

FLT3 LIGAND FUSION PROTEINS AND METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to and the benefit of U.S. provisional patent application serial number 63/062,713, filed on August 7, 2020, the contents of which are incorporated
5 herein by reference in their entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII copy, created on July 28, 2021, is hereby incorporated by reference in its entirety, is named P36298-WO_SL.txt, and is 128,413 bytes in size.

10

FIELD OF THE INVENTION

The present invention relates to Flt3 ligand fusion proteins and methods of using the same.

BACKGROUND

Numerous studies support the importance of the differential presence of immune
15 system components in cancer progression (Jochems and Schlom, Exp Biol Med, 236(5): 567-579 (2011)). Clinical data suggest that high densities of tumor-infiltrating lymphocytes are linked to improved clinical outcome (Mlecnik et al., Cancer Metastasis Rev.; 30: 5-12, (2011)). Tumor immune infiltrates include macrophages, dendritic cells (DC), mast cells, natural killer (NK) cells, nave and memory lymphocytes, B cells and effector T cells (T lymphocytes),
20 primarily responsible for the recognition of antigens expressed by tumor cells and subsequent destruction of the tumor cells by T cells.

Despite presentation of antigens by cancer cells and the presence of immune cells that could potentially react against tumor cells, in many cases the immune system does not get activated or is affirmatively suppressed. Tumors develop a number of immunomodulatory
25 mechanisms to evade antitumor immune responses. The overall result is impaired T-cell responses and induction of apoptosis or reduced anti-tumor immune capacity of CD8⁺ cytotoxic T cells. Inhibition of antigen-presenting functions and dendritic cell (DC) additionally contributes to the evasion of anti-tumor immunity (Gerlini et al. Am. J. Pathol. 165(6), 1853-1863 (2004)).

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 provides an effectorless Fc protein, fusion proteins comprising the effectorless Fc protein, including Flt3 ligand fusion proteins comprising an active Flt3 ligand
30 (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 further provides an effectorless IgG Fc protein.

In one aspect, 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 embodiments, 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 embodiments, the effectorless IgG1 Fc region comprises an amino acid sequence which is identical to SEQ ID NO:13. For clarity, in some embodiments, 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 embodiments, an effectorless Fc protein comprises the protein sequence of SEQ ID NO: 2, 4, 5, 6, 13, or 15.

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

In some aspects, 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 embodiments, the antibody binds to the Flt3L receptor.

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

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

In some aspects, 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 embodiments, the isolated nucleic acid encodes the protein sequence of SEQ ID NO: 2, 4, 5, 6, 13, or 15. In other embodiments, 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.

5 In some aspects, 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 proteins as described herein and above is provided.

 In some aspects a method of producing an effectorless Fc protein of the present
10 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
15 comprising the effectorless Fc protein so that each of the proteins is produced. In some embodiments, 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
20 effectorless Fc protein from the host cell. In some embodiments, the host cell is a eukaryotic cell or a prokaryotic cell. In other embodiments, the eukaryotic cell is a CHO cell.

 In some aspects, 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.

25 In some aspects 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
30 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 embodiments, 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 embodiments, the host cell is a eukaryotic cell or a prokaryotic cell. In other embodiments, the eukaryotic cell is a CHO cell.

5 In one aspect, 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 embodiments, the ligand modulates the target protein upon binding of the ligand to the target protein. In some
10 Fc protein. In preferred embodiments, the second protein is N-terminal to the effectorless Fc protein.

In some embodiments, 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
15 NO:13 is a glycine. In other embodiments, 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 embodiments, the
20 NO:13. For clarity, in some embodiments, 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 aspect, the fusion protein comprises a Flt3 ligand (Flt3L) and an effectorless
25 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 embodiments 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 embodiments, the Flt3L comprises the amino acid
30 sequence of SEQ ID NO:21, wherein the amino acid sequence has 1, 2, or 3 amino acid substitutions. In other embodiments, 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 embodiments, the Flt3L protein comprises the sequence of SEQ ID NO:22.

In some embodiments 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 embodiments of the Flt3L-Fc fusion protein, the Flt3L comprises
 5 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 embodiments, 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 embodiments, the Flt3L of the Flt3L-Fc fusion protein does not comprise the amino acid sequence PWSPRPLEATAPTAPQPP (SEQ ID NO:48), WSPRPLEATAPTAPQPP (SEQ ID
 10 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 embodiments 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-
 15 177, 27-178, 27-179, 27-180, 27-181, 27-182, 27-183, 27-184, or 27-185 of SEQ ID NO:21.

In some embodiments 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 embodiments 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 embodiments, the effectorless IgG1 Fc region comprises an amino acid sequence which is at least 95%, 96%,
 25 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 embodiments, the effectorless IgG1 Fc region comprises an amino acid sequence which is identical to SEQ ID NO:13. For clarity, in some embodiments, wherein residues 13-17 of SEQ ID NO:13 comprise PVAGP (SEQ ID NO:20), this may be referred to
 30 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 embodiments, 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

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

In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the primary macrophages are monocyte-derived macrophages from healthy human donors. In yet other embodiments, the target cells are human acute lymphoblastic leukemia cells, optionally, SEM cell line cells. In

some embodiments, 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 embodiments, the Flt3L-Fc fusion protein activates DC expansion in a subject that has been administered the fusion protein. In other embodiments, the DC expansion is measured in the whole blood of the subject. In still other embodiments, the DC expansion is measured by flow cytometry or FACS. In some embodiments, 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 embodiments, the heterologous protein is a wildtype human IgG Fc protein. In yet other embodiments, the wildtype human IgG Fc protein is a wildtype IgG1, IgG2, IgG3 or IgG4 Fc protein. In some embodiments, the Flt3L protein is not fused to a wildtype IgG1 Fc protein or to a human serum albumin protein. In some embodiments, the subject is a rodent, a rabbit, a cynomolgus monkey, or a human. In other embodiments, the rodent is a mouse or a rat.

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

In some aspects, an isolated nucleic acid encoding an Flt3L-Fc fusion protein as described herein and above is provided. In some embodiments, 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 embodiments, 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 some aspects, a host cell comprising a nucleic acid encoding a Flt3L-Fc fusion protein as described herein and above is provided.

In some aspects 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 embodiments, the method further comprises recovering the Flt3L-Fc fusion protein from the host cell. In some
5 embodiments, the host cell is a eukaryotic cell or a prokaryotic cell. In other embodiments, the eukaryotic cell is a CHO cell.

In some aspects, a pharmaceutical formulation is provided, comprising a Flt3L-Fc fusion protein as described herein and a pharmaceutically acceptable carrier.

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

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

In some embodiments, 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
20 mg/kg of the Flt3L-Fc fusion protein.

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

In some aspects, 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 embodiments, the method comprising administering a therapeutically effective
30 amount of the Flt3L-Fc fusion protein and administering a therapeutically effective amount of an immune checkpoint inhibitor. In other embodiments, the immune checkpoint inhibitor suppresses or inhibits the effects of PD-1 or PD-L1. In still other embodiments, the immune checkpoint inhibitor is pembrolizumab, nivolumab, pidilizumab, BMS 936559, atezolizumab,

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

In some embodiments, the method further comprises administering a dendritic cell (DC) maturation factor. In other embodiments, the DC maturation factor is selected from polyIC, poly-ICLC, a CD40 agonist, radiation therapy, and chemotherapy. In other embodiments, 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 embodiments, the DC maturation factor is radiation therapy. In some embodiments, the method comprises administering the Flt3L-Fc fusion protein to the subject after the subject has received the DC maturation factor. In other embodiments, the method comprises administering the Flt3L-Fc fusion protein to the subject before the subject has received the DC maturation factor. In still other embodiments, 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 embodiments, 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 embodiments, the subject was previously treated with a checkpoint inhibitor. In other embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the cancer in the subject has been characterized as comprising an immune desert tumor. In other embodiments, the cancer has been characterized as comprising a tumor with reduced inflammation ("cold tumor") relative to a responsive, inflamed tumor ("hot tumor").

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows different levels of ADCP activity by anti-Her2 antibodies with a wildtype or variant Fc domain. Primary M1 macrophages were incubated with SkBr3 target cells. The x-axis shows antibody concentration and the y-axis shows percent phagocytosis.

5 **FIGS. 2A-2E** show cytokine release by macrophages when the anti-Her2 antibodies with a wildtype or variant Fc domain are incubated with primary M1 macrophages and SkBr3 target cells. The x-axis shows antibody concentration and the y-axis shows pg/ml cytokine.

FIG. 2A: G-CSF; **FIG. 2B:** GM-CSF; **FIG. 2C:** IL-10; **FIG. 2D:** IL-1ra; **FIG. 2E:** IL-6.

10 **FIGS. 2F-2J** show cytokine release by macrophages when the anti-Her2 antibodies with a wildtype or variant Fc domain are incubated with primary M1 macrophages and SkBr3 target cells. The x-axis shows antibody concentration and the y-axis shows pg/ml cytokine.

FIG. 2F: IL-8; **FIG. 2G:** MIP-1alpha; **FIG. 2H:** MIP-1beta; **FIG. 2I:** RANTES; **FIG. 2J:** TNFalpha.

15 **FIG. 2K** shows ADCC activity of an anti-CD20 antibody having wildtype and variant Fc proteins.

FIG. 2L shows ADCC activity of an anti-Her2 antibody having wildtype and variant Fc proteins.

20 **FIGS. 3A-3C** show differential characteristics of Flt3L-FcNG2LH fusion proteins with respect to aggregation and disulfide bond properties. Analysis of hFLT3L.S163.no.hinge.hIgG1.NG.PVA# aggregation and dimer formation of protein expressed in CHO cells and HEK293 cells is shown by SDS-PAGE (**FIG. 3A**) and SEC-HPLC (**FIG. 3B:** 293 cells and **FIG. 3C:** HEK297 cells). For **FIGS. 3B-3C**, the x-axis is time and the y-axis is mAU.

