(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
01 October 2020 (01.10.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/198654 A1

(51) International Patent Classification:
A61K 38/00 (2006.01) *A61K 39/395* (2006.01)
A61K 38/21 (2006.01) *A61K 45/06* (2006.01)
A61K 39/00 (2006.01) *A61K 47/48* (2006.01)

(21) International Application Number:
PCT/US2020/025411

(22) International Filing Date:
27 March 2020 (27.03.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/825,569 28 March 2019 (28.03.2019) US
62/906,431 26 September 2019 (26.09.2019) US

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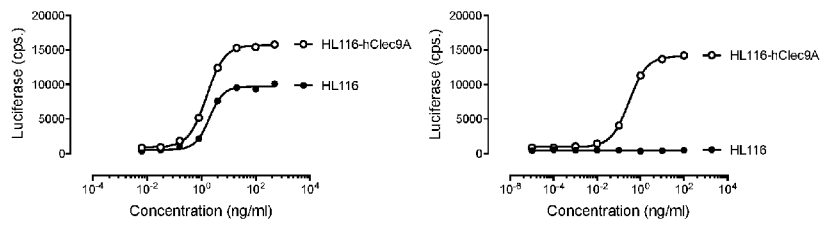
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: THERAPEUTIC INTERFERON ALPHA 1 PROTEINS

FIG. 20



(57) Abstract: The present invention relates, in part, to chimeric proteins or chimeric protein complexes, such as Fc-based chimeric protein complexes, comprising interferon alpha 1, or a variant thereof, and their use as therapeutic agents.

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THERAPEUTIC INTERFERON ALPHA 1 PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of and priority to U.S. Provisional Patent Application No. 62/906,431 filed September 26, 2019, and to U.S. Provisional Patent Application No. 62/825,569 filed March 28, 2019, the contents of which are hereby incorporated by reference in their entirety.

FIELD

The present invention relates, in part, to chimeric proteins, or chimeric protein complexes (including Fc-based chimeric protein complexes) comprising interferon alpha 1 (IFNα1) or variants thereof and their use as therapeutic agents.

SEQUENCE LISTING

The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety. A computer readable format copy of the Sequence Listing (filename: ORN-057PC_Sequence_Listing_ST25, date recorded: March 26, 2020; file size: 385,024 bytes).

BACKGROUND

Type I interferons (IFNs) form a family of multifunctional cytokines that play major roles in the immune responses and other biological processes. The human type I IFNs comprises 13 distinct non-allelic alpha subtypes, one beta subtype, and one omega subtype. Type I interferons all appear to bind a common receptor, type I interferon-α/β receptor (IFNAR), composed of the IFNAR1 and IFNAR2 subunits, albeit to different extents. Upon binding of type I IFNs, IFNAR activates the JAK-STAT signaling pathway to elicit various biological effects. Differential activities of IFN subtypes have been reported and, accordingly, differentially used in clinically for the treatment of various diseases and disorders, including, *e.g.*, viral hepatitis (IFN-α2), multiple sclerosis and cancer therapy (e.g., IFN-β or IFNα2). The assembly of a IFN-IFNAR ligand-receptor complex initiates activation of several signal transduction pathways that, depending upon the cell type, IFN subtype engaging with IFNAR and the type of receptor-activation associated signal (amplitude, duration etc.) modify cellular differentiation and/or functions. While type I interferons share the property of engaging IFNAR, they can do so to varying degrees, and, consequently, can also have non-redundant functions. These are related, at least in part, to differences in the type and quality of signal response they elicit upon engaging IFNAR. This is relevant to various type I IFN responses, such as immune stimulatory, antiproliferative, antiviral and other biological effects. IFNα2 is among the most potent IFNAR-binding ligands and IFNAR signaling activators. Various efforts have been reported in the generation of even more potent IFNAR binders for potential therapeutic use.

In contrast to other type I IFNs, Interferon alpha 1 (IFNα1) is a member of the type I interferon family that is characterized by a markedly lower affinity for the IFNAR2 receptor (20-fold lower binding affinity for IFNAR2 compared to IFN-α2; Jaks et al., J. Mol. Biol. 2007; 366:525-534). It is deemed to be the weakest, naturally occuring human IFNAR-binding type I IFN ligand and IFNAR signaling activator among the type I IFN family (Moll et al.,

Cytokine 2011; 53:52-59). These characteristics, among others, have contributed to a longtime lack of general interest in and pursuit of IFNα1 as a potential therapeutic agent. The efficacy of type I IFNs in clinical practice is limited by ineffective dosing due to significant systemic toxicity and side effects, including flu-like syndrome, depression, hepatotoxicity, autoimmune disease, thyroid dysfunction, and weight loss. It could therefore be highly worthwhile to localize and target IFN activity toward only the cellular population that should be treated with IFN (*e.g.*, infected organ or tumor mass) or activated by IFN (*e.g.*, subsets of immune cells).

Accordingly, there remains a need for safe and effective IFNα1-based therapeutics with improved pharmacokinetic and therapeutic properties and minimal toxicity profiles.

SUMMARY

Accordingly, in some aspects, the present invention relates to chimeric proteins and chimeric protein complexes, including Fc-based chimeric protein complexes, comprising wild type IFNα1, or variants thereof, as a signaling agent. The term variants as used herein includes IFNα1 mutants. In an embodiment, the IFNα1 comprises an amino acid sequence of SEQ ID NO: 1 or variants thereof.

The present disclosure concerns, in part, findings that chimeric proteins or chimeric protein complexes comprising wild type IFNα1, and variants thereof, exhibit substantially reduced IFNAR-activation signaling activity compared to wild type IFNα1. This reduced IFNAR-activation signaling activity, however, can be induced and/or restored at a target cell when directed to such a cell through a targeting moiety. Surprisingly, the induced IFNα1 activity at a target cell, achieved through targeting of chimeric proteins or chimeric protein complexes comprising IFNα1, or variants thereof, may be similar or greater at the target cell than that of wild type IFNα1. Furthermore, and equally surprising, the targeted IFNα1 activity of the chimeric protein or chimeric protein complexes comprising IFNα1, or variants thereof, may be similar to or even greater than that of wild type IFNα2, which is among the most potent natural type I IFNs (e.g., ~10-100 fold more potent than wild type IFNα1, e.g., depending on cell type). Importantly, the IFNα1 chimeric proteins and chimeric protein complexes comprising IFNα1 described herein, exhibit substantial and surprising selectivity for target cells versus non-target cells, and substantially more than, for example, achieved with targeted wild type IFNα2 chimeric protein(s). In summary, a unique combination of highly potent and highly cell target-selective IFNAR-signaling activation can be achieved with IFNα1 compositions, and variants thereof, described herein. Accordingly, in various embodiments, the present invention relates to target-selective IFNAR-activators with a high therapeutic index, as well as excellent pharmaceutical properties, for use in the treatment of various diseases, including cancer, infectious disease, and autoimmune disease.

In some embodiments the incorporation of wild type IFNα1 in a chimeric protein or chimeric protein complex, such as, for example, through genetic fusion or attachment (e.g. the formation of a complex), reduces the biological activity of IFNα1 (sometimes referred to as “attenuated by fusion”). For example, wild type IFNα1 incorporated in chimeric proteins or chimeric protein complexes may have reduced affinity and/or activity compared to wild type IFNα1 interferon for a therapeutic receptor. In an embodiment, the therapeutic receptor is the interferon-α/β receptor (IFNAR), which is composed of the IFNAR1 and IFNAR2 subunits. In some embodiments, the loss in affinity and/or activity of wild type IFNα1 for a therapeutic receptor, e.g., IFNAR, can be induced and restored upon

directing or targeting of the chimeric protein or chimeric protein complex comprising IFNα1 to a target cell through a targeting moiety. In some embodiments, the induction and restoration of IFNα1-mediated IFNAR-activation at a target cell may reach a level that is similar to or higher than IFNAR-activation achieved with wild type (non-chimeric) IFNα1. In some embodiments, the IFNα1 is a variant that comprises one or more mutations which reduce undesired disulphide pairings to improve product homogeneity and pharmaceutical properties of the chimeric protein or chimeric protein complexes, while simultaneously maintaining or avoiding substantial loss of IFNAR-activation of the modified IFNα1 compared to wild type IFNα1 in the context of chimeric proteins or chimeric protein complexes, including maintaining or avoiding substantial loss of restoration and induction of IFNAR-activation by the modified IFNα1 when directed or targeted to a target cell through a targeting moiety.

