistic antibody that binds specifically to an IL-33 receptor.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for CD16 is an agonistic antibody that binds specifically to a CD16.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for CD69 is an agonistic antibody that binds specifically to a CD69.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for CD25 or CD59 is an agonistic antibody that binds specifically to CD25 or CD59.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for CD352 is an agonistic antibody that binds specifically to a CD352.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKp80 is an agonistic antibody that binds specifically to an NKp80.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for DNAM-1 is an agonistic antibody that binds specifically to a DNAM-1.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for 2B4 is an agonistic antibody that binds specifically to a 2B4.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKp30 is an agonistic antibody that binds specifically to an NKp30.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKp44 is an agonistic antibody that binds specifically to an NKp44.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKp46 is an agonistic antibody that binds specifically to an NKp46.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKG2D is an agonistic antibody that binds specifically to an NKG2D.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DS1 is an agonistic antibody that binds specifically to a KIR2DS1.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DS2/3 is an agonistic antibody that binds specifically to a KIR2DS2/3.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DL4 is an agonistic antibody that binds specifically to a KIR2DL4.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DS4 is an agonistic antibody that binds specifically to a KIR2DS4.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DS5 is an agonistic antibody that binds specifically to a KIR2DS5.

In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR3DS1 is an agonistic antibody that binds specifically to a KIR3DS1.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) results in a decrease in the activation of one or more of: PD-1, a TGF-β receptor, TIGIT, CD1, TIM-3, Siglec-7, IRP60, Tactile, IL1R8, NKG2A/KLRD1, KIR2DL1, KIR2DL2/3, KIR2DL5, KIR3DL1, KIR3DL2, ILT2/LIR-1, and LAG-2. In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of PD-1 is an antagonistic antibody that binds specifically to PD-1, a soluble PD-1, a soluble PD-L1, or an antibody that binds specifically to PD-L1. In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of a TGF-β receptor is a soluble TGF-β receptor, an antibody that binds specifically to TGF-β, or an antagonistic antibody that binds specifically to a TGF-β receptor.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of TIGIT is an antagonistic antibody that binds specifically to TIGIT, a soluble TIGIT, or an antibody that binds specifically to a ligand of TIGIT.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of CD1 is an antagonistic antibody that binds specifically to CD1, a soluble CD1, or an antibody that binds specifically to a ligand of CD1.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of TIM-3 is an antagonistic antibody that binds specifically to TIM-3, a soluble TIM-3, or an antibody that binds specifically to a ligand of TIM-3.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of Siglec-7 is an antagonistic antibody that binds specifically to Siglec-7 or an antibody that binds specifically to a ligand of Siglec-7.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of IRP60 is an antagonistic antibody that binds specifically to IRP60 or an antibody that binds specifically to a ligand of IRP60.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of Tactile is an antagonistic antibody that binds specifically to Tactile or an antibody that binds specifically to a ligand of Tactile.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of IL1R8 is an antagonistic antibody that binds specifically to IL1R8 or an antibody that binds specifically to a ligand of IL1R8.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of NKG2A/KLRD1 is an antagonistic antibody that binds specifically to NKG2A/KLRD1 or an antibody that binds specifically to a ligand of NKG2A/KLRD1.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR2DL1 is an antagonistic antibody that binds specifically to KIR2DL1 or an antibody that binds specifically to a ligand of KIR2DL1.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of

KIR2DL2/3 is an antagonistic antibody that binds specifically to KIR2DL2/3 or an antibody that binds specifically to a ligand of KIR2DL2/3.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR2DL5 is an antagonistic antibody that binds specifically to KIR2DL5 or an antibody that binds specifically to a ligand of KIR2DL5.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR3DL1 is an antagonistic antibody that binds specifically to KIR3DL1 or an antibody that binds specifically to a ligand of KIR3DL1.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR3DL2 is an antagonistic antibody that binds specifically to KIR3DL2 or an antibody that binds specifically to a ligand of KIR3DL2.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of ILT2/LIR-1 is an antagonistic antibody that binds specifically to ILT2/LIR-1 or an antibody that binds specifically to a ligand of ILT2/LIR-1.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of LAG-2is an antagonistic antibody that binds specifically to LAG-2 or an antibody that binds specifically to a ligand of LAG-2.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) is a single-chain chimeric polypeptide that includes: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a second target-binding domain. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the soluble tissue factor domain directly abut each other. In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between the first target-binding domain and the soluble tissue factor domain. In some ~~embodiment~~examples of any of the

methods described herein, the soluble tissue factor domain and the second target-binding domain directly abut each other. In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between the soluble tissue factor domain and the second target-binding domain. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain directly abut each other. In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between the first target-binding domain and the second target-binding domain. In some ~~embodiment~~examples of any of the methods described herein, the second target-binding domain and the soluble tissue factor domain directly abut each other. In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between the second target-binding domain and the soluble tissue factor domain.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to different antigens.