25 **FIGS. 3D-3F** show differential characteristics of Flt3L-FcNG2LH fusion proteins with respect to aggregation and disulfide bond properties. Analysis of hFLT3L.P167.5aa.hinge.hIgG1.NG.PVA# aggregation and dimer formation of protein expressed in CHO cells and HEK293 cells is shown by SDS-PAGE (**FIG. 3D**) and SEC-HPLC (**FIG. 3E:** 293 cells and **FIG. 3F:** HEK297 cells). For **FIGS. 3E-3F**, the x-axis is time and the y-axis is mAU.

30 **FIGS. 4A-4B** shows that Flt3L induces proliferation of OCI-AML5 cells in vitro. **FIG. 4A** shows a dose response curve of hFLT3L.P167.hIgG1.NG2LH and gCDX-301. Data shown are the average $\pm$ SD of triplicate values. Data were normalized to 10 ug/mL hFLT3L.P167.hIgG1.NG2LH as the maximum (100%) response. **FIG. 4B** shows EC50

potency of hFLT3L.P167.hIgG1.NG2LH and gCDX-301. Results are shown from 5 independent experiments with lines marking the mean $\pm$ SEM.

FIG. 5 shows serum concentrations of Flt3L-Fc (SEQ ID NO:28) fusion protein detected in animals dosed with 0.1 mg/kg, 1 mg/kg or 10 mg/kg fusion protein.

5 **FIGS. 6A-6B** show dose-dependent cell expansion of monocytes (**FIG. 6A**) and DCs (**FIG. 6B**) in animals administered 0.1 mg/kg, 1 mg/kg or 10 mg/kg fusion protein. Anti-gD is a negative control humanized IgG antibody.

FIG. 7 show plasma concentrations of Flt3L-Fc(NG2LH) in animals administered 0.1 mg/kg, 1 mg/kg or 10 mg/kg fusion protein. Anti-gD is a negative control humanized IgG antibody.

10 **FIGS. 8A-8C** show dose-dependent cell expansion of monocytes (**FIG. 8A**), cDC1 cells (**FIG. 8B**), and cDC2 cells (**FIG. 8C**) in animals administered 0.1 mg/kg, 1 mg/kg or 10 mg/kg fusion protein. Anti-IgD is a negative control humanized IgG antibody.

FIG. 9A shows immunogenicity of select Fc-containing proteins.

15 **FIG. 9B** shows immunogenicity of select Fc-containing proteins.

FIG. 10 shows results from one representative in vitro ADCP assay as described in Example 10, comparing ADCP activity of various Flt3L-Fc proteins.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

20 I. DEFINITIONS

“Affinity” refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen).

25 The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_d). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

The term “Flt3 ligand” also referred to as “Flt3L,” refers to a protein that is capable of binding the Flt3 receptor with sufficient affinity such that the protein is useful as a diagnostic and/or therapeutic agent in targeting the Flt3 receptor (Flt3R). In one embodiment, the extent of binding of a Flt3 ligand to an unrelated, non-Flt3R protein is less than about 10% of the

binding of the protein to Flt3L as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, a protein that binds to the Flt3R has a dissociation constant (K_d) of $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or $\leq 0.001 \text{ nM}$ (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M , e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, a Flt3L binds to a Flt3R that is conserved among Flt3R from different species.

The term “Flt3L,” as used herein, refers to the cleaved, soluble Flt3L (e.g., approximately residues 27-185 of SEQ ID NO:21), but may also refer to any native Flt3L from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term can encompass “full-length,” unprocessed Flt3L as well as any form of Flt3L that results from processing in the cell, e.g., removal of a signal peptide (leader sequence) or cleavage from the TM domain. The term also encompasses naturally occurring variants of Flt3L, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human Flt3L with the endogenous signal sequence is shown in SEQ ID NO:21 and is also provided in GenBank Accession Record P49771, while in other embodiments, the amino acid sequence of mature Flt3L protein without an exogenous leader sequence is provided as residues 27-167 of SEQ ID NO:21. Minor sequence variations especially conservative amino acid substitutions of Flt3L that do not affect Flt3L's function and/or activity (e.g., binding to Flt3 receptor (GenBank Acc. No. NP_004110)) are also contemplated by the invention.

The term “Flt3L-Fc fusion protein” or alternatively, “Fc-effectorless Flt3L-Fc fusion protein,” as used herein refers to a fusion protein in which a Flt3L polypeptide is linked, directly or indirectly, to a variant IgG Fc region wherein the variant Fc region has attenuated effector function relative to its counterpart wildtype Fc region. It is noted that for any use of the term “effectorless Flt3L-Fc fusion protein” the term “effectorless” applies to the Fc portion of the fusion protein. In certain preferred embodiments, the Flt3L-Fc fusion protein of the invention comprises a human Flt3L protein or polypeptide linked to a human IgG Fc region. In certain embodiments, the human Flt3L protein comprises the amino acid sequence of SEQ ID NO:22. However, it is understood that minor sequence variations such as insertions, deletions, substitutions, especially conservative amino acid substitutions of Flt3L or Fc that do not significantly affect the function and/or activity of Flt3L or of an effectorless Flt3L-Fc fusion protein of the invention (e.g., SEQ ID NO:26) are also contemplated by the invention. The effectorless Flt3L-Fc fusion protein of the invention can bind to the Flt3 receptor protein (Flt3), which can lead to Flt3 receptor downstream signaling. As used herein, CDX-301 refers to a Flt3L having the amino acid sequence of SEQ ID NO:23.

The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

5 An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

10 The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively.

15 "Effector functions" refer to those biological activities attributable to the Fc region of an antibody, an isolated Fc region, or an Fc region fused to another protein, wherein the Fc region varies with the isotype of the source antibody. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; antibody dependent
20 cellular phagocytosis (ADCP); down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation. In certain embodiments, the Flt3L-Fc fusion protein does not exhibit any effector function or any detectable effector function. In certain other embodiments, the Flt3L-Fc fusion protein exhibits substantially reduced effector function, e.g., about 50%, 60%, 70%
25 80%, or 90% reduced effector function relative to a fusion protein comprising a Flt3L protein of the present invention fused to a wildtype Fc protein having SEQ ID NO:12.

"Dendritic cell expansion," "expansion of dendritic cells," or "increase in the number of dendritic cells" as used herein refers to an increase in the number of dendritic cells in an in vitro assay or as measured in an in vivo experiment.

30 An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG

heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as
5 described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

"T cell activation" as used herein refers to one or more cellular response of a T lymphocyte, particularly a cytotoxic T lymphocyte, selected from: proliferation, differentiation, cytokine secretion, cytotoxic effector molecule release, cytotoxic activity, and expression of
10 activation markers. The T cell activating bispecific antigen binding molecules of the invention are capable of inducing T cell activation.

The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the
15 primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

An "individual" or "subject" is a mammal. Mammals include, but are not limited to,
20 domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

An "isolated" protein or polypeptide is one which has been separated from a component of its natural environment. In some embodiments, a protein is purified to greater than 95% or
25 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated
30 from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

“Isolated nucleic acid encoding a Flt3L-Fc fusion protein” refers to one or more nucleic acid molecules encoding a Flt3L-Fc fusion protein, including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

5 “Percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity.

10 Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences

15 being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2

20 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the %

25 amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

30
$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program’s alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid

35 sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence

identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

5 The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

 A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical
10 formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

 As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the
15 individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved
20 prognosis. In some embodiments, fusion proteins of the invention are used to delay development of a disease or to slow the progression of a disease.

 The phrase "therapeutically effective amount" means an amount of a protein of the present invention that (i) treats or prevents the particular disease, condition, or disorder, (ii) attenuates, ameliorates, or eliminates one or more symptoms of the particular disease,
25 condition, or disorder, or (iii) prevents or delays the onset of one or more symptoms of the particular disease, condition, or disorder described herein. In the case of cancer, the therapeutically effective amount of the therapeutic agent or a combination of agents as described herein may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit
30 (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the therapeutic agent or a combination of agents as described herein may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer

therapy, efficacy can be measured, for example, by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

The terms "cancer" and "cancerous" as used herein refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. A "tumor" comprises one or more cancerous cells. Tumors include solid and liquid tumors. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, myeloma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g., epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer ("NSCLC"), adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, malignant brain tumors, melanoma, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, head and neck cancer, as well as acute myelogenous leukemia (AML).

The term "cold tumor" or "immune desert" as used herein refers to a cancerous tumor which is hypo-immunogenic or characterized by insufficient elicitation of tumor-specific immunity and resistance to immunogenic cytotoxicity.

The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

II. COMPOSITIONS AND METHODS

A. The Effectorless Fc Protein

In one aspect, the invention provides a novel effectorless IgG1 Fc protein that has surprisingly reduced effector activity. The Fc protein designed and described herein comprises the amino acid sequence of SEQ ID NO.2 and is also referred to herein as "FcNG2LH" or "Fc NG PVA#." As shown herein, the FcNG2LH Fc protein has significantly reduced ADCP activity compared to proteins having a wildtype IgG1 Fc domain (see, e.g., at least in Examples 1 and 10), as well as reduced activation of cytokine release by macrophages (see, e.g., Example

1) and reduced ADCC activity. Also notable is the surprising increase in thermostability of the FcNG2LH protein relative to the thermostability of an IgG1 Fc domain containing only the N297G mutation.