In some embodiments, the IFNα1 is modified, *i.e.*, is a variant and comprises one or more mutations in IFNα1. In some embodiments, the one or more mutations reduce the biological activity of the IFNα1 (sometime referred to as "attenuated by mutation"). For example, the one or more mutations may reduce the affinity and/or activity of the IFNα1 interferon for a therapeutic receptor. In an embodiment, the therapeutic receptor is the interferon-α/β receptor (IFNAR), which is composed of the IFNAR1 and IFNAR2 subunits. In an embodiment, the modified IFNα1 comprises one or more mutations that reduce its affinity and/or activity for IFNAR1. In another embodiment, the modified IFNα1 comprises one or more mutations that reduce its affinity and/or activity for IFNAR2. In an embodiment, the modified IFNα1 comprises one or more mutations that reduce its affinity and/or activity for IFNAR1 and comprises one or more mutations that reduce its affinity and/or activity for IFNAR2. In some embodiments, the loss in affinity and/or activity of the modified IFNα1 ("attenuated by mutation") for a therapeutic receptor, e.g., IFNAR1, IFNAR2 and/or IFNAR, can be induced and restored upon directing or targeting of the chimeric protein or chimeric protein complex comprising the modified IFNα1 to a target cell through a targeting moiety. In some embodiments, the modified IFNα1 variant ("attenuated by mutation") that comprises one or more mutations that reduce its affinity and/or activity for IFNAR1, IFNAR2 and/or IFNAR, further comprises one or more mutations that reduce undesired disulphide pairings to improve product homogeneity and pharmaceutical properties of the chimeric protein or chimeric protein complexes, while simultaneously maintaining or avoiding substantial loss of induction and/or restoration of IFNAR-activation activity by the modified IFNα1 ("attenuated by mutation") when directed/targeted to a target cell through a targeting moiety.

In some embodiments, the chimeric proteins and chimeric protein complexes, including Fc-based chimeric protein complexes, comprises one or more additional signaling agents, *e.g.*, without limitation, an interferon, an interleukin, and a tumor necrosis factor, that may be modified. In various embodiments, the chimeric proteins and chimeric protein complexes, including Fc-based chimeric protein complexes, of the invention provides improved safety and/or therapeutic activity and/or pharmacokinetic profiles (*e.g.*, increased serum half-life) compared to an untargeted and/or unmodified IFNα1 or an unmodified, wild type IFN-α, such as, IFN-α2a or IFN-α2b.

In various embodiments, the chimeric proteins and chimeric protein complexes, including Fc-based chimeric protein complexes, comprise one or more targeting moieties which have recognition domains (*e.g.* antigen recognition domains, including without limitation various antibody formats, inclusive of single-domain antibodies)

which specifically bind to a target (*e.g.* antigen, receptor) of interest. In various embodiments, the targeting moieties have recognition domains that specifically bind to a target (*e.g.* antigen, receptor) of interest, including those found on one or more immune cells, which can include, without limitation, T cells, cytotoxic T lymphocytes, T helper cells, T regulatory cells (Tregs), natural killer (NK) cells, natural killer T (NKT) cells, anti-tumor and tumor macrophages (*e.g.* M1 and M2 macrophages), B cells, B regulatory (Breg) cells, neutrophils, monocytes, myeloid derived cells, and dendritic cells. In various embodiments, the targeting moieties have recognition domains that specifically bind to a target (*e.g.* antigen, receptor) of interest, including those found on one or more tumor cells, endothelial cells, epithelial cells, mesenchymal cells, stromal cells or other cell types that are characteristic of and/or unique for specific organs and/or tissues, including those specifically associated with disease. In some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) of interest and effectively recruit one or more immune cells. In some embodiments, the targets (*e.g.* antigens, receptors) of interest can be found on one or more tumor cells. In some embodiments, the present chimeric proteins, chimeric protein complexes, including Fc-based chimeric protein complexes, may recruit an immune cell, *e.g.*, an immune cell that can kill and/or suppress a tumor cell, or modulate other immune cells, to a site of action (such as, by way of non-limiting example, the tumor microenvironment). In some embodiments, the present chimeric proteins, chimeric protein complexes, including Fc-based chimeric protein complexes, may modulate an immune cell at a site of action, or recruit an immune cell to a site of action that is associated with an autoimmune disease, inflammatory disease, infection, metabolic and/or cardiovascular disease (such as, by way of non-limiting example, the disease microenvironment). In some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) of interest that is part of a non-cellular structure.

In various embodiments, the present chimeric proteins and chimeric protein complexes, including Fc-based chimeric protein complexes find use in the treatment of various diseases or disorders such as cancer, infections, immune disorders, autoimmune diseases, cardiovascular diseases, wound healing, ischemia-related diseases, neurodegenerative diseases, metabolic diseases and many other diseases and disorders, and the present invention encompasses various methods of treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A-F, 2A-H, 3A-H, 4A-D, 5A-F, 6A-J, 7A-D, 8A-F, 9A-J, 10A-F, 11A-L, 12A-L, 13A-F, 14A-L, 15A-L, 16A-J, 17A-J, 18A-F, and **19A-F** show various non-limiting illustrative schematics of the Fc-based chimeric protein complexes of the present invention. In embodiments, each schematic is a composition of the present invention. Where applicable in the figures, "TM" refers to a "targeting moiety" as described herein, "SA" refers to a "signaling agent" as described herein, "⌙" is an optional "linker" as described herein, the two long parallel rectangles are human Fc domains, *e.g.* from IgG1, from IgG2, or from IgG4, as described herein and optionally with effector knock-out and/or stabilization mutations as also described herein, and the two long parallel rectangles with one having a protrusion and the other having an indentation are human Fc domains, *e.g.* from IgG1, from IgG2, or from IgG4 as described herein, with knob-in-hole and/or ionic pair (a/k/a charged pairs, ionic bond, or charged residue

pair) mutations as described herein and optionally with effector knock-out and/or stabilization mutations as also described herein.

FIGs. 1A-F show illustrative homodimeric 2-chain complexes. These figures show illustrative configurations for the homodimeric 2-chain complexes.

FIGs. 2A-H show illustrative homodimeric 2-chain complexes with two targeting moieties (TM) (as described herein, more targeting moieties may be present in some embodiments). In embodiments, the position of TM1 and TM2 are interchangeable. In embodiments, the constructs shown in the box (*i.e.*, Figs. 2G and 2H) have signaling agent (SA) between TM1 and TM2 or between TM1 and Fc.

FIGs. 3A-H show illustrative homodimeric 2-chain complexes with two signaling agents (as described herein, more signaling agents may be present in some embodiments). In embodiments, the position of SA1 and SA2 are interchangeable. In embodiments, the constructs shown in the box (*i.e.*, Figs. 3G and 3H) have TM between SA1 and SA2 or TM at N- or C-terminus.

FIGs. 4A-D show illustrative heterodimeric 2-chain complexes with split TM and SA chains, namely the TM on the knob chain of the Fc and the SA on hole chain of the Fc.

FIGs. 5A-F show illustrative heterodimeric 2-chain complexes with split TM and SA chains, namely with both TMs on the knob chain of the Fc and with SA on hole chain of the Fc, with two targeting moieties (as described herein, more targeting moieties may be present in some embodiments). In embodiments, the position of TM1 and TM2 are interchangeable. In some embodiments, TM1 and TM2 can be identical.

FIGs. 6A-J show illustrative heterodimeric 2-chain complexes with split TM and SA chains, namely with TM on the knob chain of the Fc and with a SA on the hole chain of the Fc, with two signaling agents (as described herein, more signaling agents may be present in some embodiments). In these orientations and/or configurations, one SA is on the knob chain and one SA is on the hole chain. In embodiments, the position of SA1 and SA2 are interchangeable.

FIGs. 7A-D show illustrative heterodimeric 2-chain complexes with split TM and SA chains, namely the SA on the knob chain of the Fc and the TM on hole chain of the Fc.

FIGs. 8A-F show illustrative heterodimeric 2-chain complexes with split TM and SA chains, namely with SA on the knob chain of the Fc and both TMs on hole chain of the Fc, with two targeting moieties (as described herein, more targeting moieties may be present in some embodiments). In embodiments, the position of TM1 and TM2 are interchangeable. In some embodiments, TM1 and TM2 can be identical.

FIGs. 9A-J show illustrative heterodimeric 2-chain complexes with split TM and SA chains, namely with SA on the knob chain of the Fc and TM on hole chain of the Fc, with two signaling agents (as described herein, more signaling agents may be present in some embodiments). In these orientations and/or configurations, one SA is on the knob chain and one SA is on the hole chain. In embodiments, the position of SA1 and SA2 are interchangeable.

FIGs. 10A-F show illustrative heterodimeric 2-chain complexes with TM and SA on the same chain, namely the SA and TM both on the knob chain of the Fc.

FIGs. 11A-L show illustrative heterodimeric 2-chain complexes with a TM and a SA on the same chain, namely with SA and with TM both on the knob chain of the Fc, with two targeting moieties (as described herein, more targeting moieties may be present in some embodiments). In embodiments, the position of TM1 and TM2 are interchangeable. In some embodiments, TM1 and TM2 can be identical.