In some ~~embodiment~~examples of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain are each an antigen-binding domain. In some ~~embodiment~~examples of any of the methods described herein, the antigen-binding domain includes a scFv or a single domain antibody.

In some ~~embodiment~~examples of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain bind to a target selected from the group of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

In some ~~embodiment~~examples of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein. In some ~~embodiment~~examples of any of the methods described herein, the soluble interleukin or cytokine protein is selected from the group of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some ~~embodiment~~examples of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor. In some ~~embodiment~~examples of any of the methods described herein, the soluble interleukin or cytokine receptor is a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

In some ~~embodiment~~examples of any of the methods described herein, the soluble tissue factor domain is a soluble human tissue factor domain. In some ~~embodiment~~examples of any of the methods described herein, the soluble human tissue factor domain includes a sequence that is at least 80% identical to SEQ ID NO: 93. In some ~~embodiment~~examples of any of the methods described herein, the soluble human tissue factor domain includes a sequence that is at least 90% identical to SEQ ID NO: 93. In some ~~embodiment~~examples of any

of the methods described herein, the soluble human tissue factor domain includes a sequence that is at least 95% identical to SEQ ID NO: 93. In some ~~embodiment~~examples of any of the methods described herein, the soluble human tissue factor domain does not include one or more of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

In some ~~embodiment~~examples of any of the methods described herein, the soluble human tissue factor domain does not include any of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

In some ~~embodiment~~examples of any of the methods described herein, the soluble tissue factor domain is not capable of binding Factor VIIa. In some ~~embodiment~~examples of any of the methods described herein, the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some ~~embodiment~~examples of any of the methods described herein,

the single-chain chimeric polypeptide does not blood stimulate coagulation in a mammal. In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide further includes one or more additional target-binding domains at its N- and/or C-terminus.

In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide includes one or more additional target-binding domains at its N-terminus. In some ~~embodiment~~examples of any of the methods described herein, one or more additional target-binding domains directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between one of the at least one additional target-binding domains and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide includes one or more additional target-binding domains at its C-terminus. In some ~~embodiment~~examples of any of the methods described herein, one of the one or more additional target-binding domains directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between one of the at least one additional target-binding domains and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide includes one or more additional target binding domains at its N-terminus and the C-terminus. In some ~~embodiment~~examples of any of the methods described herein, one of the one or more additional antigen binding domains at the N-terminus directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between one of the one or more additional antigen-binding domains at the N-terminus and the first

target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some ~~embodiment~~examples of any of the methods described herein, one of the one or more additional antigen binding domains at the C-terminus directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between one of the one or more additional antigen-binding domains at the C-terminus and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

In some ~~embodiment~~examples of any of the methods described herein, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen. In some ~~embodiment~~examples of any of the methods described herein, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope. In some ~~embodiment~~examples of any of the methods described herein, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains include the same amino acid sequence. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same antigen. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same epitope. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each include the same amino acid sequence.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens.

In some ~~embodiment~~examples of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more

target-binding domains is an antigen-binding domain. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain. In some ~~embodiment~~examples of any of the methods described herein, the antigen-binding domain includes a scFv or a single domain antibody.

In some ~~embodiment~~examples of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains bind specifically to a target selected from the group of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

In some ~~embodiment~~examples of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine protein. In some ~~embodiment~~examples of any of the methods described herein, the soluble interleukin or cytokine protein is selected from the group of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some ~~embodiment~~examples of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine receptor. In some ~~embodiment~~examples of any of the methods described herein, the soluble receptor is a soluble

TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) is a multi-chain chimeric polypeptide that includes: (a) a first chimeric polypeptide including: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide including: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the second chimeric polypeptide further includes a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to the same

epitope. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to different antigens.

In some ~~embodiment~~examples of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain are each antigen-binding domains. In some ~~embodiment~~examples of any of the methods described herein, the antigen-binding domain includes a scFv or a single domain antibody.