The design of an effectorless IgG1 Fc domain protein has utility in the context of many therapeutic molecules, in addition to its use in an Flt3L-Fc fusion protein as described herein. For example, antibodies designed to bind to and activate a molecule (e.g., cell surface receptor) by bridging two or more of the molecules together, antibodies that target checkpoint inhibitors, bi- or multispecific antibodies that have at least one arm that binds to and induces ADCC and/or ADCC activity. The ordinarily skilled artisan will appreciate the use of the effectorless Fc protein described herein for the generation of a fusion protein wherein the effectorless Fc protein is linked (e.g., via an amino acid bond) to the N-terminus and/or C-terminus of a second protein to the C-terminus and/or N-terminus of the effectorless Fc protein, respectively. The second protein is preferably one which has a desired therapeutic function. Effectorless Fc domains can prevent adverse reactions such as neutrophilia caused by binding of an IgG Fc receptor. There is need in the field of therapeutic proteins to continually develop ways to refine the control of Fc effector functions.

In one aspect, the invention provides antibodies in which the heavy chain constant domain comprises the FcNG2LH protein of SEQ ID NO:2. In some embodiments, the Flt3L-Fc fusion proteins described herein include immunoglobulins and antibodies. Persons having ordinary skill in the art can readily envision the format of a variety of antigen-specific antibodies which can accommodate and benefit from the presence of the NG2LH mutations and resultant lack of or decrease in effector function. For example, the human IgG1 FcNG2LH of the present disclosure could be part of a monoclonal antibody, human antibody, chimeric antibody, a humanized antibody, a bispecific antibody, or a multispecific antibody. In other words, any immunoglobulin structure that includes or can include an IgG1 Fc domain.

B. Exemplary Flt3L-Fc Fusion Proteins

In one aspect, the invention provides Flt3L-Fc fusion proteins that bind to the Flt3 receptor (Flt3R) and wherein the Fc protein has approximately no effector function or has greatly reduced effector function as compared to the wildtype Fc protein. These fusion proteins are useful for treatment of cancer through a variety of cancer immunotherapy treatment protocols, especially in cases where a patient either does not respond to checkpoint inhibitor immunotherapy or has a limited response to checkpoint inhibitor immunotherapy. It is thought that a lack of anti-tumor immune response in a “cold tumor” or “immune desert” is at least in

part due to insufficient or a lack of anti-tumor T cell response. An effective anti-tumor T cell response requires cross presentation of tumor antigens by dendritic cells (DCs).

Accordingly, one means of increasing efficacy of cancer immunotherapy is to increase the number of antigen-presenting DCs within a tumor. It is well known that Flt3L functions in part to increase DCs in a subject, through proliferation, differentiation, development and mobilization of progenitor cells. While there is evidence that a Flt3L protein administered to a healthy subject can indeed expand DCs and hematopoietic stem cells (e.g., Anandasabapathy et al., 2015, Bone Marrow Transplant., 50:924-930) one concern is the relatively short half-life of recombinant Flt3L in the blood which may result in less therapeutic efficacy and/or a need for too frequent dosing.

A well-established strategy to extend the serum half-life of a therapeutic protein is to link or fuse it to an immunoglobulin Fc domain (e.g., via a peptide bond, preferably at the N-terminus of the Fc protein) (e.g., Czajkowsky et al., 2012, EMBO, 4:1015-1028; Ha et al., 2016, Front Immunol, 7:394). However, in the case of a Flt3L-Fc fusion protein for use in, e.g., cancer immunotherapy, it may be disadvantageous to have a functional Fc domain which may activate many and unpredictable immunologic or inflammatory processes in situ. Indeed, it's important to minimize risk of administration resulting in a cytokine storm or cytokine release syndrome (CRS). Additionally, interaction between a wildtype Fc of a Flt3L-Fc fusion protein domain and a Fc receptor on an immune effector cell may lead to phagocytic destruction of the DC bound by the Flt3L-Fc fusion protein. It can be important to minimize and thereby control for effects of the Fc region of the Flt3L-Fc fusion protein while maintaining the advantages imparted by the Fc region. Described herein is a new effectorless IgG Fc region, referred to herein as "Fc-NG2LH" or "Fc-PVA# N297G," which was shown to have surprisingly low effector function (see, e.g., Example 7), especially when compared to, e.g., wildtype IgG1 Fc, IgG1 Fc-N297G, and IgG4 Fc regions. Moreover, as shown in Example 8, introduction of the NG2LH variant resulted in an unpredicted increase in the thermostability of the Fc domain as well as effects on half-life in the serum of an animal.

Additional characterization of Flt3L-Fc fusion protein variants show that substituting Flt3L W144 significantly increases immunogenicity as determined in an in vitro T cell proliferation assay, suggesting removing a potential oxidation liability at position 149 of Flt3L (SEQ ID NO:21) could be detrimental to a therapeutic effectorless Flt3L-Fc fusion protein product. In contrast, however, Example 9 shows substitution of potential glycosylation sites at positions 149 and 151 did not increase immunogenicity as determined by the in vitro T cell

proliferation assay, suggesting that mutations at these and other positions may be amenable to manufacture of a Flt3L-Fc fusion protein.

Importantly, in vivo administration of varying doses of an effectorless Flt3L-Fc fusion protein as disclosed herein produced a large expansion of dendritic cells (e.g., see Example 5).

- 5 Moreover, the level of DC expansion is arguably much greater than what has been observed with an equivalent dose of CDX-301.

The Flt3L-Fc fusion protein comprises a Flt3 ligand (Flt3L) protein linked to an IgG1 Fc region wherein the IgG1Fc has reduced or no effector function relative to the same Flt3L fused to a wildtype IgG1 Fc Fc. The Flt3L portion of the Flt3L-Fc fusion protein can bind to
10 the cell surface Flt3 receptor (Flt3R). A sequence of the Flt3L protein is described in GenBank Accession No. P49771 (provided herein as SEQ ID NO:21) as a protein 235 amino acids in length, with the signal peptide extending from approximately residues 1-26 and a transmembrane domain extending approximately from residues 185-205. Residues 29-159 are described in the GenBank record as the Flt3 ligand, while Savvides et al. (Nat Struct Bio,
15 7:486-491) state that residues 27-160 alone have been shown to be sufficient for bioactivity. In some embodiments, the present disclosure envisions Flt3 ligand domains which comprise residues of SEQ ID NO:21 with a N-terminus being any amino acid residue from 24 to 30 of SEQ ID NO:21 and a C-terminus being any amino acid residue at position 167-190 of SEQ ID NO:21. While Savvides et al. (ibid) showed that a polypeptide comprising residues 27-160
20 alone (and configured as a dimer) is sufficient for functional activity of the ligand, studies described in Example 2 below show that using a Flt3L protein which terminates N-terminal to residue 167 of SEQ ID NO:21, e.g., is covalently linked to the Fc N-terminal residue (of SEQ ID NO:13). It is understood that variants of Flt3L are also envisioned in the present disclosure, such as mature Flt3L proteins comprising a protein sequence having at least 75%, 80%, 85%,
25 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:21 residues 27-167. It shown here (e.g., Example 3 below) that the fragment size of Flt3 which is fused to an Fc protein can have a significant impact on aggregation of the fusion protein.

In one aspect, the invention provides a Flt3L-Fc fusion protein comprising a Flt3L protein comprising an amino acid sequence which is at least 95% identical to residues 27-167
30 of SEQ ID NO:21 and an IgG Fc molecule which is at least 95% identical to SEQ ID NO:13. The IgG Fc molecule has attenuated Fc effector function relative to a wildtype IgG1 Fc polypeptide (SEQ ID NO:12), wherein the Fc effector function is selected from CDC, ADC, ADCC, and/or ADCP. In some embodiments, the IgG Fc molecule has attenuated ADCP activity relative to the wildtype IgG1 Fc polypeptide. Without being bound by theory, it is

considered that the low to absent effector function of the Fc(NG2LH) protein is due to the presence of unique combination of both the N297G mutation (at position 76 of SEQ ID NO:13) and amino acids PVAGP (residues 13-17 of SEQ ID NO:13) in the lower hinge of the Fc(NG2LH) protein.

5

a) Substitution, Insertion, and Deletion Variants

In certain embodiments, protein variants having one or more amino acid substitutions are provided. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an fusion protein of interest and the products screened for a desired activity, e.g., decreased immunogenicity or decreased ADCP.

10

TABLE 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp; Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- 5 (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes
10 for another class.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include a Flt3L-Fc fusion protein with an N-terminal methionyl residue.

15 Other insertional variants of the Flt3L-Fc fusion protein molecule include the fusion to the N- or C-terminus of the Flt3L-Fc fusion protein to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the Flt3L-Fc fusion protein.

b) Glycosylation variants

In certain embodiments, a Flt3L-Fc fusion protein provided herein is altered to increase
20 or decrease the extent to which the Fc portion of the Flt3L-Fc fusion protein is glycosylated. Addition or deletion of glycosylation sites to Fc domain may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

With respect to a Fc region, the carbohydrate attached thereto may be altered. Native
25 antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary
30 oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in a Flt3L-Fc fusion protein of the invention may be made in order to create effectorless Fc variants with certain improved properties.

In one embodiment, Fc fusion variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to the Fc region. For example, the amount of

fucose may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to “defucosylated” or “fucose-deficient” antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L.; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

Fc variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the fusion protein is bisected by GlcNAc. Such Fc variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Flt3L-Fc fusion protein variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such Flt3L-Fc fusion proteins (and therefore Fc) variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Protein Derivatives

In certain embodiments, an effectorless Fc protein, an antibody or a fusion protein comprising the effectorless Fc protein, or Flt3L-Fc fusion protein provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the Flt3L-Fc fusion protein include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the Fc-fusion protein may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the Flt3L-Fc fusion protein to be improved, whether the Flt3L-Fc fusion protein derivative will be used in a therapy under defined conditions, etc.

In another embodiment, conjugates of a Flt3L-Fc fusion protein and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the fusion protein-nonproteinaceous moiety are killed.