FIGs. 12A-L show illustrative heterodimeric 2-chain complexes with a TM and a SA on the same chain, namely with SA and with TM both on the knob chain of the Fc, with two signaling agents (as described herein, more signaling agents may be present in some embodiments). In embodiments, the position of SA1 and SA2 are interchangeable.

FIGs. 13A-F show illustrative heterodimeric 2-chain complexes with TM and SA on the same chain, namely the SA and TM both on the hole chain of the Fc.

FIGs. 14A-L show illustrative heterodimeric 2-chain complexes with a TM and a SA on the same chain, namely with SA and with TM both on the hole chain of the Fc, with two targeting moieties (as described herein, more targeting moieties are present in some embodiments). In embodiments, the position of TM1 and TM2 are interchangeable. In embodiments, TM1 and TM2 can be identical.

FIGs. 15A-L show illustrative heterodimeric 2-chain complexes with a TM and a SA on the same chain, namely with SA and with TM both on the hole chain of the Fc, with two signaling agents (as described herein, more signaling agents may be present in some embodiments). In embodiments, the position of SA1 and SA2 are interchangeable.

FIGs. 16A-J show illustrative heterodimeric 2-chain complexes with two targeting moieties (as described herein, more targeting moieties may be present in some embodiments) and with SA on knob Fc and TM on each chain. In embodiments, TM1 and TM2 can be identical.

FIGs. 17A-J show illustrative heterodimeric 2-chain complexes with two targeting moieties (as described herein, more targeting moieties may be present in some embodiments) and with SA on hole Fc and TM on each chain. In embodiments, TM1 and TM2 can be identical.

FIGs. 18A-F show illustrative heterodimeric 2-chain complexes with two signaling agents (as described herein, more signaling agents may be present in some embodiments) and with split SA and TM chains: SA on knob and TM on hole Fc.

FIGs. 19A-F show illustrative heterodimeric 2-chain complexes with two signaling agents (as described herein, more signaling agents may be present in some embodiments) and with split SA and TM chains: TM on knob and SA on hole Fc.

FIG. 20 shows biological activity of IFNα1 and Clec9A VHH Fc ActaFeron (AFN) on HL116 and HL116-hClec9A cells. Parental HL116 or the derived HL116-hClec9A cells were stimulated for 6 hours with a serial dilution of Fc AFNs. Average luciferase values (± STDEV) of triplicate measurements are plotted.

FIGs. 21A-D show IFNα1 (also represented herein as IFNα1) and IFN-α2 (also represented herein as IFNα2) signaling in peripheral blood mononuclear cells (PBMC) upon targeting. PBMCs from buffy coats of healthy donors were stained for CD20 and subsequently stimulated with a serial dilution of IFNα2 (*i.e.* without a targeting moiety),

CD20 VHH-IFNα2 (a chimera of a CD20-directed VHH targeting moiety and wild type IFNα2), IFNα1 (*i.e.* without a targeting moiety), or CD20 VHH-IFNα1 (a chimera of a CD20-directed VHH targeting moiety and wild type IFNα1) for 15 minutes. STAT1 phosphorylation was quantified in FACS and plotted for CD20 positive and CD20 negative PBMCs. The data for IFN-α2 is shown in **FIG. 21A**, the data for CD20 VHH-IFNα2 is shown in **FIG. 21B**, the data for IFNα1 is shown in **FIG. 21C** and the data for CD20 VHH-IFNα1 is shown in **FIG. 21D**.

FIGs. 22A-D show IFNα1 and IFN-α2 signalling in HL116 and HL116-hCD20 cells upon targeting. Parental HL116 or the derived HL116-huCD20 cells were stimulated with a serial dilution of IFNα2 (*i.e.* without a targeting moiety), CD20 VHH-IFNα2 (a chimera of a CD20-directed VHH targeting moiety and wild type IFNα2), IFNα1 (*i.e.* without a targeting moiety), or CD20 VHH-IFNα1 for 6 hours. Average luciferase values (± STDEV) of triplicate measurements are plotted. The data for IFN-α2 is shown in **FIG. 22A**, the data for CD20 VHH-IFNα2 is shown in **FIG. 22B**, the data for IFNα1 is shown in **FIG. 22C** and the data for CD20 VHH-IFNα1 is shown in **FIG. 22D**.

FIG. 23 shows tumor growth curves in humanized mice after treatment with buffer or Flt3L-IFNα1. Average values (in mm^3) of 5 or 6 animals per time point time (+SEM) are plotted.

FIGs. 24A-G show biological activity of IFNα1 AFNs on the HL116 reporter. HL116 or HL116-Clec9A cells were stimulated for 6 hours with serial dilution wild type IFNα2 or IFNα1 AFNs. Average luciferase activities (± STDEV) are plotted.

FIGs. 25A-E show pSTAT1 activity in Clec9A-/CD141- and Clec9A+/CD141+ PBMC's by IFNα2 or IFNα1 based AFN.

FIGs. 26A-B show biological activity of PD-L1 targeted IFNα1 (**FIG. 26A**) and IFNα2 (**FIG. 26B**) AFNs on the HL116 reporter. HL116 cells were stimulated for 6 hours with serial dilution wild type IFNα2 or IFNα1 AFNs. Average luciferase activities (± STDEV) are plotted.

DETAILED DESCRIPTION

The present invention is based, in part, on the discovery that targeted chimeric proteins and chimeric protein complexes, such as Fc-based chimeric protein complexes, that include a IFNα1 exhibit substantially superior activity and/or target selectivity over a non-fused, wild type IFNα1, and exhibit beneficial therapeutic and pharmaceutical properties and reduced side effects. For example, the chimeric proteins or chimeric protein complexes, such as Fc-based chimeric protein complexes of the present invention are highly target selective, enable conditional and/or regulated modulation of IFNAR1/2 receptor signaling, and are highly active and/or long-acting active and/or long-acting while exhibiting minmal off-target effects and eliciting minimal side effects.

The present invention also provides pharmaceutical compositions that include the chimeric proteins, chimeric protein complexes (including Fc-based chimeric protein complexes), and/or nucleic acids encoding the chimeric proteins and chimeric protein complexes, including Fc-based chimeric protein complexes. The present invention also includes host cells that comprise the nucleic acids encoding the chimeric proteins and chimeric protein complexes, including Fc-based chimeric protein complexes. The present invention further includes the use of the chimeric proteins, the chimeric protein complexes (including Fc-based chimeric protein complexes), the nucleic

acids encoding the chimeric proteins and chimeric protein complexes (including Fc-based chimeric protein complexes), the pharmaceutical compositions and/or the host cells as described herein for the treatment of various diseases.

Interferon-Alpha 1 or a Variant Thereof

In one aspect, the present invention provides a chimeric protein or chimeric protein complexes, such as Fc-based chimeric protein complexes that includes an engineered interferon. In one aspect, the present invention provides a chimeric protein or chimeric protein complexes, such as Fc-based chimeric protein complexes that include a wild type IFNα1. In various embodiments, the wild-type IFNα1 comprises the following amino acid sequence:

CDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHDFGFPQEEFDGNQFQKAPAISVLHELIQQIFNLFTTKDSSAA WDEDLLDKFCTELYQQLNDLEACVMQEERVGETPLMNADSILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRS LSLSTNLQERLRRKE (SEQ ID NO: 1).

In various embodiments, the present invention provides a chimeric protein or chimeric protein complexes, such as Fc-based chimeric protein complexes that include a wild type IFNα1 fused to one or more targeting moities. In some embodiments the incorporation of wild type IFNα1 in a chimeric protein or chimeric protein complex, such as for example through genetic fusion or attachment, reduces the biological activity of IFNα1 ("attenuated by fusion IFNα1"). For example, wild type IFNα1 incorporated in chimeric proteins or chimeric protein complexes may have reduced affinity and/or activity compared to wild type IFN-α1 interferon for a therapeutic receptor. In an embodiment, the therapeutic receptor is the interferon-α/β receptor (IFNAR), which is composed of the IFNAR1 and IFNAR2 subunits. In some embodiments, the loss in affinity and/or activity of wild type IFNα1 for a therapeutic receptor, e.g., IFNAR, can be induced and restored upon directing or targeting of the chimeric protein or chimeric protein complex comprising IFNα1 to a target cell through a targeting moiety. In some embodiments, the induction and restoration of IFNα1-mediated IFNAR-activation at a target cell may reach a level that is similar to or higher than IFNAR-activation achieved with wild type (non-chimeric) IFNα1. In some embodiments, the IFNα1 is a variant that comprises one or more mutations which reduce undesired disulphide pairings to improve product homogeneity and pharmaceutical properties of the chimeric protein or chimeric protein complexes, while simultaneously maintaining or avoiding substantial loss of IFNAR-activation of the modified IFNα1 compared to wild type IFNα1 in the context of chimeric proteins or chimeric protein complexes, including maintaining or avoiding substantial loss of restoration and induction of IFNAR-activation by the modified IFNα1 when directed or targeted to a target cell through a targeting moiety. In some embodiments, the IFNα1 is a variant that comprises one or more mutations which reduce undesired disulphide pairings wherein the one or more mutations are, e.g., at amino acid positions C1, C29, C86, C99, or C139 with reference to SEQ ID NO: 1. In some embodiments, the mutation at position C86 can be, *e.g.*, C86S or C86A or C86Y. These C86 mutants of IFNα1 are called reduced cysteine-based aggregation mutants. In some embodiment, the IFNα1 variant includes mutations at positions C1, C86 and C99 with reference to SEQ ID NO: 1. In embodiments, any of C1, C86 and C99 made be deleted or substituted.