In some ~~embodiment~~examples of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain bind specifically to a target selected from the group of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

In some ~~embodiment~~examples of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein. In some ~~embodiment~~examples of any of the methods described herein, the soluble interleukin or cytokine protein is selected from the group of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some ~~embodiment~~examples of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor. In some ~~embodiment~~examples of any of the methods described herein, the soluble receptor is a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide further includes one or more additional target-binding domain(s), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence between the soluble tissue factor domain and the at least one of the one or more additional antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more additional antigen-binding domain(s) and the first domain of the pair of affinity domains.

In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide further includes one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence between the at least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains. In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more additional target-binding domains is disposed at the N- and/or C-terminus of

the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence disposed (i) between the soluble tissue factor domain and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

In some ~~embodiment~~examples of any of the methods described herein, the second chimeric polypeptide further includes one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more additional target-binding domains directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the second chimeric polypeptide further includes a linker sequence between at least one of the one or more additional target-binding domains and the second domain of the pair of affinity domains in the second chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more additional target-binding domains directly abuts the second target-binding domain in the second chimeric polypeptide. In some ~~embodiment~~examples of any of the methods described herein, the second chimeric polypeptide further includes a linker sequence between at least one of the one or more additional target-binding domains and the second target-binding domain in the second chimeric polypeptide.

In some ~~embodiment~~examples of any of the methods described herein, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen. In some ~~embodiment~~examples of any of the methods described herein, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope. In some ~~embodiment~~examples of any of the methods described herein, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains include the same amino acid sequence. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same antigen. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same epitope. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain, the second target-binding

domain, and the one or more additional target-binding domains each include the same amino acid sequence.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens. In some ~~embodiment~~examples of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains is an antigen-binding domain. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain. In some ~~embodiment~~examples of any of the methods described herein, the antigen-binding domain includes a scFv.

In some ~~embodiment~~examples of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains bind specifically to a target selected from the group of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

In some ~~embodiment~~examples of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine protein. In some ~~embodiment~~examples of any of the methods described herein, the soluble interleukin or cytokine

protein is selected from the group of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some ~~embodiment~~examples of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine receptor. In some ~~embodiment~~examples of any of the methods described herein, the soluble receptor a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

In some ~~embodiment~~examples of any of the methods described herein, the soluble tissue factor domain is a soluble human tissue factor domain. In some ~~embodiment~~examples of any of the methods described herein, the soluble human tissue factor domain includes a sequence that is at least 80% identical to SEQ ID NO: 93. In some ~~embodiment~~examples of any of the methods described herein, the soluble human tissue factor domain includes a sequence that is at least 90% identical to SEQ ID NO: 93. In some ~~embodiment~~examples of any of the methods described herein, the soluble human tissue factor domain includes a sequence that is at least 95% identical to SEQ ID NO: 93.

In some ~~embodiment~~examples of any of the methods described herein, the soluble human tissue factor domain does not include one or more of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

In some ~~embodiment~~examples of any of the methods described herein, the soluble human tissue factor domain does not include any of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

In some ~~embodiment~~examples of any of the methods described herein, the soluble tissue factor domain is not capable of binding to Factor VIIa. In some ~~embodiment~~examples of any of the methods described herein, the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some ~~embodiment~~examples of any of the methods described herein, the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal. In some ~~embodiment~~examples of any of the methods described herein, the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL-15Rα) and a soluble IL-15. In some ~~embodiment~~examples of any of the methods described herein, the soluble IL-15 has a D8N or D8A amino acid substitution. In some ~~embodiment~~examples of any of the methods described herein, the human IL-15Rα is a mature full-length IL-15Rα.

In some ~~embodiment~~examples of any of the methods described herein, the pair of affinity domains is selected from the group of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

In some ~~embodiment~~examples of any of the methods described herein, at least one of the one or more NK cell activating agent(s) is a multi-chain chimeric polypeptide that includes: (a) a first and second chimeric polypeptides, where each includes: (i) a first

target-binding domain; (ii) a Fc domain; and (iii) a first domain of a pair of affinity domains; and (b) a third and fourth chimeric polypeptide, where each includes: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where the first and second chimeric polypeptides and the third and fourth chimeric polypeptides associate through the binding of the first domain and the second domain of the pair of affinity domains, and the first and second chimeric polypeptides associate through their Fc domains.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the Fc domain directly abut each other in the first and second chimeric polypeptides. In some ~~embodiment~~examples of any of the methods described herein, the first and second chimeric polypeptides further include a linker sequence between the first target-binding domain and the Fc domain in the first and second chimeric polypeptides. In some ~~embodiment~~examples of any of the methods described herein, the Fc domain and the first domain of the pair of affinity domains directly abut each other in the first and second chimeric polypeptides. In some ~~embodiment~~examples of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence between the Fc domain and the first domain of the pair of affinity domains in the first and second chimeric polypeptides.

