

Additionally, the local immunosuppressive nature of the tumor microenvironment can lead to the escape of cancer cell subpopulations that do not express the target antigens. Thus, finding an approach that would promote the preservation and/or restoration of anti-tumor activities of the immune system would be of considerable therapeutic benefit.

5 Immune checkpoints have been implicated in the tumor-mediated downregulation of anti-tumor immunity. It has been demonstrated that T cell dysfunction occurs concurrently with an induced expression of the inhibitory receptors, CTLA-4 and programmed death 1 polypeptide (PD-1), members of the CD28 family receptors. Nevertheless, despite extensive research in recent years, the success of immunotherapy in a clinical setting has been limited.
10 Few therapeutic agents have been approved by regulatory authorities, and among those, the benefit is not experienced by a majority of patients. In recent years, immune checkpoints have been implicated in the downregulation of anti-tumor immunity and used as therapeutic targets. These observations highlight the need for development of novel therapeutic approaches for harnessing the immune system against cancer.

15 Human Flt3L (Fms-like tyrosine kinase 3 ligand), a type I transmembrane protein that stimulates the proliferation of bone marrow cells, was cloned for in 1994 (Lyman et al., 1994). The use of soluble hFlt3L has been explored in various preclinical and clinical settings including stem cell mobilization in preparation for bone marrow transplantation, cancer immunotherapy such as expansion of dendritic cells, as well as an vaccine adjuvant. Yet no
20 pharmaceutical composition based on Flt3L has progressed past Phase 2 in the clinic.

One challenge is to optimize exposure to the Flt3L ligand and identify an optimal dosing regimen while minimizing adverse side effects or potential unfavorable immunologic effects. Modulating exposure can be done, for example, by varying dosing regimens, varying dosing amounts, or changing the pharmacokinetic and/or pharmacodynamics properties of the
25 therapeutic molecule. Provided herein is a Flt3L-Fc fusion protein having beneficial PK/PD properties beneficial for enhancing immunotherapy for cancer patients.

SUMMARY

The ~~invention~~disclosure provides an effectorless Fc protein, fusion proteins comprising the effectorless Fc protein, including Flt3 ligand fusion proteins comprising an active Flt3
30 ligand (Flt3L) fused to the effectorless immunoglobulin Fc protein and methods of using the same. Methods for use of the effectorless Fc protein, fusion proteins comprising the effectorless Fc protein, and Flt3L-Fc fusion proteins include treatment of cancer, especially treatment of

cancer in patients receiving checkpoint immunotherapy. The ~~invention~~disclosure further provides an effectorless IgG Fc protein.

In one aspectexample, an effectorless Fc protein is provided, comprising an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to residues SEQ ID NO:13, wherein residues 13-17 of SEQ ID NO:13 comprise the amino acid sequence PVAGP (SEQ ID NO:20) or residue 76 of SEQ ID NO:13 is a glycine. In other embodimentexamples, the effectorless IgG1 Fc region comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:13, wherein residues 13-17 of SEQ ID NO:13 comprise the amino acid sequence PVAGP (SEQ ID NO:20) and residue 76 of SEQ ID NO:13 is a glycine. In still other embodimentexamples, the effectorless IgG1 Fc region comprises an amino acid sequence which is identical to SEQ ID NO:13. For clarity, in some embodimentexamples, wherein residues 13-17 of SEQ ID NO:13 comprise PVAGP (SEQ ID NO:20), this may be referred to herein as encompassing the PVA# variant, and wherein residue 76 of SEQ ID NO:13 is a glycine, this may be referred to herein as the N297G mutations, wherein 297 refers to EU numbering of antibodies.

In some embodimentexamples, an effectorless Fc protein comprises the protein sequence of SEQ ID NO: 2, 4, 5, 6, 13, or 15.

In still other embodimentexamples, the effectorless IgG1 Fc protein is attenuated relative to a wildtype IgG1 Fc region which comprises SEQ ID NO:12.

In some aspectexamples, an antibody is provided comprising the effectorless Fc protein which comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to residues SEQ ID NO:13, wherein residues 13-17 of SEQ ID NO:13 comprise the amino acid sequence PVAGP (SEQ ID NO:20) or residue 76 of SEQ ID NO:13 is a glycine.

In some embodimentexamples, the antibody binds to the Flt3L receptor.