In some embodiments, the IFN-α1 is modified, *i.e.*, is a variant and comprises one or more mutations in IFNα1. In some embodiments, the one or more mutations reduce the biological activity of the IFN-α1 ("attenuated by

mutation"). For example, the one or more mutations may reduce the affinity and/or activity of the IFN-α1 interferon for a therapeutic receptor. In an embodiment, the therapeutic receptor is the interferon-α/β receptor (IFNAR), which is composed of the IFNAR1 and IFNAR2 subunits. In an embodiment, the modified IFN-α1 comprises one or more mutations that reduce its affinity and/or activity for IFNAR1. In another embodiment, the modified IFN-α1 comprises one or more mutations that reduce its affinity and/or activity for IFNAR2. In an embodiment, the modified IFN-α1 comprises one or more mutations that reduce its affinity and/or activity for IFNAR1 and comprises one or more mutations that reduce its affinity and/or activity for IFNAR2. In some embodiments, the loss in affinity and/or activity of the modified IFNα1 ("attenuated by mutation") for a therapeutic receptor, e.g., IFNAR1, IFNAR2 and/or IFNAR, can be induced and restored upon directing or targeting of the chimeric protein or chimeric protein complex comprising the modified IFNα1 to a target cell through a targeting moiety, In some embodiments, the modified IFNα1 variant ("attenuated by mutation") that comprises one or more mutations that reduce its affinity and/or activity for IFNAR1, IFNAR2 and/or IFNAR, further comprises one or more mutations that reduce undesired disulphide pairings to improve product homogeneity and pharmaceutical properties of the chimeric protein or chimeric protein complexes, while simultaneously maintaining or avoiding substantial loss of induction and/or restoration of IFNAR-activation activity by the modified IFNα1 ("attenuated by mutation") when directed/targeted to a target cell through a targeting moiety. In some embodiments, the IFNα1 is a variant that comprises one or more mutations which reduce undesired disulphide pairings to improve product homogeneity and pharmaceutical properties of the chimeric protein or chimeric protein complexes, while simultaneously maintaining or avoiding substantial loss of IFNAR-activation of the modified IFNα1 compared to wild type IFNα1 in the context of chimeric proteins or chimeric protein complexes, including maintaining or avoiding substantial loss of restoration and induction of IFNAR-activation by the modified IFNα1 when directed or targeted to a target cell through a targeting moiety. In some embodiments, the IFNα1 is a variant that comprises one or more mutations which reduce undesired disulphide pairings wherein the one or more mutations are, e.g., at amino acid positions C1, C29, C86, C99, or C139 with reference to SEQ ID NO: 1. In some embodiments, the mutation at position C86 can be, *e.g.*, C86S or C86A or C86Y. These C86 mutants of IFNα1 are called reduced cysteine-based aggregation mutants. In some embodiment, the IFNα1 variant includes mutations at positions C1, C86 and C99 with reference to SEQ ID NO: 1.

In various embodiments, the chimeric protein or chimeric protein complexes, such as Fc-based chimeric protein complexes of the invention comprises a modified version of IFNα1, *i.e.*, a IFNα1 variant including a IFNα1 mutant, as a signaling agent. In various embodiments, the IFNα1 variant encompasses mutants, functional derivatives, analogs, precursors, isoforms, splice variants, or fragments of the interferon.

Additional IFNα1 variant sequences are known in the art. In various embodiments the modified IFNα1 comprises an amino acid sequence that has at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least

about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with any known amino acid sequences of a IFNα1 interferon variant (*e.g.*, about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% sequence identity).

In some embodiments, the IFNα1 variant comprises an amino acid sequence that has at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with any of the IFNα1 or IFNα1 variant sequences disclosed herein, *e.g.*, SEQ ID NO: 1 (*e.g.* about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% sequence identity).

In various embodiments, the IFNα1 variant comprises an amino acid sequence having one or more amino acid mutations. In some embodiments, the one or more amino acid mutations may be independently selected from substitutions, insertions, deletions, and truncations.

In some embodiments, the amino acid mutations are amino acid substitutions, and may include conservative and/or non-conservative substitutions.

"Conservative substitutions" may be made, for instance, on the basis of similarity in polarity, charge, size, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the amino acid residues involved. The 20 naturally occurring amino acids can be grouped into the following six standard amino acid groups: (1) hydrophobic: Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr; Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe.

As used herein, "conservative substitutions" are defined as exchanges of an amino acid by another amino acid listed within the same group of the six standard amino acid groups shown above. For example, the exchange of Asp by Glu retains one negative charge in the so modified polypeptide. In addition, glycine and proline may be substituted for one another based on their ability to disrupt α-helices.

As used herein, "non-conservative substitutions" are defined as exchanges of an amino acid by another amino acid listed in a different group of the six standard amino acid groups (1) to (6) shown above.

In various embodiments, the substitutions may also include non-classical amino acids (*e.g.* selenocysteine, pyrrolysine, *N*-formylmethionine β-alanine, GABA and δ-Aminolevulinic acid, 4-aminobenzoic acid (PABA), D-isomers of the common amino acids, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ-Abu, ε-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosme, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β methyl amino acids, C α-methyl amino acids, N α-methyl amino acids, and amino acid analogs in general).

In various embodiments, the IFNα1 is modified to have one or more mutations. In some embodiments, the mutations allow for the IFNα1 variant to have one or more of attenuated activity such as one or more of reduced binding affinity, reduced endogenous activity, and reduced specific bioactivity relative to unmutated, *e.g.,* the wild type form of IFNα1 (*e.g.*, the IFNα1 having an amino acid sequence of SEQ ID NO: 1). For instance, the one or more of attenuated activity such as reduced binding affinity, reduced endogenous activity, and reduced specific bioactivity relative to unmutated, *e.g.* the wild type form of IFNα1, may be at a therapeutic receptor such as IFNAR. Consequentially, in various embodiments, the mutations allow for the IFNα1 variant to have reduced systemic toxicity, reduced side effects, and reduced off-target effects relative to unmutated, *e.g.* the wild type form of IFNα1.

In various embodiments, IFNα1 is modified to have a mutation that reduces its binding affinity or activity at a therapeutic receptor such as IFNAR. In some embodiments, the activity provided by IFNα1 is agonism at the therapeutic receptor (*e.g.* activation of a cellular effect at a site of therapy). For example, the IFNα1 may activate the therapeutic receptor. In such embodiments, the mutation results in IFNα1 variant to have reduced activating activity at the therapeutic receptor.

In some embodiments, the reduced affinity or activity of the modified IFNα1 at the therapeutic receptor is inducible or restorable by attachment to a targeting moiety or upon inclusion of a targeting moiety in a chimeric protein or a chimeric protein complex, e.g., a Fc-based chimeric protein complex as disclosed herein. In some embodiments, the activity of IFNα1 is reduced or attenuated by virtue of its fusion with another protein, including, in some instances, by fusion with targeting moieties as described herein. In other embodiments, the activty of IFNα1 is reduced or attenuated by modifying the IFNα1, e.g., by introducing mutations as described herein. In some embodiments, attenuation of the activity can be restored by attaching the IFNα1 to a targeting moiety or by the action of the attached targeting moiety. In embodiments, the targeting moiety—by virtue of its attachment or by its activity—induces IFNα1's activity.

In other embodiments, the reduced affinity or activity at the therapeutic receptor is not substantially inducible or restorable by attachment with the targeting moiety or upon inclusion in a chimeric protein or a chimeric protein complex, e.g., a Fc-based chimeric protein complex as disclosed herein. In various embodiments, the therapeutic chimeric proteins, the chimeric protein complexes, or Fc-based chimeric protein complexes of the present invention reduce off-target effects because the wild type IFNα1 or IFNα1 variant having one or more mutations, exhibit weak binding affinity or activity at a therapeutic receptor compared to wild type IFNα1 (non-fused). In various embodiments, this reduces side effects observed with, for example, the wild type form of IFNα1 or other type I interferons. In various embodiments, the IFNα1 construct and/or IFNα1variant is substantially inactive *en route* to the site of therapeutic activity and has its effect substantially on specifically targeted cell types, which greatly reduces undesired cross-reactivities and side effects.