C. Recombinant Methods and Compositions

Flt3L fusion proteins may be produced using recombinant methods and compositions readily known to the ordinarily skilled artisan. In one embodiment, isolated nucleic acid encoding a Flt3L-Fc fusion protein described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the Flt3L and Fc portions of the Flt3L-Fc fusion protein. In a further embodiment, one or more vectors (e.g., expression vectors) comprising

such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes a Flt3L polypeptide and an Fc polypeptide according to the present invention. In preferred embodiments, the nucleic acid encoding the Flt3L polypeptide is upstream of the nucleic acid encoding the Fc polypeptide. Moreover, the 2 nucleic acids are in a single operon. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In one embodiment, a method of making a Flt3L-Fc fusion protein is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the Flt3L-Fc fusion protein, as provided above, under conditions suitable for expression of the Flt3L-Fc fusion protein, and optionally recovering the Flt3L-Fc fusion protein from the host cell (or host cell culture medium).

For recombinant production of a Flt3L-Fc fusion protein, nucleic acid encoding a Flt3L-Fc fusion protein, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the fusion protein).

Suitable host cells for cloning or expression of effectorless Fc proteins, antibodies or fusion proteins containing the Fc protein, or e.g., Flt3L-Fc fusion protein-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, Flt3L-Fc fusion proteins may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the Flt3L-Fc fusion protein may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for a Flt3L-Fc fusion protein-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of a Flt3L-Fc fusion protein with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

Suitable host cells for the expression of glycosylated Flt3L-Fc fusion protein are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate

cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures can also be utilized as hosts. See, e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR⁺ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)), and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

D. Assays

Flt3L-Fc fusion proteins which can facilitate expansion of DCs as provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

E. Immunoconjugates

The invention also provides immunoconjugates comprising a Flt3L-Fc protein herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

In one embodiment, an immunoconjugate of the Flt3L-Fc protein is generated in a manner similar to generation of an antibody-drug conjugate (ADC) in which an antibody is

conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP0425235B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

In another embodiment, an immunoconjugate comprises a Flt3L-Fc protein as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In another embodiment, an immunoconjugate comprises a Flt3L-Fc protein as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

Conjugates of a Flt3L-Fc protein and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido

compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238:1098 (1987).

- 5 Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992);
10 U.S. Patent No. 5,208,020) may be used.

The immunoconjugates herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB,
15 and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A.).

F. Pharmaceutical Formulations

Pharmaceutical formulations of a Flt3L-Fc fusion protein as described herein are prepared by mixing such protein having the desired degree of purity with one or more optional
20 pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as
25 octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids
30 such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic

surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX[®], Baxter International, Inc.). Certain exemplary
5 sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that
10 do not adversely affect each other. For example, it may be desirable to further provide a dendritic cell maturation factor and/or adjuvant. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose
15 or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-
20 release preparations include semipermeable matrices of solid hydrophobic polymers containing the Fc fusion protein, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

25 **G. Therapeutic Methods and Compositions**

Any of the Flt3L-Fc proteins provided herein may be used in therapeutic methods.

In one aspect, a Flt3L-Fc protein for use as a medicament is provided. In further aspects, a Flt3L-Fc protein for use in a cancer is provided. In certain embodiments, a Flt3L-Fc protein for use in a method of treatment is provided. In certain embodiments, the invention
30 provides a Flt3L-Fc protein for use in a method of treating an individual having cancer comprising administering to the individual an effective amount of the Flt3L-Fc protein. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In further

embodiments, the invention provides a Flt3L-Fc protein for use in expanding dendritic cells. In certain embodiments, the invention provides a Flt3L-Fc protein for use in a method of expanding dendritic cells in an individual comprising administering to the individual an effective amount of the Flt3L-Fc protein to expand dendritic cells. An “individual” according to any of the above embodiments is preferably a human.

In a further aspect, the invention provides for the use of a Flt3L-Fc protein in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of cancer. In a further embodiment, the medicament is for use in a method of treating cancer comprising administering to an individual having cancer an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In a further embodiment, the medicament is for expanding dendritic cells. In a further embodiment, the medicament is for use in a method of expanding dendritic cells in an individual comprising administering to the individual an amount effective of the medicament to expand dendritic cells. An “individual” according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for treating a cancer. In one embodiment, the method comprises administering to an individual having such cancer an effective amount of a Flt3L-Fc protein. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An “individual” according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for expanding dendritic cells in an individual. In one embodiment, the method comprises administering to the individual an effective amount of a Flt3L-Fc protein to expand dendritic cells. In one embodiment, an “individual” is a human.

In a further aspect, the invention provides pharmaceutical formulations comprising any of the a Flt3L-Fc proteins provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the a Flt3L-Fc proteins provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the Flt3L-Fc proteins provided herein and at least one additional therapeutic agent, e.g., as described below.

Flt3L-Fc proteins of the invention can be used either alone or in combination with other agents in a therapy. For instance, a Flt3L-Fc protein of the invention may be co-administered with at least one additional therapeutic agent.

Such combination therapies noted above encompass combined administration (where
5 two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the Flt3L-Fc protein of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents. In one embodiment, administration of the Flt3L-Fc protein and administration of an additional therapeutic agent occur within about one month, or within about one, two or three
10 weeks, or within about one, two, three, four, five, or six days, of each other. Flt3L-Fc proteins of the invention can also be used in combination with radiation therapy.

A Flt3L-Fc fusion protein of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include
15 intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Flt3L-Fc fusion proteins of the invention would be formulated, dosed, and administered
20 in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The Flt3L-Fc fusion protein need not be, but is optionally formulated
25 with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of Flt3L-Fc fusion protein present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described
30 herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of a Flt3L-Fc fusion protein of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of Flt3L-Fc fusion

protein, the severity and course of the disease, whether the Flt3L-Fc fusion protein is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the fusion protein, and the discretion of the attending physician. The Flt3L-Fc fusion protein is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1 mg/kg-10 mg/kg) of Flt3L-Fc fusion protein can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the Flt3L-Fc fusion protein would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (*e.g.* such that the patient receives from about two to about twenty, or *e.g.* about six doses of the fusion protein). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful.

It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to a Flt3L-Fc fusion protein.

H. Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a Fc fusion protein of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of

manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises a Fc fusion protein of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWHI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to a Flt3L-Fc fusion protein.

III. EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1. Design of an Effectorless Fc Protein

The term “NG2LH” refers to a human IgG1 Fc domain that comprises the IgG1 N297G substitution and that “swaps” the lower hinge residues 233-236 (ELLG, SEQ ID NO:19) of IgG1 with residues 233-236 (PVA; no amino acid at position 236, also referred to herein as “PVA#”) of human IgG2, wherein positions are numbered according to the EU index as described in Kabat. The effectorless nature of the NG2LH Fc variant protein (also referred to herein as “PVA# NG”) was first shown in the context of a full-length antibody and in comparison to wildtype, N297G and other Fc variant IgG1's, as well as IgG2 and IgG4 antibodies, as defined in **Table 2** below.

TABLE 2

SEQ ID NO:	Description of Heavy Chain Constant Region (CH1 – CH3)	Substitution(s) (EU Numbering)
2	IgG1 NG2LH, aka IgG1 NG PVA#	N297G/E233P/L234V/L235A/G236#
3	IgG1 NG	N297G
4	IgG1 PVA#	E233P/L234V/L235A/G236#

5	IgG1 NG PVA# G	N297G/E233P/L234V/L235A/G236#/A327G
6	IgG1 NG PVA# GSS	N297G/E233P/L234V/L235A/G236#/A327G/A330S/P331S
7	IgG1 PG	P329G
8	IgG1 LALA	L234A/L235A
9	IgG1 LALAPG	L234A/L235A/P329G

Full length antibodies comprising the heavy chain constant regions (CH1-CH3) listed in Table 2 were constructed that encoded the light chain and heavy chain variable and domains of an anti-Her2 antibody (trastuzumab (HERCEPTIN®, Genentech, Inc., South San Francisco, Calif., USA) (also referred to in U.S. Pat. No. 6,407,213 and Lee et al., J. Mol. Biol. (2004), 340(5):1073-93; herein as SEQ ID NO:47). The light chain for these anti-Her2 antibodies comprised a native human C kappa constant region. Nucleic acids encoding the full length light chain and various full length heavy chains were cloned into the pRK mammalian expression vector (Eaton et al., 1986, Biochemistry 25:8343-47). Light and heavy chain nucleic acid vectors were co-expressed in CHO cells, and the antibodies were purified via protein A affinity chromatography followed by size exclusion chromatography.