In some embodimentexamples, the antibody binds to a checkpoint inhibitor protein. In other embodimentexamples, the checkpoint inhibitor protein is PD-L1, PD-1, and/or CTLA-4. In some embodimentexamples, the antibody is a bispecific antibody.

In some aspectexamples, a heterodimeric protein is provided comprising the effectorless Fc protein of the present disclosure and a second protein. In some embodimentexamples, the effectorless Fc protein and the second protein are covalently linked to each other. In other embodimentexamples, the effectorless Fc protein and the second protein are linked by a disulfide bond.

In some aspectexamples, an isolated nucleic acid encoding an effectorless Fc protein of the present disclosure, an antibody that comprises the effectorless Fc protein, and/or the heterodimeric protein comprising the effectorless Fc protein as described herein and above is

provided. In some embodimentexamples, the isolated nucleic acid encodes the protein sequence of SEQ ID NO: 2, 4, 5, 6, 13, or 15. In other embodimentexamples, the isolated nucleic acid further encodes a signal sequence at the N-terminal end of the effectorless Fc protein. In a preferred example, the isolated nucleic acid encodes the protein sequence of SEQ ID NO:13.

In some aspectexamples, a host cell comprising a nucleic acid encoding the effectorless Fc protein of the present disclosure, a fusion protein comprising the effectorless Fc protein, an antibody that comprises the effectorless Fc protein, and/or the heterodimeric protein comprising the effectorless Fc protein as described herein and above is provided.

In some aspectexamples a method of producing an effectorless Fc protein of the present disclosure, a fusion protein comprising the effectorless Fc protein, an antibody that comprises the effectorless Fc protein, and/or the heterodimeric protein comprising the effectorless Fc protein is provided, comprising culturing the host cell comprising a nucleic acid encoding the effectorless Fc protein of the present disclosure, a fusion protein comprising the effectorless Fc protein, an antibody that comprises the effectorless Fc protein, and/or the heterodimeric protein comprising the effectorless Fc protein so that each of the proteins is produced. In some embodimentexamples, the method further comprises recovering the effectorless Fc protein of the present disclosure, a fusion protein comprising the effectorless Fc protein, an antibody that comprises the effectorless Fc protein, and/or the heterodimeric protein comprising the effectorless Fc protein from the host cell. In some embodimentexamples, the host cell is a eukaryotic cell or a prokaryotic cell. In other embodimentexamples, the eukaryotic cell is a CHO cell.

In some aspectexamples, a host cell comprising a nucleic acid encoding an effectorless Fc protein of the present disclosure, a fusion protein comprising the effectorless Fc protein, an antibody that comprises the effectorless Fc protein, and/or the heterodimeric protein comprising the effectorless Fc protein is provided.

In some aspectexamples a method of producing an effectorless Fc protein of the present disclosure, a fusion protein comprising the effectorless Fc protein, an antibody that comprises the effectorless Fc protein, and/or the heterodimeric protein comprising the effectorless Fc protein is provided, comprising culturing the host cell comprising a nucleic acid encoding the effectorless Fc protein of the present disclosure, a fusion protein comprising the effectorless Fc protein, an antibody that comprises the effectorless Fc protein, and/or the heterodimeric protein comprising the effectorless Fc protein so that the effectorless Fc protein of the present disclosure, a fusion protein comprising the effectorless Fc protein, an antibody that comprises the effectorless Fc protein, and/or the heterodimeric protein comprising the effectorless Fc protein is produced. In some embodimentexample, the method further comprises recovering the

effectorless Fc protein of the present disclosure, a fusion protein comprising the effectorless Fc protein, an antibody that comprises the effectorless Fc protein, and/or the heterodimeric protein comprising the effectorless Fc protein from the host cell. In some embodimentexamples, the host cell is a eukaryotic cell or a prokaryotic cell. In other embodimentexamples, the eukaryotic cell is a CHO cell.

In one aspectexample, a fusion protein comprising the effectorless Fc protein is provided comprising the effectorless Fc protein (SEQ ID NO:13 or variant thereof) and a second protein, wherein the second protein is a ligand to a target protein. In some embodimentexamples, the ligand modulates the target protein upon binding of the ligand to the target protein. In some embodimentexamples, the second protein is a Flt3L. In other embodimentexample, the target protein is the Flt3L receptor. In preferred embodimentexample, the second protein is N-terminal to the effectorless Fc protein.