In various embodiments, the IFNα1 variant has one or more mutations that cause the IFNα1 variant to have attenuated or reduced affinity, *e.g.* binding (*e.g.* K_D) and/or activation (measurable as, for example, K_A and/or EC_{50}) for one or more therapeutic receptors. In various embodiments, the reduced affinity at the therapeutic receptor allows for attenuation of activity and/or signaling from the therapeutic receptor.

In various embodiments, the IFNα1 variant has one or more mutations that reduce its binding to or its affinity for the IFNAR1 subunit of IFNAR. In one embodiment, the IFNα1 variant has reduced affinity and/or activity at IFNAR1. In some embodiments, the IFNα1 variant has one or more mutations that reduce its binding to or its affinity for the IFNAR2 subunit of IFNAR. In some embodiments, the IFNα1 variant has one or more mutations that reduce its binding to or its affinity for both IFNAR1 and IFNAR2 subunits.

In some embodiments, the IFNα1 variant has one or more mutations that reduce its binding to or its affinity for IFNAR1 and one or more mutations that substantially reduce or ablate binding to or its affinity for IFNAR2. In some embodiments, chimeric proteins and chimeric protein complexes, such as or Fc-based chimeric protein complexes with such IFNα1 variant can provide target-selective IFNAR1 activity (*e.g.* IFNAR1 activity is inducible or restorable via targeting through the targeting moiety or upon inclusion in the Fc-based chimeric protein complex disclosed herein).

In some embodiments, the IFNα1 variant has one or more mutations that reduce its binding to or its affinity for IFNAR2 and one or more mutations that substantially reduce or ablate binding to or its affinity for IFNAR1. In some embodiments, chimeric proteins or chimeric protein complexes, such as Fc-based chimeric protein complexes with such IFNα1 variant can provide target-selective IFNAR2 activity (*e.g.* IFNAR2 activity is inducible or restorable via targeting through the targeting moiety or upon inclusion in the Fc-based chimeric protein complex disclosed herein).

In some embodiments, the IFNα1 variant has one or more mutations that reduce its binding to or its affinity for IFNAR1 and one or more mutations that reduce its binding to or its affinity for IFNAR2. In some embodiments, chimeric proteins or chimeric protein complexes, such as Fc-based chimeric protein complexes with such IFNα1 variant can provide target-selective IFNAR1 and/or IFNAR2 activity (*e.g.* IFNAR1 and/IFNAR2 activity is inducible

or restorable via targeting through the targeting moiety or upon inclusion in the Fc-based chimeric protein complex disclosed herein).

In various embodiments, the IFNα1 variant has about 1%, or about 3%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 10%-20%, about 20%-40%, about 50%, about 40%-60%, about 60%-80%, about 80%-100% of the affinity for the therapeutic receptor (*e.g.*, IFNAR or any one of its subunits IFNAR1 and/or IFNAR2) relative to the wild type IFNα1. In some embodiments, the binding affinity is at least about 2-fold lower, about 3-fold lower, about 4-fold lower, about 5-fold lower, about 6-fold lower, about 7-fold lower, about 8-fold lower, about 9-fold lower, at least about 10-fold lower, at least about 15-fold lower, at least about 20-fold lower, at least about 25-fold lower, at least about 30-fold lower, at least about 35-fold lower, at least about 40-fold lower, at least about 45-fold lower, at least about 50-fold lower, at least about 100-fold lower, at least about 150-fold lower, or about 10-50-fold lower, about 50-100-fold lower, about 100-150-fold lower, about 150-200-fold lower, or more than 200-fold lower relative to the wild type IFNα1.

In some embodiments, the IFNα1 variant comprises one or more mutations that cause the IFNα1 variant to have reduced affinity for a receptor. In some embodiments, the IFNα1 variant's binding affinity for a receptor is lower than the binding affinity of the targeting moiety for its receptor. In some embodiments, this binding affinity differential is between the IFNα1 variant/receptor and targeting moiety/receptor on the same cell. In some embodiments, this binding affinity, differential allows for the IFNα1 variant to have localized, on-target effects and to minimize off-target effects that underlie side effects that are observed with wild type IFNα1. In some embodiments, this binding affinity is at least about 2-fold, or at least about 5-fold, or at least about 10-fold, or at least about 15-fold lower, or at least about 25-fold, or at least about 50-fold lower, or at least about 100-fold, or at least about 150-fold less.

Receptor binding activity may be measured using methods known in the art. For example, affinity and/or binding activity may be assessed by Scatchard plot analysis and computer-fitting of binding data (*e.g.* Scatchard, 1949) or by reflectometric interference spectroscopy under flow through conditions, as described by Brecht *et al.* (1993), the entire contents of all of which are hereby incorporated by reference.

In various embodiments, the chimeric protein complexes of the present invention include (a) an interferon alpha 1 (IFNα1) or a variant thereof, and (b) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to antigens or receptors of interest; wherein the IFNα1 or the variant thereof, and the one or more targeting moieties are connected with a domain that causes complexation (e.g. a complexation domain). In some embodiments, the chimeric protein complexes of the present invention further include one or more proteins or peptides that interact with each other (e.g. a complexation domain), e.g., using electrostatic interactions, hydrogen bonding, and/or the hydrophobic effect. In some embodiments, the chimeric protein complexes are homomers (e.g., that include two or more chimeric proteins as described herein comprising, e.g., interferon alpha 1 (IFNα1) or a variant thereof and one or more targeting moieties connected with one or more linkers). In some embodiments, the chimeric protein complexes are heteromers (e.g., that include one chimeric protein comprising interferon alpha 1 (IFNα1) or a variant thereof and one or more targeting moieties connected

with one or more linkers and another protein). A variety of protein interaction domains (e.g. a complexation domains) have been employed to generate protein complexes and can be used for the purposes of making chimeric protein complexes of the present invention. In some embodiments, the chimeric protein complexes can be made by using leucine zippers, Jun and Fos family of proteins, helix-turn-helix self dimerizing peptides, tri- and tetrameric subdomains of collagen and p53 (see, e.g. methods of making protein complexes as described in U.S. Patent No. 8507222, which is hereby incorporated by reference in its entirety). Other methods to make heteromeric complexes include charge based heterodimers as e.g. described by Chang et al. (PNAS 1984;91:11408-11412) or heterodimerizing leucine zippers as described e.g. by Deng et al. (Chemistry & Biology 2008;15:908-919) or designed heterodimers as described by Chen et al. (Nature 2019;565:106-111). In various embodiments, these chimeric protein complexes, are not Fc-based. In some embodiments, the variety of protein interaction domains can be used in place of Fc-domains described herein (in the context of Fc-based chimeric protein complexes) to form protein complexes.

In various embodiments, the chimeric protein complexes, such as Fc-based chimeric protein complex comprises a wild type signaling agent that has improved target selectivity and safety relative to a signaling agent which is not fused to an Fc, or a signaling agent which is not in the context of a complex, *e.g.*, without limitation, a heterodimeric complex. In various embodiments, the chimeric protein complexes, such as Fc-based chimeric protein complex comprises a wild type signaling agent that has improved target selective activity relative to a signaling agent which is not fused to an Fc, or a signaling agent which is not in the context of a complex, *e.g.*, without limitation, a heterodimeric complex. In various embodiments, the chimeric protein complexes, such as Fc-based chimeric protein complex allows for conditional activity.

In various embodiments, the chimeric protein complexes, such as Fc-based chimeric protein complex comprises a wild type signaling agent that has improved safety, *e.g.* reduced systemic toxicity, reduced side effects, and reduced off-target effects relative to a signaling agent which is not fused to an Fc, or a signaling agent which is not in the context of a complex, *e.g.*, without limitation, a heterodimeric complex. In various embodiments, improved safety means that the present chimeric protein complexes, such as Fc-based chimeric protein provides lower toxicity (*e.g.* systemic toxicity and/or tissue/organ-associated toxicities); and/or lessened or substantially eliminated side effects; and/or increased tolerability, lessened or substantially eliminated adverse events; and/or reduced or substantially eliminated off-target effects; and/or an increased therapeutic window of the wild type signaling agent as compared to the signaling agent which is not fused to an Fc, or a signaling agent which is not in the context of a complex, *e.g.*, without limitation, a heterodimeric complex.

In some embodiments, the reduced affinity or activity at the receptor is inducible or restorable by attachment with one or more of the targeting moieties as described herein or upon inclusion in the chimeric protein complexes, such as Fc-based chimeric protein complex disclosed herein.