The antibodies were then tested for their effects on antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cytokine release (ADCR) using primary macrophages and SK-BR-3 human breast cancer cell line that overexpresses Her2 (ATCC® HTB-30). An anti-gD antibody was used as a negative control antibody. Monocytes were isolated from donor PBMCs and stimulated with 25 ng/ml human MCSF-1 and allowed to differentiate to macrophages for 5-7 days in T-175 tissue culture flasks. The day before the ADCP and cytokine release experiments, all macrophages were stimulated with 50 ng/ml IFN γ (R&D Systems 285-IF-100/CF) to differentiate to M1 macrophages. Macrophages and SK-BR-3 GFP cells were harvested by accutase (Millipore SCR005) treatment. Dislodged macrophages and SK-BR-3 cells were resuspended in media (X-Vivo 10; Lonza 04-743Q, 10% HI FBS; Gibco 10438-026) at concentration of 2×10^6 and 0.5×10^6 cells/ml, respectively. A 96-well plate map was designed to determine the test conditions (antibody variants and treatment concentration). 2X serial dilutions of each of the antibody variants were prepared in media (6 points 10-fold dilution series: 2000, 200, 20, 2, 0.2, 0.02 ng/ml). Following the plate map, 50 μ l of macrophage and 50 μ l of SK-BR-3 were added to each well, resulting in 100k macrophages and 25k SK-BR-3 cells in each well. 100 μ L of the 2X antibody serial diluted solution were combined the well of 96-well plates according to the plate map. The final

antibody treatment concentrations were 1000, 100, 10, 1, 0.1, 0.01 ng/ml. 100 uL of media was added to 3 wells containing the macrophages and SK-BR-3 cells as “media only” control treatment. The 96-well plate was centrifuged at 20 x g for 2 min then incubated at 37°C for about 24 h. Cells were centrifuged at 400 x g for 4 min and supernatant was collected for

5 ADCR analyzed by Luminex Reader Millipore Multiplex. Cells were dislodged from the wells by accutase treatment. Cells in each well were washed once with 200 ul of FACS Buffer (BD, 554657). Staining solution was prepared with anti-CD11b (BD, 555385) and anti-CD14 (BD, 555396) conjugated with Alexa 647 (Invitrogen, Alexa Fluor 647 Antibody Labeling Kit, A20186) (1:100 antibody conjugate in FACS buffer). 50 uL of the staining solution were added

10 to all the wells and incubate at 4°C for 30 - 60 min. Cells were washed twice with FACS Buffer (200 ul per well). Cells in each well were resuspended in 50 ul of FACS Buffer. One plate of cells was analyzed by FACS for ADCP efficiency. FACS data were analyzed with software FlowJo. Cells were gated in the Forward and Side Scatter plot then plotted on GFP-expression on Y-axis and Alexa 647 on the X-axis. Cell percentage in each quadrant were

15 calculated. SK-BR-3 cells were largely in quadrant 1 (Q1, GFP positive) and macrophages were largely in quadrant 3 (Q3, Alexa 647 positive). % Phagocytosis of each well were calculated:

$$\% \text{ Phagocytosis} = 100\% - (Q1 \text{ of each well}) / (\text{average Q1 of 3 media only control wells})$$

% Phagocytosis of the antibody variants with respect to the antibody treatment concentration

20 plotted on a graph.

Two plates were analyzed by Luminex (Luminex Corp). As shown in **FIG. 1**, approximately no phagocytosis was observed in the presence of increasing concentrations of the LALAPG or NGLH2 Fc anti-Her2 antibodies. The highest levels of ADCP were observed with the IgG1 wildtype and IgG4 antibody variants. Intermediate levels of phagocytosis were

25 observed for IgG2 wildtype Fc and IgG1 PVA# Fc. Accordingly, **FIG. 1** shows that the IgG1 NG2LH and IgG1 LALAPG Fc domains lack ADCP activity in an in vitro assay. The experiment shows a similar lack of ADCP activity in the presence of IgG1 NG PVAG and IgG1 NG PVA# GSS (data not shown).

Results of the cytokine release profiles from the ADCP assay are provided in **FIGS. 2A-2J**. Consistent with the phagocytosis data, these results demonstrate that in contrast to

30 native IgG1, IgG4, and to a lesser extent N297G, the NG2LH variant mediates no ADCR by macrophages in the presence of target cells.

These data contrast to data from an ADCC reporter assay of the variants compared to wildtype IgG1 Fc. The variable domains from ocrelizumab (anti-CD20) or trastuzumab (anti-

Her2) were fused to the wildtype and variant domains described above and tested in an ADCC assay. In this assay, a NK cells was engineered as a NFAT-RE-Luciferase reporter cell, reading out RcyRIIIa binding, then assayed in the presence of WIL2S CD20+ cells or SkBr3 Her2+ cells. For both the anti-CD20 and anti-Her2 constructs with wildtype or variant domains, all non-wildtype Fc molecules had minimal activity as compared to wildtype IgG1 Fc (see FIGS. 2K and 2L).

Example 2. Design of Flt3L Fc Fusion Proteins

The Flt3L-Fc fusion proteins were generated using routine nucleic acid cloning, protein expression and protein purification techniques.

PCR was used to amplify cDNA containing the nucleotide sequence encoding the Flt3L and Fc portions of desired chimeric Flt3L-Fc proteins (see Table 3, SEQ ID NOS:10-31). For expression and production of the Flt3L-Fc proteins described in Table 3 (sequences provided in Table 10), the cDNA was generated to include the native signal sequence of human Flt3L (residues 1-27 of GenBank Acc. No. P49771; SEQ ID NO:21). As an example, the cDNA generated for expression of the hFLT3L.P167.5aa.hinge.hIgG1.NG.PVA# protein comprised a cDNA sequence encoding the fusion protein with the Flt3L signal sequence as provided in Table 10 as SEQ ID NO:45.

TABLE 3

SEQ ID NO:	Construct Name
25	hFLT3L.P168.5aa.hinge.hIgG1.NG.PVA#
26	hFLT3L.P167.5aa.hinge.hIgG1.NG.PVA#
27	hFLT3L.P167.5aa.hinge.hIgG1
28	hFLT3L.P167.5aa.hinge.hIgG1.NG
29	hFLT3L.P167.5aa.hinge.hIgG1.PVA#
30	hFLT3L.P167.5aa.hinge.hIgG1.LALAPG
31	hFLT3L.P167.7aa.hinge.hIgG4
32	hFLT3L.P166.5aa.hinge.hIgG1.NG.PVA#
33	hFLT3L.L165.5aa.hinge.hIgG1.NG.PVA#
34	hFLT3L.L164.5aa.hinge.hIgG1.NG.PVA#
35	hFLT3L.C158.5aa.hinge.hIgG1.NG.PVA#
36	hFLT3L.P179.hIgG1.N297G
37	hFLT3L.Q159.hIgG1.N297G
38	hFTL3L.C158.3aa.hinge.hIgG1.NG.PVA#

39	hFTL3L.C158.no.hinge.hIgG1.NG.PVA#
40	hFTL3L.D161.no.hinge.hIgG1.NG.PVA#
41	hFTL3L.S163.no.hinge.hIgG1.NG.PVA#
42	Hs_FLT3LG.M1-L165.W144D.O.pRK-5aa.hIgG1.NG2LH
43	Hs_FLT3LG.M1-L165.S151D.O.pRK-5aa.hIgG1.NG2LH
44	Hs_FLT3LG.M1-L165.S151E.O.pRK-5aa.hIgG1.NG2LH
46	Flt3L-Fc(IgG4)

The cDNA encoding Flt3L-Fc fusions was subcloned into a mammalian expression vector pRK5 (Gorman, et al., DNA and Protein Engineering Techniques 2:1 (1990); U.S. Pat. No. 6,232,117) and expressed under the control of the CMV promoter.

5 Either CHO or HEK293 cells were transiently transfected with a desired expression construct, grown in the appropriate culture media 10 or 6 days, respectively, and harvested for purification.

Purification of Flt3L-Fc fusion constructs was achieved using HiTrap MabSelect SuRe affinity capture chromatography (GE), followed by Superdex 200 SEC, and finally
10 concentration and buffer exchange via dialysis.

Example 3. Characterization of Flt3L-Fc Fusion Proteins

The fusion proteins generated as described in Example 2 were characterized in terms of susceptibility to aggregation in solution as well as reduction and loss of disulfide bonds, each of which was analyzed by SDS-PAGE (3-5 ug per well) and HPLC SEC (SuperDex 200; equilibration buffer: 200 mM arginine, 137 mM succinic acid, pH 5.0). Data from experiments
15 performed using Flt3L-Fc fusion proteins purified from CHO cell cultures are summarized in Table 4 below with exemplary data from Flt3L-Fc fusion construct hFLT3L.S163.no.hinge.hIgG1.NG.PVA# (SEQ ID NO:41) expressed in and purified from CHO cells and HEK293 cells shown in **FIGS. 3A-3C**, and data from Flt3L-Fc fusion construct
20 hFLT3L.P167.5aa.hinge.hIgG1.NG.PVA# (SEQ ID NO:26) expressed in and purified from CHO cells and HEK293 cells shown in **FIGS. 3D-3F**.

TABLE 4

SEQ ID NO:	Construct	% Aggregation	Incomplete hinge disulfide
25	hFLT3L.P168.5aa.hinge.hIgG1.NG.PVA#	24	ND*
26	hFLT3L.P167.5aa.hinge.hIgG1.NG.PVA#	26	ND
32	hFLT3L.P166.5aa.hinge.hIgG1.NG.PVA#	26	+
33	hFLT3L.L165.5aa.hinge.hIgG1.NG.PVA#	33	++
34	hFLT3L.L164.5aa.hinge.hIgG1.NG.PVA#	35	+++
35	hFLT3L.C158.5aa.hinge.hIgG1.NG.PVA#	86	++++

38	hFTL3L.C158.3aa.hinge.hIgG1.NG.PVA#	90	+++
39	hFTL3L.C158.no.hinge.hIgG1.NG.PVA#	77	++
41	hFTL3L.S163.no.hinge.hIgG1.NG.PVA#	85	++++

* ND = Not detected

The data analysis of the variants, summarized in Table 4 above, showed that the number of amino acid residues present between the last cysteine of the Flt3L molecule and the first cysteine of the Fc(NG2LH) molecule (junction length) can impact the level of aggregation and/or disulfide bond formation. When the junction length is less than 14 residues, up to 30% of each construct appears to lack intermolecular hinge disulfides. A junction length of 5 amino acid residues or less resulted in very high aggregation. The lowest levels of aggregation were observed for fusion proteins of SEQ ID NOS. 24, 26, 32, 33, and 34. The lowest levels of incomplete hinge disulfide bonding was observed for fusion proteins of SEQ ID NOS. 25, 26, and 32, with SEQ ID NOS. 33 and 39 also having relatively low levels of incomplete hinge disulfide. The data observed here was somewhat unexpected considering that the structures of both the Flt3L and Fc proteins are well characterized and shown to be capable of folding as independent domains. It is noted, however, that each protein domain contains cysteine residues important for disulfide bonding and structural integrity. Accordingly, the data show that design of a Flt3L-Fc(NG2LH) fusion molecule requires careful assessment of the protein product to ensure sufficient covalent Fc-dimerization and minimal or lack of aggregation.