In some ~~embodiment~~examples of any of the methods described herein, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the third and fourth chimeric polypeptides. In some ~~embodiment~~examples of any of the methods described herein, the third and fourth chimeric polypeptides further include a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the third and fourth chimeric polypeptides.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some ~~embodiment~~examples of any of the methods described herein, the first target-

binding domain and the second target-binding domain include the same amino acid sequence.

In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to different antigens. In some ~~embodiment~~examples of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain. In some ~~embodiment~~examples of any of the methods described herein, the first target-binding domain and the second target-binding domain are each antigen-binding domains. In some ~~embodiment~~examples of any of the methods described herein, the antigen-binding domain includes a scFv or a single domain antibody.

In some ~~embodiment~~examples of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain bind specifically to a target selected from the group of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

In some ~~embodiment~~examples of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein. In some ~~embodiment~~examples of any of the methods described herein, the soluble interleukin or cytokine protein is selected from the group of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some ~~embodiment~~examples of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin

or cytokine receptor. In some ~~embodiment~~examples of any of the methods described herein, the soluble receptor is a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

In some ~~embodiment~~examples of any of the methods described herein, the soluble tissue factor domain is a soluble human tissue factor domain that does not stimulate blood coagulation. In some ~~embodiment~~examples of any of the methods described herein, the soluble tissue factor domain comprises or consists of a sequence from a wildtype soluble human tissue factor.

Provided herein are methods of killing or reducing the number of naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more common gamma-chain family cytokine receptor activating agent(s). In one aspect, the present disclosure refers to the use of one or more common gamma-chain family cytokine receptor activating agent(s) in the manufacture of a medicament for killing or reducing the number of naturally-occurring and/or treatment-induced senescent cells in a subject.

Also provided herein are methods of decreasing the accumulation of naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more common gamma-chain family cytokine receptor activating agent(s). In one aspect, the present disclosure refers to the use of one or more common gamma-chain family cytokine receptor activating agent(s) in the manufacture of a medicament for decreasing the accumulation of naturally-occurring and/or treatment-induced senescent cells in a subject.

Also provided herein are methods of decreasing a level of a marker of naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more common gamma-chain family cytokine receptor activating agent(s). In one aspect, the present disclosure refers to the use of one or more common gamma-chain family cytokine receptor activating agent(s) in the manufacture of a medicament for decreasing a level of a marker of naturally-occurring and/or treatment-induced senescent cells in a subject.

Also provided herein are methods of reducing the activity of naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more common gamma-chain family cytokine receptor activating agent(s). In one aspect, the present disclosure refers to the use of one or more common gamma-chain family cytokine receptor activating agent(s) in the manufacture of a medicament for reducing the activity of naturally-occurring and/or treatment-induced senescent cells in a subject.

Also provided herein are methods of decreasing levels and/or activity of one or more SASP factor(s) derived from naturally-occurring and/or treatment-induced senescent cells in a subject that include administering to the subject a therapeutically effective amount of one or more common gamma-chain family cytokine receptor activating agent(s).

In one aspect, the present disclosure refers to the use of one or more common gamma-chain family cytokine receptor activating agent(s) in the manufacture of a medicament for decreasing levels or activity of SASP factors derived from naturally-occurring and/or treatment-induced senescent cells in a subject.

In some ~~embodiment~~examples, the subject has been previously diagnosed or identified as having an aging-related disease or an inflammatory disease. In some ~~embodiment~~examples, the aging-related disease is inflamm-aging related.

In some ~~embodiment~~examples, the aging-related disease is selected from the group of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, age-related macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, neurodegeneration, stroke, cancer, dementia, vascular disease, infection susceptibility, chronic inflammation, and renal dysfunction.

In some ~~embodiment~~examples, the aging-related disease is a cancer selected from the group of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some ~~embodiment~~examples, the inflammatory disease is selected from the group of: rheumatoid arthritis, inflammatory bowel disease, lupus erythematosus, lupus nephritis, diabetic nephropathy, CNS injury, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Crohn's disease, multiple sclerosis, Guillain-Barre syndrome, psoriasis,

Grave's disease, ulcerative colitis, nonalcoholic steatohepatitis, mood disorders and cancer treatment-related cognitive impairment.