In some embodimentexamples, the fusion protein comprises a Fc protein which is at least about 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:13, wherein residues 13-17 of SEQ ID NO:13 comprise the amino acid sequence PVAGP (SEQ ID NO:20) or residue 76 of SEQ ID NO:13 is a glycine. In one aspect, the disclosure provides a Fc fusion protein comprising an effectorless Fc protein which comprises a Flt3 ligand (Flt3L) protein and the effectorless Fc protein, wherein the effectorless Fc protein is at least 95%, 96%, 97%, 98%, or 99% identical to residues SEQ ID NO:13, and wherein residues 13-17 of SEQ ID NO:13 comprise the amino acid sequence PVAGP (SEQ ID NO:20) and residue 76 of SEQ ID NO:13 is a glycine. In other embodimentexamples, the effectorless IgG1 Fc region comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:13, wherein residues 13-17 of SEQ ID NO:13 comprise the amino acid sequence PVAGP (SEQ ID NO:20) and residue 76 of SEQ ID NO:13 is a glycine. In still other embodimentexamples, the effectorless IgG1 Fc region comprises an amino acid sequence which is identical to SEQ ID NO:13. For clarity, in some embodimentexamples, wherein residues 13-17 of SEQ ID NO:13 comprise PVAGP (SEQ ID NO:20), this may be referred to herein as encompassing the PVA# variant, and wherein residue 76 of SEQ ID NO:13 is a glycine, this may be referred to herein as the N297G mutations, wherein 297 refers to EU numbering of antibodies.

In one aspectexample, the fusion protein comprises a Flt3 ligand (Flt3L) and an effectorless IgG1 Fc region, wherein the Flt3L-Fc fusion protein has an effector function which is attenuated relative to a wildtype IgG1 Fc region which comprises SEQ ID NO:12.

In some embodimentexamples of the Flt3L-Fc fusion protein, the Flt3L comprises a protein comprising an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to residues 27-167 of SEQ ID NO:21. In other embodimentexamples, the Flt3L comprises the amino acid sequence of SEQ ID NO:21, wherein the amino acid sequence has 1, 2, or 3 amino acid substitutions. In other embodimentexamples, the 1, 2, or 3 amino acid substitutions are not present in a region of the Flt3L that binds to an FcRn protein. In other embodimentexample, the Flt3L protein comprises the sequence of SEQ ID NO:22.

In some embodimentexamples of the Flt3L-Fc fusion protein, the Flt3L comprises a protein comprising amino acids 27-167, 27-168, 27-169, 27-170, 27-171, 27-172, 27-173, 27-174, 27-175, 27-176, 27-177, 27-178, 27-179, 27-180, 27-181, 27-182, 27-183, 27-184, or 27-185 of SEQ ID NO:21. In preferred embodimentexamples of the Flt3L-Fc fusion protein, the Flt3L comprises an amino acid sequence consisting of amino acids 27-167 of SEQ ID NO:21 or amino acids 27-168 of SEQ ID NO:21. In other embodimentexamples, the Flt3L of the Flt3L-Fc fusion protein does not comprise 168-235, 169-235, 170-235, 171-235, 172-235, 173-235, of SEQ ID NO:21. In still other embodimentexamples, the Flt3L of the Flt3L-Fc fusion protein does not comprise the amino acid sequence PWSRPLEATAPTAPQPP (SEQ ID NO:48), WSPRPLEATAPTAPQPP (SEQ ID NO:49), SPRPLEATAPTAPQPP (SEQ ID NO:50), PRPLEATAPTAPQPP (SEQ ID NO:51), RPLEATAPTAPQPP (SEQ ID NO:52), or PLEATAPTAPQPP (SEQ ID NO:53).

In some embodimentexamples of the Flt3L-Fc fusion protein, the Flt3L comprises a protein which is at least about 95%, 96%, 97%, 98%, or 99% identical to a protein comprising amino acids 27-167, 27-168, 27-169, 27-170, 27-171, 27-172, 27-173, 27-174, 27-175, 27-176, 27-177, 27-178, 27-179, 27-180, 27-181, 27-182, 27-183, 27-184, or 27-185 of SEQ ID NO:21.

In some embodimentexamples of the Flt3L-Fc fusion protein, the Flt3L comprises a protein which is at least about 95%, 96%, 97%, 98%, 99%, or 100% identical to a protein comprising amino acids 24-167, 25-167, 26-167, 24-168, 25-168, 26-168, 24-169, 25-169, or 26-169 of SEQ ID NO:21.