In various embodiments, the chimeric protein complexes, such as Fc-based chimeric protein complex comprises a wild type signaling agent that has reduced, substantially reduced, or ablated affinity, *e.g.* binding (*e.g.* K_D) and/or activation (for instance, when the modified signaling agent is an agonist of its receptor, measurable as, for example,

K_A and/or EC_{50}) and/or inhibition (for instance, when the modified signaling agent is an antagonist of its receptor, measurable as, for example, K_I and/or IC_{50}), for one or more of its receptors. In various embodiments, the reduced affinity at the signaling agent's receptor allows for attenuation of activity. In such embodiments, the modified signaling agent has about 1%, or about 3%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 10%-20%, about 20%-40%, about 50%, about 40%-60%, about 60%-80%, about 80%-100% of the affinity for the receptor as compared to the signaling agent which is not fused to an Fc, or a signaling agent which is not in the context of a complex, *e.g.*, without limitation, a heterodimeric complex. In some embodiments, the binding affinity is at least about 2-fold lower, about 3-fold lower, about 4-fold lower, about 5-fold lower, about 6-fold lower, about 7-fold lower, about 8-fold lower, about 9-fold lower, at least about 10-fold lower, at least about 15-fold lower, at least about 20-fold lower, at least about 25-fold lower, at least about 30-fold lower, at least about 35-fold lower, at least about 40-fold lower, at least about 45-fold lower, at least about 50-fold lower, at least about 100-fold lower, at least about 150-fold lower, or about 10-50-fold lower, about 50-100-fold lower, about 100-150-fold lower, about 150-200-fold lower, or more than 200-fold lower as compared to the signaling agent which is not fused to an Fc, or a signaling agent which is not in the context of a complex, *e.g.*, without limitation, a heterodimeric complex.

In various embodiments, the chimeric protein complexes, such as Fc-based chimeric protein complex comprises a wild type signaling agent that has reduced endogenous activity of the signaling agent to about 75%, or about 70%, or about 60%, or about 50%, or about 40%, or about 30%, or about 25%, or about 20%, or about 10%, or about 5%, or about 3%, or about 1%, *e.g.*, as compared to the signaling agent which is not fused to an Fc, or a signaling agent which is not in the context of a complex, *e.g.*, without limitation, a heterodimeric complex.

In various embodiments, the attenuated activity at the therapeutic receptor, the weakened affinity at the therapeutic receptor is inducible or restorable by attachment with a targeting moiety or upon inclusion in the chimeric protein complexes, such as Fc-based chimeric protein complex disclosed herein, having high affinity for an antigen at the site of therapeutic activity (*e.g.* an antibody or antibody format described herein). The targeting is realized by linking the IFNα1 or a variant thereof to a targeting moiety or upon its inclusion in the chimeric protein complexes, such as Fc-based chimeric protein complex as disclosed herein. In an embodiment, the IFNα1 or a variant thereof is linked to a targeting moiety through its amino-terminus. In another embodiment, the IFNα1 or a variant thereof is linked to a targeting moiety through its carboxy-terminus. In this way, the present chimeric proteins or chimeric protein complexes, such as Fc-based chimeric protein complexes provide, in some embodiments, localized, on-target, and controlled therapeutic action at the therapeutic receptor.

In some embodiments, the IFNα1 interferon is modified to have a mutation at one or more amino acids at positions L15, A19, R23, S25, L30, D32, R33, H34, Q40, D115, L118, K121, R126, E133, K134, K135, R145, A146, M149, R150, S153, L154, and N157 with reference to SEQ ID NO: 1. The mutations can optionally be a hydrophobic mutation and can be, *e.g.*, selected from alanine, valine, leucine, and isoleucine. In some embodiments, the IFNα1 interferon is modified to have a one or more mutations selected from L15A, A19W, R23A, S25A, L30A, L30V,

D32A, R33K, R33A, R33Q, H34A, Q40A, D115R, L118A, K121A, K121E, R126A, R126E, E133A, K134A, K135A, R145A, R145D, R145E, R145G, R145H, R145I, R145K, R145L, R145N, R145Q, R145S, R145T, R145V, R145Y, A146D, A146E, A146G, A146H, A146I, A146K, A146L, A146M, A146N, A146Q, A146R, A146S, A146T, A146V, A146Y, M149A, M149V, R150A, S153A, L154A, and N157A with reference to SEQ ID NO: 1. In some embodiments, the IFNα1 mutant comprises one or more multiple mutations selected from L30A/H58Y/E59N_Q62S, R33A/H58Y/E59N/Q62S, M149A/H58Y/E59N/Q62S, L154A/H58Y/E59N/Q62S, R145A/H58Y/E59N/Q62S, D115A/R121A, L118A/R121A, L118A/R121A/K122A, R121A/K122A, and R121E/K122E with reference to SEQ ID NO: 1.

In an embodiment, the IFNα1 interferon, or variant thereof, is modified to have one or more mutations at amino acid positions C1, C29, C86, C99, or C139 with reference to SEQ ID NO: 1. In this regard, Beilharz et al., Journal of interferon research 6.6 (1986): 677-685 (which is hereby incorporated by reference in its entirety) describes various mutations of IFNα1 that may be used introduced in the modified IFNα1 of the present invention. The mutation at position C86 can be, *e.g.*, C86S or C86A or C86Y. These C86 mutants of IFNα1 are called reduced cysteine-based aggregation mutants. In some embodiment, the IFNα1 variant includes mutations at positions C1, C86 and C99 with reference to SEQ ID NO: 1.

Therapeutic Agents Comprising the Interferon or a Variant Thereof

Targeting Moiety Cellular Recruitment

In various embodiments, the chimeric proteins or chimeric protein complexes, such as Fc-based chimeric protein complexes of the present invention additionally comprise one or more targeting moieties having recognition domains which specifically bind to a target (*e.g.* antigen, receptor) of interest. In some embodiments, the chimeric protein or chimeric protein complexes, such as Fc-based chimeric protein complexes may comprise two, three, four, five, six, seven, eight, nine, ten or more targeting moieties. In illustrative embodiments, the chimeric proteins or chimeric protein complexes, such as Fc-based chimeric protein complexes of the invention comprise two or more targeting moieties. In such embodiments, the chimeric proteins or chimeric protein complexes, such as Fc-based chimeric protein complexes can target two different cells (*e.g.* to make a synapse) or the same cell (*e.g.* to get a more concentrated signaling agent effect). In some embodiments, the chimeric proteins or chimeric protein complexes, such as Fc-based chimeric protein complexes of the invention comprise IFNα1 or a variant thereof, a targeting moiety that is Flt3L and one targeting moiety that recognizes PD-1 or PD-L1. In some embodiments, the chimeric proteins or chimeric protein complexes, such as Fc-based chimeric protein complexes of the invention comprise IFNα1 or a variant thereof, a targeting moiety that is Flt3L and two targeting moieties that recognizes PD-1 or PD-L1.

In some embodiments, the chimeric proteins or chimeric protein complexes, such as Fc-based chimeric protein complexes of the invention comprise IFNα2 or a variant thereof, a targeting moiety that is Flt3L and one targeting moiety that recognizes PD-1 or PD-L1. In some embodiments, the chimeric proteins or chimeric protein complexes, such as Fc-based chimeric protein complexes of the invention comprise IFNα2 or a variant thereof, a targeting moiety that is Flt3L and two targeting moieties that recognizes PD-1 or PD-L1.

In various embodiments, the target (*e.g.* antigen, receptor) of interest can be found on one or more immune cells, which can include, without limitation, T cells, cytotoxic T lymphocytes, T helper cells, natural killer (NK) cells, natural killer T (NKT) cells, anti-tumor or tumor-associated macrophages (*e.g.* M1 or M2 macrophages), B cells, Breg cells, dendritic cells, or subsets thereof. In some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) of interest and effectively, directly or indirectly, recruit one of more immune cells. In some embodiments, the target (*e.g.* antigen, receptor) of interest can be found on one or more tumor cells. In some embodiments, the present chimeric proteins or chimeric protein complexes, such as Fc-based chimeric protein complexes may directly or indirectly recruit an immune cell, *e.g.*, in some embodiments, to a therapeutic site (*e.g.* a locus with one or more disease cell or cell to be modulated for a therapeutic effect). In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes may directly or indirectly recruit an immune cell, *e.g.* an immune cell that can kill and/or suppress a tumor cell, to a site of action (such as, by way of non-limiting example, the tumor microenvironment).

In various embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have targeting moieties having recognition domains which specifically bind to a target (*e.g.* antigen, receptor) which is part of a non-cellular structure. In some embodiments, the antigen or receptor is not an integral component of an intact cell or cellular structure. In some embodiments, the antigen or receptor is an extracellular antigen or receptor. In some embodiments, the target is a non-proteinaceous, non-cellular marker, including, without limitation, nucleic acids, inclusive of DNA or RNA, such as, for example, DNA released from necrotic tumor cells or extracellular deposits such as cholesterol.