In some ~~embodiment~~examples, the treatment-induced senescent cells are chemotherapy-induced senescent cells. In some ~~embodiment~~examples, the administration of the one or more common gamma-chain family cytokine receptor activating agent(s) results in a decrease in the number of naturally-occurring senescent cells and/or treatment-induced senescent cells in a target tissue in the subject. In some ~~embodiment~~examples, the target tissue is selected from the group of: adipose tissue, pancreatic tissue, liver tissue, kidney tissue, lung tissue, vasculature, bone tissue, central nervous system (CNS) tissue, eye tissue, skin tissue, muscle tissue, and secondary lympho-organ tissue.

In some ~~embodiment~~examples, at least one of the one or more common gamma-chain family cytokine receptor activating agent(s) is a complex of a common gamma-chain family cytokine or a functional fragment thereof and an antibody or antibody fragment that binds specifically to the common gamma-chain family cytokine or the functional fragment thereof.

In some ~~embodiment~~examples, at least one of the one or more common gamma-chain family cytokine receptor activating agent(s) is a single-chain chimeric polypeptide comprising: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a second target-binding domain, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble common gamma-chain family cytokine, an agonistic antigen-binding domain that binds specifically to a common gamma-chain family cytokine receptor, a soluble common gamma-chain family cytokine receptor, or an antigen-binding domain that binds specifically to a common gamma-chain family cytokine.

In some ~~embodiment~~examples, one or both of the first target-binding domain and the second target-binding domain comprises a soluble common gamma-chain family cytokine. In some ~~embodiment~~examples, the soluble common gamma-chain family cytokine is selected from the group consisting of: soluble IL-2, soluble IL-4, soluble IL-7, soluble IL-9, soluble IL-15, and soluble IL-21. In some ~~embodiment~~examples, one or both of the first target-binding domain and the second target-binding domain comprises an agonistic

antigen-binding domain that binds specifically to a common gamma-chain family cytokine receptor. In some ~~embodiment~~examples, the common gamma-chain family cytokine receptor is a receptor for one or more of IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. In some ~~embodiment~~examples, the agonistic antigen-binding domain is an scFv, a VHH, or a VNAR.

In some ~~embodiment~~examples, the first target-binding domain and the soluble tissue factor domain directly abut each other. In some ~~embodiment~~examples, the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain. In some ~~embodiment~~examples, the soluble tissue factor domain and the second target-binding domain directly abut each other. In some ~~embodiment~~examples, the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain. In some ~~embodiment~~examples, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some ~~embodiment~~examples, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some ~~embodiment~~examples, the first target-binding domain and the second target-binding domain comprise the same amino acid sequence. In some ~~embodiment~~examples, the first target-binding domain and the second target-binding domain bind specifically to different antigens.

In some ~~embodiment~~examples, the soluble tissue factor domain is a soluble human tissue factor domain. In some ~~embodiment~~examples, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 93. In some ~~embodiment~~examples, the single-chain chimeric polypeptide further comprises one or more additional target-binding domains at its N- and/or C-terminus.

In some ~~embodiment~~examples, at least one of the one or more common gamma-chain family cytokine receptor activating agent(s) is a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble common gamma-chain family cytokine, an agonistic antigen-binding domain that binds specifically to a common

gamma-chain family cytokine receptor, a soluble common gamma-chain family cytokine receptor, or an antigen-binding domain that binds specifically to a common gamma-chain family cytokine.

In some ~~embodiment~~examples, one or both of the first target-binding domain and the second target-binding domain comprises a soluble common gamma-chain family cytokine. In some ~~embodiment~~examples, the soluble common gamma-chain family cytokine is selected from the group of: soluble IL-2, soluble IL-4, soluble IL-7, soluble IL-9, soluble IL-15, and soluble IL-21. In some ~~embodiment~~examples, one or both of the first target-binding domain and the second target-binding domain comprises an agonistic antigen-binding domain that binds specifically to a common gamma-chain family cytokine receptor. In some ~~embodiment~~examples, the common gamma-chain family cytokine receptor is a receptor for one or more of IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. In some ~~embodiment~~examples, the agonistic antigen-binding domain is an scFv, a VHH, or a VNAR.

In some ~~embodiment~~examples, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some ~~embodiment~~examples, the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide. In some ~~embodiment~~examples, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some ~~embodiment~~examples, the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide. In some ~~embodiment~~examples, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some ~~embodiment~~examples, the second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some ~~embodiment~~examples, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some ~~embodiment~~examples, the first target-binding domain and the second target-binding domain bind specifically to different

antigens. In some ~~embodiment~~examples, the first chimeric polypeptide further comprises one or more additional target-binding domain(s). In some ~~embodiment~~examples, the second chimeric polypeptide further comprises one or more additional target-binding domains.