In some embodimentexamples of the Flt3L-Fc fusion protein, the effectorless IgG1 Fc region comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:13, wherein residues 13-17 of SEQ ID NO:13 comprise the amino acid sequence PVAGP (SEQ ID NO:20) or residue 76 of SEQ ID NO:13 is a glycine. In other embodimentexamples, the effectorless IgG1 Fc region comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:13, wherein residues 13-17 of SEQ ID NO:13 comprise the amino acid sequence PVAGP (SEQ ID NO:20) and residue 76 of SEQ ID NO:13 is a glycine. In still other embodimentexamples, the effectorless IgG1 Fc region comprises an amino acid sequence which is identical to SEQ ID NO:13. For clarity, in some embodimentexamples, wherein residues 13-17 of SEQ ID NO:13 comprise PVAGP (SEQ ID NO:20), this may be referred to herein as encompassing the PVA# variant, and wherein residue 76 of SEQ ID NO:13 is a glycine, this may be referred to herein as the N297G mutations, wherein 297 refers to EU numbering of antibodies.

In some embodimentexamples, the Flt3L-Fc fusion protein comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:26. In other

embodimentexamples, the Flt3L-Fc fusion protein comprises the amino acid sequence of SEQ ID NO:26.

In some embodimentexamples, the Flt3L-Fc fusion protein comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:26, wherein residues 154-158 of SEQ ID NO:26 comprise the amino acid sequence PVAGP (SEQ ID NO:20) and residue 217 of SEQ ID NO:26 is a glycine.

In some embodimentexamples, the Flt3L-Fc fusion protein comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:25, wherein residues 155-159 of SEQ ID NO:25 comprise the amino acid sequence PVAGP (SEQ ID NO:20) and residue 218 of SEQ ID NO:25 is a glycine.

In some embodimentexamples, the Flt3L-Fc fusion protein comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:32, wherein residues 153-157 of SEQ ID NO:32 comprise the amino acid sequence PVAGP (SEQ ID NO:20) and residue 216 of SEQ ID NO:32 is a glycine.

In some embodimentexamples, the Flt3L-Fc fusion protein comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:33, wherein residues 152-156 of SEQ ID NO:33 comprise the amino acid sequence PVAGP (SEQ ID NO:20) and residue 215 of SEQ ID NO:33 is a glycine.

In some embodimentexamples, the Flt3L-Fc fusion protein comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:34, wherein residues 151-154 of SEQ ID NO:34 comprise the amino acid sequence PVAGP (SEQ ID NO:20) and residue 214 of SEQ ID NO:34 is a glycine.

In some embodimentexamples, the Flt3L-Fc fusion protein comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, 99%, or 100% identical to a protein selected from the group consisting of SEQ ID NO:27 to SEQ ID NO:31 and SEQ ID NO:35 to SEQ ID NO:44.

In some embodimentexamples, the Flt3L-Fc fusion protein activates antibody-dependent cellular phagocytosis (ADCP) in an in vitro assay at an activity level that is less than about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the activity level by a Flt3L-Fc fusion protein comprising wildtype Flt3L fused to wildtype IgG1 Fc (SEQ ID NO:12) in the in vitro assay. In other embodimentexamples, ADCP in vitro assay comprises use of primary macrophages as effector cells and a cell line that expresses the Flt3L receptor as target cells. In still other embodimentexamples, the primary macrophages are monocyte-derived macrophages from healthy human donors. In yet other embodimentexamples, the target cells are human acute lymphoblastic leukemia cells, optionally, SEM cell line cells. In

some embodimentexamples, the activity is measured as percent phagocytosis (% ADCP), wherein phagocytosis is determined by measuring uptake of a visual marker from the target cells.