Example 4. In vitro Functional Assays for Flt3L Activity

In vitro assays were done to assess the functional properties of an Flt3L-Fc fusion protein compared to the function of the Flt3L protein (no Fc fusion; SEQ ID NO:23). The in vitro assay performed as described here can be used to measure the potency of a Flt3L protein to induce proliferation of a human cell line expressing Flt3 receptor.

Costar 96-well flat bottom plates (Cat#3610 Thermo Fisher) were coated overnight at room temperature with 100 uL of Poly-L-ornithine (Cat#A-004-C EMD Millipore), washed 3x with PBS and allowed to dry. Human OCI-AML5 cells (No. ACC 247, DSMZ) were seeded at 3000 cells per well in 100 uL of assay media (RPMI 1640 containing 5% heat inactivated FBS, 1X Glutamax). Flt3L test articles were diluted to 20 ug/mL in assay media and serially diluted 1:10. 100 uL of diluted test article was added to each well (in triplicate) to achieve a final starting concentration of 10 ug/mL. Maximum and minimum control wells included 10 ug/mL hFTL3L.P167.hIgG1.NG2LH (SEQ ID NO:26) or assay media alone. Assay plates were incubated at 37°C, 5% CO₂ for 7 days. 100 uL of supernatant was removed from each well and

then 100 μ L of Cell Titer Glo (Cat#PR-G7572 Thermo Fisher) was added to each well and plates were incubated for 8-10 min in the dark. Luminescence was measured with a SpectraMax i3 plate reader (Molecular Devices). Raw values were normalized to the maximum and minimum control wells. A dose response of each test article was plotted with XLfit (IDBS) and EC₅₀ values determined using a four parameter logistic curve. Graphs shown in **FIGS. 4A-4B** were generated with GraphPad Prism 7.

These data show that both hFLT3L.P167.hIgG1.NG2LH and gCDX-301 induced dose-dependent proliferation of OCI-AML5 cells in vitro. Average EC₅₀ potency values were comparable: 2.5 $\pm$ 0.5 pM for hFLT3L.P167.hIgG1.NG2LH; 2.3 $\pm$ 0.5 pM for gCDX-301. Moreover, hFLT3L.P167.hIgG1.NG2LH and gCDX-301 can induce the proliferation of OCI-AML5 cells in vitro with at least equivalent potency.

Example 5. PKPD Studies in Mouse

Given the complexities of the immune system, it can be important to show pharmacodynamics in mammalian subjects that can provide information for this human Fc molecule. PK/PD studies were performed with mice as described here.

The study was approved by Genentech's Institutional Animal Care and Use Committee and was conducted using SCID mice. Animals were divided into 3 groups. Animals in Group 1, 2, and 3 were given FLT3L-Fc (SEQ ID NO:37--Flt3L.Q159.Fc.NG) at 0.1, 1.0, and 10 mg/kg, respectively. Whole blood was collected at selected time points for cell population counts by FACS. Plasma was collected and assayed using a huFlt3L ELISA to determine the amount of test article in each sample.

Group mean PK parameters are summarized in Table 5 below.

TABLE 5

Nominal Dose	C _{max} (μ g/mL)	C _{max} /Dose (μ g/mL)/(mg/kg)	AUC _{inf} (μ g/mL*day)	AUC _{inf} /Dose (μ g/mL*day)/(mg/kg)	CL (mL/day/kg)
0.1 mg/kg	2.68	26.8	4.42	22.6	22.6
1 mg/kg	27.9	27.9	77.9	77.9	12.8
10 mg/kg	273	27.3	768	76.8	13

25

The NCA parameters reported in Table 5 include C_{max} (Observed maximum serum concentration after the first dose); C_{max}/Dose (Dose normalized C_{max}); AUC_{inf} (Area under the serum concentration–time curve from Day 0 to infinity); AUC_{inf}/Dose (Dose normalized AUC); and CL (Clearance calculated using the serum concentration–time curve).

30 The results are graphically presented in **FIG. 5**.

In Groups 1-3, C_{max} increased nearly dose-proportionally. A more than dose-proportional increase in AUC was observed, suggesting target-mediated drug disposition (TMDD) impacted PK profiles at all dose groups. In addition, total clearance (CL) was observed to be dose-dependent, suggesting target-mediated CL.

The mean monocyte and DC counts from the peripheral blood samples are shown in FIG. 6A (monocytes) and FIG. 6B (DCs). In all three groups, robust dose-dependent cell expansion was noted in peripheral blood at 0.1-10 mg/kg. The initial kinetics of DC expansion was similar across doses while the duration of expansion was dose/exposure dependent.

Example 6. PKPD Studies in Cynomolgus Monkeys

Additional PKPD studies were performed using varying doses of the Flt3L-Fc (NG2LH) protein (SEQ ID NO:26) administered to cynos. This study was conducted at Charles River Laboratories (Reno, NV) using naive, cynomolgus monkeys. Animals were divided into 4 groups (2 males and 1 female/group). Animals in Group 1 were given Anti-gD (anti-glycoprotein D human IgG1 antibody with N297G mutation) at 10 mg/kg, while animals in Groups 2, 3, and 4 were given FLT3L-Fc at 0.1, 1.0, and 10 mg/kg, respectively. Animals in Group 2 received a single dose whereas animals in other groups were administered two doses (Study Days 1 and 22). Whole blood was collected at selected time points for cell population counts by FACS. Serum was collected and assayed using an anti-huFlt3L-huFc ELISA to determine the amount of test article in each serum sample.

Table 6A below details the dosing of each animal group. Group mean PK parameters are summarized in Table 6B.

TABLE 6A

Group	Nominal Dose	Treatment
1	10 mg/kg	Anti-gD (N297G)
2	0.1 mg/kg	FLT3L-Fc(NG2LH)
3	1 mg/kg	FLT3L-Fc(NG2LH)
4	10 mg/kg	FLT3L-Fc(NG2LH)

TABLE 6B

Group	C_{max} (ug/ml)	$C_{max}/Dose$ (ug/ml)/(mg/kg)	AUC_{0-21} (ug/ml*day)	$AUC_{0-21}/Dose$ (ug/ml*day)/(mg/kg)	CL_{0-14}^* (ml/day/kg)
1	286 ± 26.6	28.6	2140 ± 45.9	214	3.65
2	2.63 ± 0.189	26.3	6.4 ± 0.285	64	13.7
3	29.2 ± 0.413	29.2	141 ± 16.7	141	6.83
4	280 ± 33.1	28	1630 ± 386	163	4.92

The results are graphically presented in **FIG. 7**.

In Groups 2-3, C_{max} increase was nearly dose-proportional. Anti-drug-antibody (ADA) was detected in all dosed animals on or after Day 14 and exposures were impacted in animals with high ADA titers. More than dose-proportional increase in AUC_{0-21} was observed after the first dose, suggesting target-mediated drug disposition (TMDD) impacting PK profiles, particularly at ≤ 1 mg/kg. In addition, total clearance (CL) was observed to be dose-dependent, suggesting target-mediated CL. At 10 mg/kg, the anti-GD and FLT3L-Fc groups had similar PK profiles and the total CL for FLT3L-Fc was 4.92 mL/day/kg.

The mean monocyte, cDC1, and cDC2 counts for all four groups is shown in **FIGS. 8A-8C**. For FLT3L-Fc treated animals, robust dose-dependent cell expansion (monocytes, cDC1, and cDC2) was noted in peripheral blood after 1st dose at 0.1-1 mg/kg. At 10 mg/kg, less expansion was observed. Without being bound by theory, a possible explanation could be that high concentrations of a Flt3 ligand prevent the receptor dimerization required for proliferation and differentiation. At 1-10 mg/kg, lower but sustained cell expansion is observed after the 2nd dose (possibly due to the impact of ADA).

Example 7. Fc γ R and FcRn Binding of Fc(NG2LH)

Effector function of a Fc domain can be affected by certain amino acid changes within the domain. Accordingly, binding of Fc(NG2LH) to Fc γ R and FcRn receptors was measured by Biacore. For these assays, Fc γ Rs were captured through anti-His antibody on the Biacore chip or FcRn was directly immobilized on the chip. Data were collected at the end of the injection (association phase) and the relative binding activity (%) was calculated by normalizing the value of the sample divided by the value of the standard.

A summary of the PTD Biocore data is provided in Table 7 below. The values show relative binding level (%) at 10 ug/ml.

TABLE 7

Antibody	Fc γ RI		Fc γ RI		Fc γ RIIIa V158		FcRn	
	α CD20	α Her2	α CD20	α Her2	α CD20	α Her2	α CD20	α Her2
IgG1 wildtyp	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
IgG1 LALAP	1.74	1.92	-0.34	-0.62	1.36	1.58	95.93	97.86
IgG1 PVA#	2.69	2.51	25.11	26.77	5.16	5.57	104.45	102.81
IgG1 NG	4.50	6.12	0.06	0.385	2.12	2.95	86.73	86.27
IgG1 NG PV (NG2LH)	2.79	2.58	0.09	-0.21	2.09	2.02	94.17	94.72
IgG1 NG PV	2.77	2.17	0.59	-0.40	2.16	1.74	94.65	91.12

IgG1 NG PV	4.00	1.99	0.81	-0.47	2.24	1.46	92.92	93.24
IgG2 wildtype	2.43	2.10	179.04	157.56	3.85	3.33	99.42	95.35
IgG4 SP	70.59	63.59	14.82	18.22	3.41	3.12	66.49	65.08

Example 8. Thermostability of Fc(NG2LH)

Thermostability of the FLT3L ligand fusion protein is one important aspect of developing a therapeutic biologic. After design of the effectorless Fc(NG2LH) protein, differential scanning fluorimetry (DSF) was performed to understand effects of the amino acid changes on thermostability of the Fc protein. DSF monitors thermal unfolding of proteins in the presence of a fluorescent dye and is typically performed by using a real-time PCR instrument (e.g., Bio-Rad CFX). SYPRO orange dye (Invitrogen, cat. no. S6650) is diluted 1:20 in PBS. One μ l of diluted dye is added to 24 μ l Fab protein (~100 μ g/ml) in a well. As the temperature increases from 20°C. to 100°C in a real-time PCR instrument (Bio-Rad CFX), the fluorescence intensity is plotted and the inflection point of the transition curve (T_m) is calculated using, for example, the Boltzmann equation. See Nature Protocols, 2007, 2:2212-2221.