In some ~~embodiment~~examples, the soluble tissue factor domain is a soluble human tissue factor domain. In some ~~embodiment~~examples, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 93. In some ~~embodiment~~examples, the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL15Rα) and a soluble IL-15. In some ~~embodiment~~examples, the pair of affinity domains is selected from the group of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

In some ~~embodiment~~examples, the first domain or the second domain of a pair of affinity domains is a soluble common gamma-chain family cytokine or an antigen-binding domain that binds specifically to a common gamma-chain family cytokine receptor. In some ~~embodiment~~examples, at least one of the one or more common gamma-chain family cytokine receptor activating agent(s) is soluble IL-15 or an IL-15 agonist. In some ~~embodiment~~examples, the soluble IL-15 is at least 90% identical to SEQ ID NO: 82. In some ~~embodiment~~examples, the IL-15 agonist comprises a complex of IL-15 and all or a portion of a soluble IL-15 receptor (IL-15R). In some ~~embodiment~~examples, the portion of the soluble IL-15R is a portion of IL-15Rα. In some ~~embodiment~~examples, the portion of the soluble IL-15Rα is a sushi domain of IL-15Rα. In some ~~embodiment~~examples, the IL-15 agonist further comprises an Fc domain. In some ~~embodiment~~examples, the IL-15 agonist comprises a fusion protein comprising IL-15 and a sushi domain from an IL-15Rα. In some ~~embodiment~~examples, one of the one or more common gamma-chain family cytokine receptor activating agent(s) is a soluble IL-2 or an IL-2 agonist. In some ~~embodiment~~examples, one of the one or more common gamma-chain family cytokine receptor activating agent(s) is an antibody or an antigen-binding antibody fragment that binds specifically to a common gamma-chain family cytokine.

In some ~~embodiment~~examples, the method comprises administering one, two or more doses of the one or more common gamma-chain family cytokine receptor activating

agent(s) to the subject. In some ~~embodiment~~examples, any two consecutive doses of the two or more doses are administered about 1 week to about one year apart. In some ~~embodiment~~examples, any two consecutive doses of the two or more doses are administered about 1 week to about 6 months apart. In some ~~embodiment~~examples, any two consecutive doses of the two or more doses are administered about 1 week to about 2 months apart. In some ~~embodiment~~examples, any two consecutive doses of the two or more doses are administered about 1 week to about 1 month apart.

In some ~~embodiment~~examples, the one, two or more doses are administered by subcutaneous administration. In some ~~embodiment~~examples, the two or more doses are administered by intramuscular administration. In some ~~embodiment~~examples, the two or more doses are administered over a period of time of about 1 year to about 60 years. In some ~~embodiment~~examples, the two or more doses are administered over a period of time of about 1 year to about 50 years. In some ~~embodiment~~examples, the two or more doses are administered over a period of time of about 1 year to about 40 years. In some ~~embodiment~~examples, the two or more doses are administered over a period of time of about 1 year to about 30 years. In some ~~embodiment~~examples, the two or more doses are administered over a period of time of about 1 year to about 20 years. In some ~~embodiment~~examples, the two or more doses are administered over a period of time of about 1 year to about 10 years.

In some ~~embodiment~~examples, each of the two or more doses are administered at a dosage of about 0.01 mg of each common gamma-chain family cytokine receptor activating agent/kg to about 10 mg of each common gamma-chain family cytokine receptor activating agent/kg. In some ~~embodiment~~examples, each of the two or more doses are administered at a dosage of about 0.02 mg of each common gamma-chain family cytokine receptor activating agent/kg to about 5 mg of each common gamma-chain family cytokine receptor activating agent/kg.

In some ~~embodiment~~examples, a first dose of the one or more common gamma-chain family cytokine receptor activating agent(s) begins when the subject reaches an age of at least 30 years. In some ~~embodiment~~examples, a first dose of the one or more common gamma-chain family cytokine receptor activating agent(s) begins when the subject reaches an age of at least 40 years. In some ~~embodiment~~examples, a first dose of the one or more common

gamma-chain family cytokine receptor activating agent(s) begins when the subject reaches an age of at least 50 years. In some ~~embodiment~~examples, a first dose of the one or more common gamma-chain family cytokine receptor activating agent(s) begins when the subject reaches an age of at least 60 years.