In some embodimentexamples, the Flt3L-Fc fusion protein activates DC expansion in a subject that has been administered the fusion protein. In other embodimentexamples, the DC expansion is measured in the whole blood of the subject. In still other embodimentexamples, the DC expansion is measured by flow cytometry or FACS. In some embodimentexamples, the DC expansion is at least 5-fold, 10-fold, 15-fold, 20-fold, 50-fold, 100-fold, 500-fold, 1000-fold, 2000-fold, 5000-fold, or 10,000-fold greater than the DC expansion resulting from administration of a Flt3L protein which is not fused to a heterologous protein. In other embodimentexamples, the heterologous protein is a wildtype human IgG Fc protein. In yet other embodimentexamples, the wildtype human IgG Fc protein is a wildtype IgG1, IgG2, IgG3 or IgG4 Fc protein. In some embodimentexamples, the Flt3L protein is not fused to a wildtype IgG1 Fc protein or to a human serum albumin protein. In some embodimentexamples, the subject is a rodent, a rabbit, a cynomolgus monkey, or a human. In other embodimentexamples, the rodent is a mouse or a rat.

In some embodimentexamples, the Flt3L-Fc fusion protein has an increased thermostability as compared to the thermostability of a Flt3L-Fc fusion protein having SEQ ID NO:14. In other embodimentexamples, the thermostability is measured using differential scanning fluorimetry. In still other embodimentexamples, the Flt3L-Fc fusion protein has a melting temperature (T_m) which is at least 1.5°C, 2°C, 3°C, 4°C or 5°C greater than the T_m of the Flt3L-Fc fusion protein having SEQ ID NO:14.

In some embodimentexamples, the Flt3L-Fc fusion protein contains a single amino acid substitution in the Flt3L domain. In other embodimentexamples, the substitution reduces in vivo immunogenicity but does not reduce functional activity by more than 10%, 20%, or 30%. In other embodimentexamples, the functional activity assay is the expansion of dendritic cells in an animal.

In some aspectexamples, an isolated nucleic acid encoding an Flt3L-Fc fusion protein as described herein and above is provided. In one aspect, the disclosure provides an isolated nucleic acid encoding the Fc fusion protein as disclosed herein. In some embodimentexamples, the isolated nucleic acid encodes the protein sequence of SEQ ID NO: 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, or 44. In other embodimentexamples, the isolated nucleic acid further encodes a signal sequence which is at the N-terminal end of the Flt3L-Fc fusion protein. In a preferred example, the isolated nucleic acid encodes the protein sequence of SEQ ID NO:45. In one aspect, the disclosure provides an isolated nucleic acid encoding the Flt3L-Fc fusion protein, wherein the Flt3L-Fc fusion protein comprises SEQ ID NO:45.

In some aspectexamples, a host cell comprising a nucleic acid encoding a Flt3L-Fc fusion protein as described herein and above is provided. In one aspect, the disclosure provides a host cell comprising the nucleic acid as disclosed herein.

In some aspectexamples a method of producing a Flt3L-Fc fusion protein is provided, comprising culturing the host cell comprising a nucleic acid encoding the effectorless Flt3L-Fc fusion protein so that the Flt3L-Fc fusion protein is produced. In some embodimentexamples, the method further comprises recovering the Flt3L-Fc fusion protein from the host cell. In one aspect, the disclosure provides a method of producing the Fc fusion protein comprising culturing the host cell as disclosed herein. In some embodimentexamples, the host cell is a eukaryotic cell or a prokaryotic cell. In other embodimentexamples, the eukaryotic cell is a CHO cell.

In some aspectexamples, a pharmaceutical formulation is provided, comprising a Flt3L-Fc fusion protein as described herein and a pharmaceutically acceptable carrier. In one aspect, the disclosure provides a pharmaceutical formulation comprising the Fc fusion protein as disclosed herein and a pharmaceutically acceptable carrier.

In some embodimentexamples, the pharmaceutical formulation further comprises an additional therapeutic agent. In still other embodimentexamples, the additional pharmaceutical agent is an adjuvant, a dendritic cell maturation factor, and/or a checkpoint inhibitor.

In one aspect, the disclosure provides use of the Flt3L-Fc fusion protein as disclosed herein in the manufacture of a medicament for expanding the number of dendritic cells (DCs) in a subject.

In some aspectexamples, a method for expanding the number of dendritic cells (DCs) in a subject administered the Flt3L-Fc fusion protein is provided. In some embodimentexamples, the DCs are cDC1 and/or cDC2 cells. In other embodimentexamples, the method comprises administering to the subject a Flt3L-Fc fusion protein of the present inventiondisclosure.