In some embodiments, the target (*e.g.* antigen, receptor) of interest is part of the non-cellular component of the stroma or the extracellular matrix (ECM) or the markers associated therewith. As used herein, stroma refers to the connective and supportive framework of a tissue or organ. Stroma may include a compilation of cells such as fibroblasts/myofibroblasts, glial, epithelia, fat, immune, vascular, smooth muscle, and immune cells along with the extracellular matrix (ECM) and extracellular molecules. In various embodiments, the target (*e.g.* antigen, receptor) of interest is part of the non-cellular component of the stroma such as the extracellular matrix and extracellular molecules. As used herein, the ECM refers to the non-cellular components present within all tissues and organs. The ECM is composed of a large collection of biochemically distinct components including, without limitation, proteins, glycoproteins, proteoglycans, and polysaccharides. These components of the ECM are usually produced by adjacent cells and secreted into the ECM via exocytosis. Once secreted, the ECM components often aggregate to form a complex network of macromolecules. In various embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein chimeric proteins of the invention comprises a targeting moiety that recognizes a target (*e.g.*, an antigen or receptor or non-proteinaceous molecule) located on any component of the ECM. Illustrative components of the ECM include, without limitation, the proteoglycans, the non-proteoglycan polysaccharides, fibers, and other ECM proteins or ECM non-proteins, *e.g.* polysaccharides and/or lipids, or ECM associated molecules (*e.g.* proteins or non-proteins, *e.g.* polysaccharides, nucleic acids and/or lipids).

In some embodiments, the targeting moiety recognizes a target (*e.g.* antigen, receptor) on ECM proteoglycans. Proteoglycans are glycosylated proteins. The basic proteoglycan unit includes a core protein with one or more covalently attached glycosaminoglycan (GAG) chains. Proteoglycans have a net negative charge that attracts positively charged sodium ions (Na+), which attracts water molecules via osmosis, keeping the ECM and resident cells hydrated. Proteoglycans may also help to trap and store growth factors within the ECM. Illustrative proteoglycans that may be targeted by the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes of the invention include, but are not limited to, heparan sulfate, chondroitin sulfate, and keratan sulfate. In an embodiment, the targeting moiety recognizes a target (*e.g.* antigen, receptor) on non-proteoglycan polysaccharides such as hyaluronic acid.

In some embodiments, the targeting moiety recognizes a target (*e.g.* antigen, receptor) on ECM fibers. ECM fibers include collagen fibers and elastin fibers. In some embodiments, the targeting moiety recognizes one or more epitopes on collagens or collagen fibers. Collagens are the most abundant proteins in the ECM. Collagens are present in the ECM as fibrillar proteins and provide structural support to resident cells. In one or more embodiments, the targeting moiety recognizes and binds to various types of collagens present within the ECM including, without limitation, fibrillar collagens (types I, II, III, V, XI), facit collagens (types IX, XII, XIV), short chain collagens (types VIII, X), basement membrane collagens (type IV), and/or collagen types VI, VII, or XIII. Elastin fibers provide elasticity to tissues, allowing them to stretch when needed and then return to their original state. In some embodiments, the target moiety recognizes one or more epitopes on elastins or elastin fibers.

In some embodiments, the targeting moiety recognizes one or more ECM proteins including, but not limited to, a tenascin, a fibronectin, a fibrin, a laminin, or a nidogen/entactin.

In an embodiment, the targeting moiety recognizes and binds to tenascin. The tenascin (TN) family of glycoproteins includes at least four members, tenascin-C, tenascin-R, tenascin-X, and tenascin W. The primary structures of tenascin proteins include several common motifs ordered in the same consecutive sequence: amino-terminal heptad repeats, epidermal growth factor (EGF)-like repeats, fibronectin type III domain repeats, and a carboxyl-terminal fibrinogen-like globular domain. Each protein member is associated with typical variations in the number and nature of EGF-like and fibronectin type III repeats. Isoform variants also exist particularly with respect to tenascin-C. Over 27 splice variants and/or isoforms of tenascin-C are known. In a particular embodiment, the targeting moiety recognizes and binds to tenascin-CA1. Similarly, tenascin-R also has various splice variants and isoforms. Tenascin-R usually exists as dimers or trimers. Tenascin-X is the largest member of the tenascin family and is known to exist as trimers. Tenascin-W exists as trimers. In some embodiments, the targeting moiety recognizes one or more epitopes on a tenascin protein. In some embodiments, the targeting moiety recognizes the monomeric and/or the dimeric and/or the trimeric and/or the hexameric forms of a tenascin protein.

In an embodiment, the targeting moieties recognize and bind to fibronectin. Fibronectins are glycoproteins that connect cells with collagen fibers in the ECM, allowing cells to move through the ECM. Upon binding to integrins, fibronectins unfolds to form functional dimers. In some embodiments, the targeting moiety recognizes the monomeric and/or the dimeric forms of fibronectin. In some embodiments, the targeting moiety recognizes one or

more epitopes on fibronectin. In illustrative embodiments, the targeting moiety recognizes fibronectin extracellular domain A (EDA) or fibronectin extracellular domain B (EDB). Elevated levels of EDA are associated with various diseases and disorders including psoriasis, rheumatoid arthritis, diabetes, and cancer. In some embodiments, the targeting moiety recognizes fibronectin that contains the EDA isoform and may be utilized to target the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes to diseased cells including cancer cells. In some embodiments, the targeting moiety recognizes fibronectin that contains the EDB isoform. In various embodiments, such targeting moieties may be utilized to target the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes to tumor cells including the tumor neovasculature.

In an embodiment, the targeting moiety recognizes and binds to fibrin. Fibrin is another protein substance often found in the matrix network of the ECM. Fibrin is formed by the action of the protease thrombin on fibrinogen which causes the fibrin to polymerize. In some embodiments, the targeting moiety recognizes one or more epitopes on fibrin. In some embodiments, the targeting moiety recognizes the monomeric as well as the polymerized forms of fibrin.

In an embodiment, the targeting moiety recognizes and binds to laminin. Laminin is a major component of the basal lamina, which is a protein network foundation for cells and organs. Laminins are heterotrimeric proteins that contain an α-chain, a β-chain, and a γ-chain. In some embodiments, the targeting moiety recognizes one or more epitopes on laminin. In some embodiments, the targeting moiety recognizes the monomeric, the dimeric as well as the trimeric forms of laminin.

In an embodiment, the targeting moiety recognizes and binds to a nidogen or entactin. Nidogens/entactins are a family of highly conserved, sulfated glycoproteins. They make up the major structural component of the basement membranes and function to link laminin and collagen IV networks in basement membranes. Members of this family include nidogen-1 and nidogen-2. In various embodiments, the targeting moiety recognizes an epitope on nidogen-1 and/or nidogen-2.

In various embodiments, the targeting moiety comprises an antigen recognition domain that recognizes an epitope present on any of the targets described herein. In an embodiment, the antigen-recognition domain recognizes one or more linear epitopes present on the protein. As used herein, a linear epitope refers to any continuous sequence of amino acids present on the protein. In another embodiment, the antigen-recognition domain recognizes one or more conformational epitopes present on the protein. As used herein, a conformation epitope refers to one or more sections of amino acids (which may be discontinuous) which form a three-dimensional surface with features and/or shapes and/or tertiary structures capable of being recognized by an antigen recognition domain.

In various embodiments, the targeting moiety may bind to the full-length and/or mature forms and/or isoforms and/or splice variants and/or fragments and/or any other naturally occurring or synthetic analogs, variants, or mutants of any of the targets described herein. In various embodiments, the targeting moiety may bind to any forms of the proteins described herein, including monomeric, dimeric, trimeric, tetrameric, heterodimeric, multimeric and associated forms. In various embodiments, the targeting moiety may bind to any post-translationally modified forms of the proteins described herein, such as glycosylated and/or phosphorylated forms.

In various embodiments, the targeting moiety comprises an antigen recognition domain that recognizes extracellular molecules such as DNA. In some embodiments, the targeting moiety comprises an antigen recognition domain that recognizes DNA. In an embodiment, the DNA is shed into the extracellular space from necrotic or apoptotic tumor cells or other diseased cells.

In various embodiments, the targeting moiety comprises an antigen recognition domain that recognizes one or more non-cellular structures associated with atherosclerotic plaques. Two types of atherosclerotic plaques are known. The fibro-lipid (fibro-fatty) plaque is characterized by an accumulation of lipid-laden cells underneath the intima of the arteries. Beneath the endothelium there is a fibrous cap covering the atheromatous core of the plaque. The core includes lipid-laden cells (macrophages and smooth muscle cells) with elevated tissue cholesterol and cholesterol ester content, fibrin, proteoglycans, collagen, elastin, and cellular debris. In advanced plaques, the central core of the plaque usually contains extracellular cholesterol deposits (released from dead cells), which form areas of cholesterol crystals with empty, needle-like clefts. At the periphery of the plaque are younger foamy cells and capillaries. A fibrous plaque is also localized under the intima, within the wall of the artery resulting in thickening and expansion of the wall and, sometimes, spotty localized narrowing of the lumen with some atrophy of the muscular layer. The fibrous plaque contains collagen fibers (eosinophilic), precipitates of calcium (hematoxylinophilic) and lipid-laden cells. In some embodiments, the targeting moiety recognizes and binds to one or more of the non-cellular components of these plaques such as the fibrin, proteoglycans, collagen, elastin, cellular debris, and calcium or other mineral deposits or precipitates. In some embodiments, the cellular debris is a nucleic acid, *e.g.* DNA or RNA, released from dead cells.