Variant Fc proteins were analyzed in the context of full-length antibodies, specifically, as an anti-CD20 antibody derived from ocrelizumab and as an anti-Her antibody derived from trastuzumab. The stability data for these variant antibodies are provided in Table 8 below. In addition, variant Fc proteins were also tested in the context of an isolated Fc region comprising the sequences described in Table 2 but absent the CH1 domain and thus beginning N-terminally at the hinge sequence DKTHT. The stability data for these variant Fc proteins are provided in Table 9 below.

TABLE 8

Construct	Heavy Chain Constant Region SEQ ID NO	α -CD20 IgG ($^{\circ}$ C)	α -Her2 IgG ($^{\circ}$ C)	Ave. ($^{\circ}$ C)	Δ IgG1 ($^{\circ}$ C)
IgG2 wildtype (wt)	8	57	57	57	-4.3
IgG4 SP	9	59	59	59	-2.3
IgG1 wt	1	61.2	61.4	61.3	0
IgG1 LALAPG	7	59.7	59.7	59.7	-1.6
IgG1 PVA#	4	61.9	61.95	61.9	+0.6
IgG1 NG	3	52.8	53.2	53	-8.3
IgG1 NG PVA#	2	55.2	55.6	55.4	-5.9
IgG1 NG PVA# G	5	54.4	54.2	54.3	-7.0
IgG1 NG PVA# GSS	6	50.4	50	50.2	-11.1

TABLE 9

Construct	SEQ ID NO	T _m (°C)	Δ IgG1 (°C)
Fc N297G	14	59.3	-7.1
Fc NG2LH	13	61.6	-4.8
Fc PG	16	63.4	-3.0
Fc LALAPG	18	65.5	-0.9
Fc wt	12	66.4	0.0
LALA	17	67.0	+0.6
Fc PVA#	15	68.5	+2.1

The data show that for both a full-length IgG and an isolated Fc protein, combining the PVA# variant with the N297G substitution results in an increase in thermostability of about 2°C relative to the thermostability of the Fc construct having the N297G mutation alone, which was unexpected.

Example 9. Variants of an Effectorless Flt3L-Fc Fusion Protein

Experiments were performed to assess immunogenicity of various Flt3L-Fc fusion proteins. Specifically, immunogenicity of certain proteins was tested in a T cell proliferation assay. As shown in FIG. 9A, neither Flt3L.P167.Fc.NG2LH (SEQ ID NO:26) nor Flt3L.L165.Fc.NG2LH (SEQ ID NO:33), had detectable immunogenicity. Unexpectedly, there was significant immunogenicity detected with the introduction of the Flt3L W144D mutation (SEQ ID NO:42).

Another experiment assessed immunogenicity of glycosylation variants of the Flt3L protein. As shown in FIG. 9A, introduction of the N149E, S151D or S151E mutations did not cause any detectable increase in CD4 T cell proliferation. N149E, S151D and S151E indicate the amino acid change and position relative to SEQ ID NO:21.

Example 10. Effectorless Flt3L-Fc Fusion Protein in an ADCP Assay

Experiments were performed to compare the antibody dependent cellular phagocytosis (ADCP) activity of 3 variants of a human recombinant protein Flt3L-Fc construct: 1) Flt3L-Fc-wildtype IgG1 (SEQ ID NO:27) Flt3L with Fc of hIgG1.NG2LH (SEQ ID NO:26) and 3) Flt3L with hIgG1.N297G (SEQ ID NO:28). EB10.hIgG1, an anti-Flt3 monoclonal antibody (Piloto et al., 2006, Cancer Res. 66:4843-4851) was used as a positive control. ADCP assays were carried out using primary monocyte-derived macrophages from healthy human donors as effector cells and SEM, human acute lymphoblastic leukemia cells as target cells. Briefly, primary human macrophages were generated by isolating CD14 positive cells from healthy human PBMCs by positive selection (Miltenyi Biotec Inc, Auburn, California) and cultured

in macrophage differentiation media (RPMI 1640, 10% FBS, 1% Glutamax, 1% penicillin/streptomycin, and 20 ng/mL M-CSF (R&D Systems, Minneapolis, MN)) at 37 °C in a humidified incubator with 5% CO₂. On day 3, cells are stimulated with 50ng/mL of M-CSF and were cultured for 4 more days. On day 7, macrophages were stained with 10 μM Cell Trace Violet (Thermo Fisher Scientific, Eugene, OR), diluted to 1×10^6 cells/mL in ADCP assay media (IMDM, 10% FBS, 1% Glutamax, 1% penicillin/streptomycin), and added at 50 uL/well to a 96 well low adherent U-bottom plate (Costar, Corning, NY). Target SEM cells were diluted in ADCP assay media at 2×10^6 cells/mL, pre-labeled with pHrodo (Thermo Fisher Scientific, Eugene, OR), and added (50 uL/well) to the assay plate containing macrophages. Then 100 uL of serial dilutions of test antibodies (table 1) were added to each well containing macrophages and SEM cells, followed by incubation at 37°C with 5% carbon dioxide for 4.5 hours. The final concentrations of antibodies ranged from 0.457-3000 ng/mL following 3-fold serial dilutions for a total of 11 samples per test antibody. After incubation, the cells were centrifuged at 1200 rpm for 5 min and washed in PBS before being fixed in 4% paraformaldehyde for 10 min at 4 °C. The cells were analyzed with a flow cytometer (BD Biosciences FACSCanto IVD 10). Phagocytosis was analyzed via FlowJo (Tree Star, Inc.; Ashland, OR). Cell Trace Violet fluorescence was used to gate the macrophages in the sample population. Phagocytosis was determined by measuring the percentage of pHrodo Green positive macrophages. The degree of phagocytosis was normalized by subtracting the percent pHrodo Green positive macrophages from the control condition (no antibody present). All data points were collected in duplicate. Percent phagocytosis (%ADCP) was plotted opposite antibody concentration and fitted to a four-parameter model using GraphPad Prism (LaJolla, CA). This procedure was conducted in independent experiments with 3 donors.

In this study, the Flt3-expressing SEM cells were used as target cells. SEM target cells were pre-labeled with pHrodo AM that fluorescence brightly in acidic pH. As the phagosome containing the target cells becomes increasingly acidic, the pHrodo green fluorescent signal increases, which can be detected on a flow cytometer. ADCP activity induced by Flt3L with Fc of hIgG1.NG2LH (SEQ ID NO:26, 5.1 mg/mL), Flt3L with hIgG1.N297G (SEQ ID NO:28, 1.92 mg/mL), Flt3L with WT IgG1 (SEQ ID NO:27, 2.5 mg/mL) and EB10.hIgG1 (3.17 mg/mL) were examined and compared in three independent experiments using monocyte derived macrophages from three different donors.

All data points were collected in duplicate, and the mean of the % phagocytosis (%ADCP) was plotted against the concentration of the tested molecule (drug concentration: 3 ug/mL-0.457 ng/mL). The data were fitted with a four-parameter model. A representative

dose-response ADCP curve is shown in **FIG. 10**. Subject to the inherent limitations of the ADCP assay, in all three experiments ADCP activity induced by Flt3L with WT IgG1 and EB10.hIgG1 was observed in Flt3 expressing cells. In addition, FLT3L-Fc-IgG-N297G induced relatively low ADCP while little to no ADCP activity was detected with FLT 3L-Fc-IgG

5 NG2LH.

Additional studies can be done to measure the ADCP activity of additional Flt3L-Fc fusion proteins which comprise the Fc NG2LH (SEQ ID NO:13), such as Flt3L-Fc fusion protein which contain 1, 2, or 3 amino acid substitutions in the Flt3L portion. Such studies can show additional Flt3L-Fc fusion proteins which have little to no ADCP activity, but which can

10 activate expansion of DCs in vivo.