In some embodimentexamples, the method comprises administering to the subject a dose of about 0.1 mg/kg to 50 mg/kg, 0.1 mg/kg to 40 mg/kg, 0.1 mg/kg to 25 mg/kg, 0.1 mg/kg to 20 mg/kg, 0.1 mg/kg to 15 mg/kg, 0.1 mg/kg to 10 mg/kg, 1 mg/kg to 50 mg/kg, 1 mg/kg to 40 mg/kg, 1 mg/kg to 25 mg/kg, 1 mg/kg to 20 mg/kg, 1 mg/kg to 15 mg/kg, or 1 mg/kg to 10 mg/kg of the Flt3L-Fc fusion protein.

In some embodimentexamples, the method comprises administering to the subject the Flt3L fusion protein about once per day, once per week, twice per week, once every 2 weeks, once every 3 weeks, once per month, or once every other month.

In some embodimentexamples, the method comprises administering to the subject the Flt3L fusion protein by intravenous administration, parenteral administration, intramuscular injection, or subcutaneous injection.

In one aspect, the disclosure provides use of the Flt3L-Fc fusion protein as disclosed herein or the pharmaceutical formulation as disclosed herein, in the manufacture of a medicament for treating a cancer.

In some aspectexamples, a method for treating a cancer is provided, wherein the method comprises administering to a subject in need thereof a Flt3L-Fc fusion protein as described herein. In some embodimentexamples, the method comprising administering a therapeutically effective amount of the Flt3L-Fc fusion protein and administering a therapeutically effective amount of an immune checkpoint inhibitor. In other embodimentexamples, the immune checkpoint inhibitor suppresses or inhibits the effects of PD-1 or PD-L1. In still other embodimentexamples, the immune checkpoint inhibitor is pembrolizumab, nivolumab, pidilizumab, BMS 936559, atezolizumab,

or avelumab. In other embodimentexamples, the checkpoint inhibitor is administered before, simultaneously with, or after administration of the Flt3L-Fc fusion protein.

In some embodimentexamples, the method further comprises administering a dendritic cell (DC) maturation factor. In other embodimentexamples, the DC maturation factor is selected from polyIC, poly-ICLC, a CD40 agonist, radiation therapy, and chemotherapy. In other embodimentexamples, the DC maturation factor is administered to the individual prior to, simultaneously with, and/or subsequent to administering the Flt3L-Fc fusion protein. In particular embodimentexamples, the DC maturation factor is radiation therapy. In some embodimentexamples, the method comprises administering the Flt3L-Fc fusion protein to the subject after the subject has received the DC maturation factor. In other embodimentexamples, the method comprises administering the Flt3L-Fc fusion protein to the subject before the subject has received the DC maturation factor. In still other embodimentexamples, method comprises administering the Flt3L-Fc fusion protein to the subject at about the same time that the subject receives the DC maturation factor

In some embodimentexamples, the cancer is selected from the group consisting of non small cell lung cancer (NSCLC), small cell lung cancer (SCLC), melanoma, Pancreatic ductal adenocarcinoma, (PDAC), triple negative breast cancer (TNBC), non-Hodgkin lymphoma (NHL), colorectal cancer (CRC), breast cancer, bladder cancer, kidney cancer, or a combination thereof.

In some embodimentexamples, the subject was previously treated with a checkpoint inhibitor. In other embodimentexamples, the subject did not respond to the checkpoint inhibitor therapy, wherein the checkpoint inhibitor therapy was administered over a period of at least or about 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 1 year, 1.5 years, or 2.

In some embodimentexamples, the subject was treated with radiation therapy, wherein the radiation therapy was administered over a period of at least or about 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 1 year, 1.5 years, or 2 years.

In some embodimentexamples, the subject was treated with chemotherapy, wherein the chemotherapy was administered over a period of at least or about 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 1 year, 1.5 years, or 2 years.

In some embodimentexamples, the cancer in the subject has been characterized as comprising an immune desert tumor. In other embodimentexamples, the cancer has been characterized as comprising a tumor with reduced inflammation (“cold tumor”) relative to a responsive, inflamed tumor (“hot tumor”).