In some ~~embodiment~~examples, the subject is not diagnosed or identified as having an aging-related disease or an inflammatory disease. In some ~~embodiment~~examples, the subject has not been previously treated with a chemotherapeutic agent. In some ~~embodiment~~examples, the subject has not been previously treated with a therapeutic agent that induces cellular senescence. In some ~~embodiment~~examples, the method further comprises administering to the subject at least one or more agent(s) that results in a decrease in the activation of a TGF-β receptor. In some ~~embodiment~~examples, the agent that results in a decrease in the activation of a TGF-β receptor is a soluble TGF-β receptor, an extracellular domain of TGF-β receptor, an antibody that binds specifically to TGF-β, an antagonistic antibody that binds to a TGF-β receptor, an agent that binds to a LAP, or an agent that binds to a TGF-β/LAP complex. In some ~~embodiment~~examples, the one or more agent(s) that result(s) in a decrease in the activation of a TGF-β decrease(s) the activation of a TGF-β receptor through binding to a LAP, or to a TGF-β/LAP complex.

In some ~~embodiment~~examples, the soluble human tissue factor domain does not initiate blood coagulation. In some ~~embodiment~~examples, the method further comprises administering an additional therapeutic agent selected from the group of: combinations of agents, such as checkpoint inhibitors, chemotherapy drugs, and therapeutic antibodies.

In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide is stable in human serum for at least 10 days at 37 °C. In some ~~embodiment~~examples of any of the methods described herein, the multi-chain chimeric polypeptide is stable in human serum for at least 10 days at 37 °C. In some ~~embodiment~~examples of any of the methods described herein, the single-chain chimeric polypeptide does not have significant clotting activity. In some ~~embodiment~~examples of any of the methods described herein, the multi-chain chimeric polypeptide does not have significant clotting activity.

In some ~~embodiment~~examples of any of the methods described herein, the method results in rejuvenation of aged immune cells in the subject. In some ~~embodiment~~examples of any of the

methods described herein, the rejuvenation of the aged immune cells results in a reduction of number of diseased cells or infectious agents in the subject. In some ~~embodiment~~examples of any of the methods described herein, the aged immune cells include one or more of aged NK cells, aged NKT cells, aged T cells, aged B cells, aged monocytes, aged macrophages, aged neutrophils, aged basophils, aged eosinophils, aged Kupffer cells, and aged microgial cells. In some ~~embodiment~~examples of any of the methods described herein, the diseased cells include cancer cells, virally-infected cells, and intracellularly-bacterially-infected cells. In some ~~embodiment~~examples of any of the methods described herein, the infectious agents include virus, bacterium, fungus, and parasite.

As used herein, the term "chimeric" refers to a polypeptide that includes amino acid sequences (e.g., domains) originally derived from two different sources (e.g., two different naturally-occurring proteins, e.g., from the same or different species). For example, a chimeric polypeptide can include domains from at least two different naturally occurring human proteins. In some examples, a chimeric polypeptide can include a domain that is a synthetic sequence (e.g., a scFv) and a domain that is derived from a naturally-occurring protein (e.g., a naturally-occurring human protein). In some ~~embodiment~~examples, a chimeric polypeptide can include at least two different domains that are synthetic sequences (e.g., two different scFvs).

An "activated NK cell" is a NK cell demonstrating increased expression levels of two or more (e.g., three, four, five, or six) of CD25, CD69, MTOR-C1, SREBP, IFN-γ, and a granzyme (e.g., granzyme B), e.g., as compared to a resting NK cell. Exemplary methods for identifying the expression levels of CD25, CD69, MTOR-C1, SREBP, IFN-γ, and a granzyme (e.g., granzyme B) are described herein.

A "resting NK cell" is a NK cell that has a reduced expression of two or more (e.g., three, four, five, or six) of CD25, CD69, MTOR-C1, SREBP, IFN-γ, and a granzyme (e.g., granzyme B), e.g., as compared to an activated NK cell.

An "NK cell activating agent" is an agent that induces or promotes (alone or in combination with additional NK cell activating agents) a resting NK cell to develop into an activated NK cell. Non-limiting examples and aspects of NK cell activating agents are described herein.

An "antigen-binding domain" is one or more protein domain(s) (e.g., formed from amino acids from a single polypeptide or formed from amino acids from two or more polypeptides (e.g., the same or different polypeptides) that is capable of specifically binding to one or more different antigen(s). In some examples, an antigen-binding domain can bind to an antigen or epitope with specificity and affinity similar to that of naturally-occurring antibodies. In some ~~embodiment~~examples, the antigen-binding domain can be an antibody or a fragment thereof. In some ~~embodiment~~examples, an antigen-binding domain can include an alternative scaffold. Non-limiting examples of antigen-binding domains are described herein. Additional examples of antigen-binding domains are known in the art.