Table 10
SEQUENCE LISTING

SEQ ID NO:	Description of Heavy Chain Constant Region (CH1 – CH3)	Substitution(s) (EU Numbering)	Sequence
1	IgG1 WT		ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK
2	IgG1 NG2LH, aka IgG1 NG PVA# (NG2LH)	N297G/E233P/L234V/L235A/G236#	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVL HQQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK
3	IgG1 NG	N297G	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK
4	IgG1 PVA#	E233P/L234V/L235A/G236#	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK

5	IgG1 NG PVA# G	N297G/E233P/ L234V/L235A/ G236#/A327G	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVL HQDWLNGKEYKCKVSNKGLPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
6	IgG1 NG PVA# GSS	N297G/E233P/ L234V/L235A/ G236#/A327G/ A330S/P331S	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVL HQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
7	IgG1 PG	P329G	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL LHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
8	IgG1 LALA	L234A/L235A	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
9	IgG1 LALAPG	L234A/L235A/ P329G	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL LHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
10	IgG2 WT		ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKT VERKCCVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV HEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKGLPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
11	IgG4 SP	S228P	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKR VESKYGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSREEMTKN NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS RLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
12	IgG1 Fc region		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
13	Fc(NG2LH)		DKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:	Construct Name	Sequence
14	IgG1 N297G Fc region	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSLSPGK
15	NVA# Fc region	DKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSLSPGK
16	IgG1 PG Fc domain	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSLSPGK
17	IgG1 LALA Fc domain	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSLSPGK
18	LALAPG Fc region	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSLSPGK
19	LH residues (wt)	ELLG
20	PVAGP (non-variant part of Fc)	PVAGP
21	P49771	MTVLAPAWSPTTYLLLLLLLLSSGLSGTQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQDEELCGGLWRLVLAQRWMERLKTAVAGSKMQGLLERVNTIEHFVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPWITRQNFSCRLELQCQPDSSSTLPPPWSPRPLEATAPAPQPLLLLLLPPVGLLLAAAWCLHWQTRRRTRPRPGEQVPPVPSQDLLLLVEH
22	Flt3L 27-167	TQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQDEELCGGLWRLVLAQRWMERLKTAVAGSKMQGLLERVNTIEHFVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPWITRQNFSCRLELQCQPDSSSTLPP
23	CDX-301	TQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQDEELCGGLWRLVLAQRWMERLKTAVAGSKMQGLLERVNTIEHFVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPWITRQNFSCRLELQCQPDSSSTLPPPWSPRPLEATAP
24	Flt3L Signal Sequence	MTVLAPAWSPTTYLLLLLLLLSSGLSG
25	hFLT3L.P168.5aa.hinge.hlgG1.NG.PVA#	TQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQDEELCGGLWRLVLAQRWMERLKTAVAGSKMQGLLERVNTIEHFVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPWITRQNFSCRLELQCQPDSSSTLPPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSLSPGK
26	hFLT3L.P167.5aa.hinge.hlgG1.NG.PVA#	TQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQD

	Please confirm this sequence	EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIHFTVKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNFRCLELQCQPDSSSTLPPDKTHTCPPCPAPPVAGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP PVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN HYTQKSLSLSPGK
27	hFLT3L.P167.5aa.hinge.hlgG1	TQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIHFTVKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNFRCLELQCQPDSSSTLPPDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGK
28	hFLT3L.P167.5aa.hinge.hlgG1.NG	TQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIHFTVKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNFRCLELQCQPDSSSTLPPDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGK
29	hFLT3L.P167.5aa.hinge.hlgG1.PVA#	TQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIHFTVKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNFRCLELQCQPDSSSTLPPDKTHTCPPCPAPPVAGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP PVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN HYTQKSLSLSPGK
30	hFLT3L.P167.5aa.hinge.hlgG1.LALAPG	TQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIHFTVKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNFRCLELQCQPDSSSTLPPDKTHTCPPCPAPEAAGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGK
31	hFLT3L.P167.7aa.hinge.hlgG4	TQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIHFTVKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNFRCLELQCQPDSSSTLPPESKYGPPCPSCPAEFLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSEQDEPQFN WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSGDSFFLYSRLTVDKSRWQEGNVFCFSVMHEA LHNHYTQKSLSLSLGK
32	hFLT3L.P166.5aa.hinge.hlgG1.NG.PVA#	TQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQD

		EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIH FVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNF SRCL ELQCQPD SSTLPDKTHTCPPCPAPPVAGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK
33	hFLT3L.L165.5aa.hinge.hlgG1.NG.PVA#	TQDCSFQHSPISSDFAVKIRELS DYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIH FVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNF SRCL ELQCQPD SSTLPDKTHTCPPCPAPPVAGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
34	hFLT3L.L164.5aa.hinge.hlgG1.NG.PVA#	TQDCSFQHSPISSDFAVKIRELS DYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIH FVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNF SRCL ELQCQPD SSTLPDKTHTCPPCPAPPVAGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
35	hFLT3L.C158.5aa.hinge.hlgG1.NG.PVA#	TQDCSFQHSPISSDFAVKIRELS DYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIH FVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNF SRCL ELQCQPD SSTLPDKTHTCPPCPAPPVAGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
36	hFLT3L.P179.hlgG1.N297G	TQDCSFQHSPISSDFAVKIRELS DYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIH FVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNF SRCL ELQCQPD SSTLPDPWSPRPLEATAPGGGSVT DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
37	hFLT3L.Q159.hlgG1.N297G	TQDCSFQHSPISSDFAVKIRELS DYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIH FVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNF SRCL ELQCQGGGSVTDKTHTCPPCPAPPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK
38	hFLT3L.C158.3aa.hinge.hlgG1.NG.PVA#	TQDCSFQHSPISSDFAVKIRELS DYLLQDYPVTVASNLQD

		EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIH FVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNF SRCL ELQC TH TCP PCAPPVAGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSG SFFL YSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLS PGK
39	hFTL3L.C158.no.hinge.hlgG1.NG.PVA#	TQDCSFQHSPISDFAVKIRELS DYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIH FVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNF SRCL ELQC TH TCP PCAPPVAGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPVLDSG SFFLYSKL TVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK
40	hFTL3L.D161.no.hinge.hlgG1.NG.PVA#	TQDCSFQHSPISDFAVKIRELS DYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIH FVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNF SRCL ELQC TH TCP PCAPPVAGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSG SFFL YSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLS PGK
41	hFTL3L.S163.no.hinge.hlgG1.NG.PVA#	TQDCSFQHSPISDFAVKIRELS DYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIH FVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNF SRCL ELQC TH TCP PCAPPVAGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSG S FFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLS LSPGK
42	Hs_FLT3LG.M1-L165.W144D.O.pRK- 5aa.hlgG1.NG2LH	TQDCSFQHSPISDFAVKIRELS DYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIH FVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPD ITRQNF SRCL ELQC TH TCP PCAPPVAGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSG SFFL YSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK
43	Hs_FLT3LG.M1-L165.S151D.O.pRK- 5aa.hlgG1.NG2LH	TQDCSFQHSPISDFAVKIRELS DYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT EIH FVTKCAFQPPPSCLRFVQTNISRLQETSEQLVALKPW ITRQNF DRCL ELQC TH TCP PCAPPVAGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSG SFFL YSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK
44	Hs_FLT3LG.M1-L165.S151E.O.pRK- 5aa.hlgG1.NG2LH	TQDCSFQHSPISDFAVKIRELS DYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKT VAGSKMQGLLERVNT

		EIHFTKCAFPQPPSCLRFVQTNISRLLOETSEQLVALKPW ITRQNFDRCLELQCQPDSSSTLDKTHTCPPCPAPPVAGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDDSGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
45	hFLT3L.P167.5aa.hinge.hlgG1.NG.PVA# with native signal sequence	MTVLAPAWSPTTYLLLLLLLLSSGLSGTQDCSFQHSPISSDF AVKIRELSDYLLQDYPVTVASNLQDEELCGGLWRLVLAQ RWMERLKTVAGSKMQGLLERNTEIHFTKCAFPQPPSC LRFVQTNISRLLOETSEQLVALKPWITRQNFDRCLELQCQ PDSSSTLPPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIS RPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
46	Flt3L-Fc(IgG4)	TQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQD EELCGGLWRLVLAQRWMERLKTVAGSKMQGLLERNTE EIHFTKCAFPQPPSCLRFVQTNISRLLOETSEQLVALKPW ITRQNFDRCLELQCQPDSSSTLPPWSPRPLEATAPTAPQP RSPPCPSCAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKA KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW QEGNVFSCSVMHEALHNHYTQKSLSLSPGK
47	huMAb4D5-8	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQK PGKAPKLLIYSASFYSGVPSRFSGSRSGTFTLTISSLQPE DFATYYCQGHYTTTPPTFGQGTKVEIK

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all

5 patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

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 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), 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.
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, 27-179, 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 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.
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 not 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 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

5 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
10 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-
15 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. A method for expanding the number of dendritic cells (DCs) in a subject, comprising
20 administering to the subject the Flt3L-Fc fusion protein of any one of claims 1-9.

22. The method of claim 21, comprising administering to the subject a dose of about 0.1 mg/kg to 50 mg/kg of the Flt3L-Fc fusion protein.

23. A method for treating a cancer, comprising administering to a subject in need thereof
25 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 of claim 23, comprising administering to the subject a dose of about 0.1 mg/kg to 50 mg/kg of the Flt3L-Fc fusion protein.

25. The method of claim 23 or 24, further comprising administering to the subject a second
30 therapeutic agent.

26. The method of claim 25, wherein the second therapeutic agent is an adjuvant, a dendritic maturation factor, and/or a checkpoint inhibitor.

27. The method of any one of claims 21 to 26, wherein the method comprises administering a DC maturation factor.

28. The method of claim 27, wherein the DC maturation factor is selected from polyIC, polyICLC, DC40, radiation therapy, and chemotherapy.
29. The method of any one of claims 21 to 28, comprising administering an immune checkpoint inhibitor.
- 5 30. The method of claim 29, wherein the checkpoint inhibitor is administered before, simultaneously with, or after administration of the Fc-effectorless Flt3L-Fc fusion protein.
31. The method 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.
- 10 32. The method of any one of claims 21 to 31, wherein 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.
- 15 33. The method of any one of claims 21-32, wherein the subject was previously treated with a checkpoint inhibitor.
34. The method 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
- 20 years after receiving a last dose of checkpoint immunotherapy.
35. The method of any one of claims 21-34, wherein the cancer in the subject has 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
- 25 ID NO:13 comprise the amino acid sequence PVAGP (SEQ ID NO:20) or residue 76 of SEQ ID NO:13 is a glycine.
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.
- 30 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.
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
5 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 humanized antibody, or a chimeric antibody.
45. The antibody of claim 41 or 42, wherein the antibody is a bispecific or multispecific
10 antibody.

15