WHAT IS CLAIMED IS:

1. A Fc fusion protein comprising an effectorless Fc protein which comprises a Flt3 ligand (Flt3L) protein and the effectorless Fc protein, wherein the effectorless Fc protein is at least 95%, 96%, 97%, 98%, or 99% identical to residues SEQ ID NO:13, and wherein residues
5 13-17 of SEQ ID NO:13 comprise the amino acid sequence PVAGP (SEQ ID NO:20) and residue 76 of SEQ ID NO:13 is a glycine.
2. The Fc fusion protein of claim 1, wherein the Flt3L protein does not comprise the amino acid sequence of PWSPRPLEATAPTAPQPP (SEQ ID NO:48), WSPRPLEATAPTAPQPP (SEQ ID NO:49), SPRPLEATAPTAPQPP (SEQ ID NO:50),
10 PRPLEATAPTAPQPP (SEQ ID NO:51), RPLEATAPTAPQPP (SEQ ID NO:52), or PLEATAPTAPQPP (SEQ ID NO:53).
3. The Fc fusion protein of claim 1 or 2, wherein the Flt3L comprises a protein comprising an amino acid sequence which is at least 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO:22.
- 15 4. The Fc fusion protein of any one of claims 1 to 3, wherein the Flt3L comprises a protein comprising the amino acid sequence of SEQ ID NO:22.
5. The Fc fusion protein of any one of claims 1 to 4, wherein the Flt3L comprises a protein which is at least about 90% identical to a protein comprising amino acids 27-167, 27-168, 27-169, 27-170, 27-171, 27-172, 27-173, 27-174, 27-175, 27-176, 27-177, 27-178, 27-
20 179, 27-180, 27-181, 27-182, 27-183, 27-184, or 27-185 of SEQ ID NO:21.
6. The Fc fusion protein of any one of claims 1 to 5, wherein the N-terminus of the effectorless Fc protein is linked to the C-terminus of the Flt3L protein via a peptide bond.
7. The Fc fusion protein of any one of claims 1 to 6, wherein the fusion protein comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, 99%, or 100% identical to
25 SEQ ID NO:26 and wherein the fusion protein comprises an amino acid sequence identical to SEQ ID NO:13.
8. The Fc fusion protein of any one of claims 1 to 7, wherein the Fc fusion protein has an Fc effector function which is attenuated relative to the Fc effector function of a wildtype IgG1 Fc region which comprises SEQ ID NO:12.
- 30 9. The Fc fusion protein of any one of claims 1 to 8, wherein the the Fc-effectorless Flt3L-Fc fusion protein activates antibody-dependent cellular phagocytosis (ADCP) in an in vitro assay at an activity level which is nor more than 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the activity level by a Flt3L-Fc fusion protein comprising wildtype Flt3L fused to wildtype IgG1 Fc (SEQ ID NO:12) in the in
35 vitro assay.

10. An isolated nucleic acid encoding the Fc fusion protein of any one of claims 1 to 9.
11. The isolated nucleic acid according to claim 10, wherein the isolated nucleic acid further encodes a signal sequence at the N-terminal end of the Fc fusion protein.
12. An isolated nucleic acid encoding the Flt3L-Fc fusion protein, wherein the Flt3L-Fc fusion protein comprises SEQ ID NO:45.
13. A host cell comprising the nucleic acid according to any one of claims 10-12.
14. A method of producing the Fc fusion protein comprising culturing the host cell of claim 13.
15. The method of claim 14, wherein the host cell is a eukaryotic cell or a prokaryotic cell.
16. The method of claim 14 or 15, wherein the host cell is *E. coli*.
17. The method of claim 14 or 15, wherein the host cell is a CHO cell.
18. A pharmaceutical formulation comprising the Fc fusion protein of any one of claims 1-8 and a pharmaceutically acceptable carrier.
19. The pharmaceutical formulation of claim 18, further comprising a second therapeutic agent.
20. The pharmaceutical formulation of claim 18 or 19, wherein the second therapeutic agent is an adjuvant, a dendritic maturation factor, and/or a checkpoint inhibitor.
21. Use of the Flt3L-Fc fusion protein of any one of claims 1-9 in the manufacture of a medicament ~~A method~~ for expanding the number of dendritic cells (DCs) in a subject, ~~comprising administering to the subject the Flt3L-Fc fusion protein of any one of claims 1-9.~~
22. The ~~method-use~~ of claim 21, wherein the Flt3L-Fc fusion protein is to be administered ~~comprising administering~~ to the subject at a dose of about 0.1 mg/kg to 50 mg/kg ~~of the Flt3L-Fc fusion protein.~~
23. Use of the Flt3L-Fc fusion protein of any one of claims 1 to 9 or the pharmaceutical formulation of any one of claims 18 to 20, in the manufacture of a medicament ~~A method~~ for treating a cancer, ~~comprising administering to a subject in need thereof the Flt3L-Fc fusion protein of any one of claims 1 to 9 or the pharmaceutical formulation of any one of claims 18 to 20.~~
24. The ~~method-use~~ of claim 23, wherein the Flt3L-Fc fusion protein is to be administered ~~comprising administering~~ to the subject at a dose of about 0.1 mg/kg to 50 mg/kg ~~of the Flt3L-Fc fusion protein.~~
25. The ~~method-use~~ of claim 23 or 24, wherein the medicament is to be administered ~~further comprising administering to the subject with~~ a second therapeutic agent.
26. The ~~method-use~~ of claim 25, wherein the second therapeutic agent is an adjuvant, a dendritic maturation factor, and/or a checkpoint inhibitor.