A "soluble tissue factor domain" refers to a polypeptide having at least 70% identity (e.g., at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 99% identity, or 100% identical) to a segment of a wildtype mammalian tissue factor protein (e.g., a wildtype human tissue factor protein) that lacks the transmembrane domain and the intracellular domain. Non-limiting examples of soluble tissue factor domains are described herein.

The term "soluble interleukin protein" is used herein to refer to a mature and secreted interleukin protein or a biologically active fragment thereof. In some examples, a soluble interleukin protein can include a sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to a wildtype mature and secreted mammalian interleukin protein (e.g., a wildtype human interleukin protein) and retains its biological activity. Non-limiting examples of soluble interleukin proteins are described herein.

The term "soluble cytokine protein" is used herein to refer to a mature and secreted cytokine protein or a biologically active fragment thereof. In some examples, a soluble cytokine protein can include a sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to a wildtype mature and secreted mammalian interleukin protein (e.g., a wildtype human interleukin protein) and retains its

formed by a VH -VL dimer. Additional examples of an antibody are described herein. Additional examples of an antibody are known in the art.

"Affinity" refers to the strength of the sum total of non-covalent interactions between an antigen-binding site and its binding partner (e.g., an antigen or epitope). Unless indicated otherwise, as used herein, "affinity" refers to intrinsic binding affinity, which reflects a 1:1 interaction between members of an antigen-binding domain and an antigen or epitope. The affinity of a molecule X for its partner Y can be represented by the dissociation equilibrium constant (K_D). The kinetic components that contribute to the dissociation equilibrium constant are described in more detail below. Affinity can be measured by common methods known in the art, including those described herein. Affinity can be determined, for example, using surface plasmon resonance (SPR) technology (e.g., BIACORE®) or biolayer interferometry (e.g., FORTEBIO®). Additional methods for determining the affinity for an antigen-binding domain and its corresponding antigen or epitope are known in the art.

A "single-chain polypeptide" as used herein to refers to a single protein chain.

A "multi-chain polypeptide" as used herein to refers to a polypeptide comprising two or more (e.g., three, four, five, six, seven, eight, nine, or ten) protein chains (e.g., at least a first chimeric polypeptide and a second polypeptide), where the two or more proteins chains associate through non-covalent bonds to form a quaternary structure.

The term "pair of affinity domains" is two different protein domain(s) that bind specifically to each other with a K_D of less than of less than 1×10^{-7} M (e.g., less than 1×10^{-8} M, less than 1×10^{-9} M, less than 1×10^{-10} M, or less than 1×10^{-11} M). In some examples, a pair of affinity domains can be a pair of naturally-occurring proteins. In some ~~embodiment~~examples, a pair of affinity domains can be a pair of synthetic proteins. Non-limiting examples of pairs of affinity domains are described herein.

The term "epitope" means a portion of an antigen that specifically binds to an antigen-binding domain. Epitopes can, e.g., consist of surface-accessible amino acid residues and/or sugar side chains and may have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the

latter may be lost in the presence of denaturing solvents. An epitope may comprise amino acid residues that are directly involved in the binding, and other amino acid residues, which are not directly involved in the binding. Methods for identifying an epitope to which an antigen-binding domain binds are known in the art.

The term "treatment" means to ameliorate at least one symptom of a disorder. In some examples, the disorder being treated is cancer and to ameliorate at least one symptom of cancer includes reducing aberrant proliferation, gene expression, signaling, translation, and/or secretion of factors. Generally, the methods of treatment include administering a therapeutically effective amount of a composition that reduces at least one symptom of a disorder to a subject who is in need of, or who has been determined to be in need of such treatment.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this ~~invention~~disclosure belongs. Methods and materials are described herein for use in the present ~~invention~~disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the ~~invention~~ disclosure will be apparent from the following detailed description and figures, and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1A-1B show the results of immunostimulation of an exemplary multi-chain polypeptide in C57BL/6 mice. Figure 1A shows the spleen weight of mice treated with increasing dosage of the exemplary multi-chain polypeptide as compared to mice treated with the control solution. Figure 1B shows the percentages of immune cell types present in the spleen of mice treated with increasing dosage of the exemplary multi-chain polypeptide as compared to mice treated with the control solution.