27. The ~~method-use~~ of any one of claims 21 to 26, wherein the medicament is to be administered with method-comprises-administering a DC maturation factor.
28. The ~~method-use~~ of claim 27, wherein the DC maturation factor is selected from polyIC, polyICLC, DC40, radiation therapy, and chemotherapy.
- 5 29. The ~~method-use~~ of any one of claims 21 to 28, wherein the medicament is to be administered with comprising-administering an immune checkpoint inhibitor.
30. The ~~method-use~~ of claim 29, wherein the checkpoint inhibitor is to be administered before, simultaneously with, or after administration of the Fc-effectorless Flt3L-Fc fusion protein.
- 10 31. The ~~method-use~~ of claim 29 or 30, wherein the immune checkpoint inhibitor is selected from the group consisting of pembrolizumab, nivolumab, pidilizumab, BMS 936559, atezolizumab, and avelumab.
32. The ~~method-use~~ of any one of claims ~~21-23~~ to 31, wherein the cancer is selected from the group consisting of non small cell lung cancer (NSCLC), small cell lung cancer (SCLC),
15 melanoma, Pancreatic ductal adenocarcinoma, (PDAC), triple negative breast cancer (TNBC), non-Hodgkin lymphoma (NHL), colorectal cancer (CRC), breast cancer, bladder cancer, kidney cancer, or a combination thereof.
33. The ~~method-use~~ of any one of claims 21-32, wherein the subject was previously treated with a checkpoint inhibitor.
- 20 34. The ~~method-use~~ of claim 33, wherein the subject did not respond to the checkpoint inhibitor therapy and/or did not respond to the checkpoint inhibitor therapy for longer than 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 1 year, 1.5 years, or 2 years after receiving a last dose of checkpoint immunotherapy.
35. The ~~method-use~~ of any one of claims ~~21-23~~-34, wherein the cancer in the subject has
25 been characterized as comprising an immune desert tumor.
- ~~36.—An effectorless Fc protein, comprising an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to residues SEQ ID NO:13, wherein residues 13-17 of SEQ ID NO:13 comprise the amino acid sequence PVAGP (SEQ ID NO:20) or residue 76 of SEQ ID NO:13 is a glycine.~~
- 30 ~~37.—The effectorless Fc protein of claim 36, wherein residues 13-17 of SEQ ID NO:13 comprise the amino acid sequence PVAGP (SEQ ID NO:20) and residue 76 of SEQ ID NO:13 is a glycine.~~
- ~~38.—The effectorless Fc protein of claim 36 or 37, which comprises an amino acid sequence identical to SEQ ID NO:13.~~

39. — ~~An effectorless Fc protein which comprises an amino acid sequence identical to SEQ ID NO: 2, 4, 5, 6, 13, or 15.~~
40. — ~~The effectorless Fc protein according to any one of claim 36 to 39, wherein the Fc protein is attenuated relative to a wildtype IgG1 Fc region which comprises SEQ ID NO: 12.~~
- 5 41. — ~~An antibody which comprises the effectorless Fc protein of any one of claims 1-9.~~
42. — ~~The antibody of claim 40, which comprises a heavy chain variable domain and a light chain variable domain.~~
43. — ~~The antibody of claim 41 or 42, wherein the antibody is a monoclonal antibody.~~
44. — ~~The antibody of claim 41 or 42, wherein the antibody is a human antibody, a~~
- 10 ~~humanized antibody, or a chimeric antibody.~~
45. — ~~The antibody of claim 41 or 42, wherein the antibody is a bispecific or multispecific antibody.~~