In various embodiments, the targeting moiety comprises an antigen recognition domain that recognizes one or more non-cellular structures found in the brain plaques associated with neurodegenerative diseases. In some embodiments, the targeting moiety recognizes and binds to one or more non-cellular structures located in the amyloid plaques found in the brains of patients with Alzheimer's disease. For example, the targeting moiety may recognize and bind to the peptide amyloid beta, which is a major component of the amyloid plaques. In some embodiments, the targeting moiety recognizes and binds to one or more non-cellular structures located in the brains plaques found in patients with Huntington's disease. In various embodiments, the targeting moiety recognizes and binds to one or more non-cellular structures found in plaques associated with other neurodegenerative or musculoskeletal diseases such as Lewy body dementia and inclusion body myositis.

In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes of the invention may have two or more targeting moieties that bind to non-cellular structures. In some embodiments, there are two targeting moieties and one targets a cell while the other targets a non-cellular structure. In various embodiments, the targeting moieties can directly or indirectly recruit cells, such as disease cells and/or effector cells. In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes are capable of, or find use in methods involving, shifting the balance of immune cells in favor of immune attack of a tumor. For instance, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes can shift the ratio of immune cells at a site of clinical

importance in favor of cells that can kill and/or suppress a tumor (*e.g.* T cells, cytotoxic T lymphocytes, T helper cells, natural killer (NK) cells, natural killer T (NKT) cells, anti-tumor macrophages (*e.g.* M1 macrophages), B cells, dendritic cells, or subsets thereof) and in opposition to cells that protect tumors (*e.g.* myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs); tumor associated neutrophils (TANs), M2 macrophages, tumor associated macrophages (TAMs), or subsets thereof). In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes are capable of increasing a ratio of effector T cells to regulatory T cells.

For example, in some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) associated with T cells. In some embodiments, the recognition domains directly or indirectly recruit T cells. In an embodiment, the recognition domains specifically bind to effector T cells. In some embodiments, the recognition domain directly or indirectly recruits effector T cells, *e.g.*, in some embodiments, to a therapeutic site (*e.g.* a locus with one or more disease cell or cell to be modulated for a therapeutic effect). Illustrative effector T cells include cytotoxic T cells (*e.g.* αβ TCR, $CD3^+$, $CD8^+$, $CD45RO^+$); $CD4^+$ effector T cells (*e.g.* αβ TCR, $CD3^+$, $CD4^+$, $CCR7^+$, CD62Lhi, $IL\text{-}7R/CD127^+$); $CD8^+$ effector T cells (*e.g.* αβ TCR, $CD3^+$, $CD8^+$, $CCR7^+$, CD62Lhi, $IL\text{-}7R/CD127^+$); effector memory T cells (*e.g.* CD62Llow, $CD44^+$, TCR, $CD3^+$, $IL\text{-}7R/CD127^+$, $IL\text{-}15R^+$, CCR7low); central memory T cells (*e.g.* $CCR7^+$, $CD62L^+$, $CD27^+$; or CCR7hi, $CD44^+$, CD62Lhi, TCR, $CD3^+$, IL-7R/$CD127^+$, $IL\text{-}15R^+$); $CD62L^+$ effector T cells; $CD8^+$ effector memory T cells (TEM) including early effector memory T cells ($CD27^+$ $CD62L^-$) and late effector memory T cells ($CD27^-$ $CD62L^-$) (TemE and TemL, respectively); $CD127(^+)CD25(low/-)$ effector T cells; $CD127(^-)CD25(^-)$ effector T cells; $CD8^+$ stem cell memory effector cells (TSCM) (*e.g.* CD44(low)CD62L(high)CD122(high)$sca(^+)$); TH1 effector T-cells (*e.g.* $CXCR3^+$, $CXCR6^+$ and $CCR5^+$; or αβ TCR, $CD3^+$, $CD4^+$, $IL\text{-}12R^+$, $IFN\gamma R^+$, $CXCR3^+$), TH2 effector T cells (*e.g.* $CCR3^+$, $CCR4^+$ and $CCR8^+$; or αβ TCR, $CD3^+$, $CD4^+$, $IL\text{-}4R^+$, $IL\text{-}33R^+$, $CCR4^+$, $IL\text{-}17RB^+$, $CRTH2^+$); TH9 effector T cells (*e.g.* αβ TCR, $CD3^+$, $CD4^+$); TH17 effector T cells (*e.g.* αβ TCR, $CD3^+$, $CD4^+$, $IL\text{-}23R^+$, $CCR6^+$, $IL\text{-}1R^+$); $CD4^+CD45RO^+CCR7^+$ effector T cells, $ICOS^+$ effector T cells; $CD4^+CD45RO^+CCR7(^-)$ effector T cells; and effector T cells secreting IL-2, IL-4 and/or IFN-γ.

Illustrative T cell antigens of interest include, for example (and inclusive of the extracellular domains, where applicable): CD8, CD3, SLAMF4, IL-2Rα, 4-1BB/TNFRSF9, IL-2 R β, ALCAM, B7-1, IL-4 R, B7-H3, BLAME/SLAMFS, CEACAM1, IL-6 R, CCR3, IL-7 Rα, CCR4, CXCRI/IL-S RA, CCR5, CCR6, IL-10R α, CCR 7, IL-l 0 R β, CCRS, IL-12 R β 1, CCR9, IL-12 R β 2, CD2, IL-13 R α 1, IL-13, CD3, CD4, ILT2/CDS5j, ILT3/CDS5k, ILT4/CDS5d, ILT5/CDS5a, lutegrin α 4/CD49d, CDS, Integrin α E/CD103, CD6, Integrin α M/CD 11 b, CDS, Integrin α X/CD11c, Integrin β 2/CDIS, KIR/CD15S, CD27/TNFRSF7, KIR2DL1, CD2S, KIR2DL3, CD30/TNFRSFS, KIR2DL4/CD15Sd, CD31/PECAM-1, KIR2DS4, CD40 Ligand/TNFSF5, LAG-3, CD43, LAIR1, CD45, LAIR2, CDS3, Leukotriene B4-R1, CDS4/SLAMF5, NCAM-L1, CD94, NKG2A, CD97, NKG2C, CD229/SLAMF3, NKG2D, CD2F-10/SLAMF9, NT-4, CD69, NTB-A/SLAMF6, Common γ Chain/IL-2 R γ, Osteopontin, CRACC/SLAMF7, PD-1, CRTAM, PSGL-1, CTLA-4, RANK/TNFRSF11A, CX3CR1, CX3CL1, L-Selectin, CXCR3, SIRP β 1, CXCR4, SLAM, CXCR6, TCCR/WSX-1, DNAM-1, Thymopoietin, EMMPRIN/CD147, TIM-1, EphB6, TIM-2, Fas/TNFRSF6, TIM-3, Fas Ligand/TNFSF6, TIM-4, Fcγ RIII/CD16, TIM-6,

TNFR1/TNFRSF1A, Granulysin, TNF RIII/TNFRSF1B, TRAIL RI/TNFRSFIOA, ICAM-1/CD54, TRAIL R2/TNFRSF10B, ICAM-2/CD102, TRAILR3/TNFRSF10C,IFN-γR1, TRAILR4/TNFRSF10D, IFN-γ R2, TSLP, IL-1 R1 and TSLP R. In various embodiments, a targeting moiety of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds one or more of these illustrative T cell antigens.

By way of non-limiting example, in various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have a targeting moiety directed against a checkpoint marker expressed on a T cell, *e.g.* one or more of PD-1, CD28, CTLA4, ICOS, BTLA, KIR, LAG3, CD137, OX40, CD27, CD40L, TIM3, and A2aR.

For example, in some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) associated with B cells. In some embodiments, the recognition domains directly or indirectly recruit B cells, *e.g.*, in some embodiments, to a therapeutic site (*e.g.* a locus with one or more disease cell or cell to be modulated for a therapeutic effect). Illustrative B cell antigens of interest include, for example, CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD38, CD39, CD40, CD70, CD72, CD73, CD74, CDw75, CDw76, CD77, CD78, CD79a/b, CD80, CD81, CD82, CD83, CD84, CD85, CD86, CD89, CD98, CD126, CD127, CDw130, CD138, CDw150, CS1, and B-cell maturation antigen (BCMA). In various embodiments, a targeting moiety of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds one or more of these illustrative B cell antigens.

By way of further example, in some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) associated with Natural Killer cells. In some embodiments, the recognition domains directly or indirectly recruit Natural Killer cells, *e.g.*, in some embodiments, to a therapeutic site (*e.g.* a locus with one or more disease cell or cell to be modulated for a therapeutic effect). Illustrative Natural Killer cell antigens of interest include, for example TIGIT, 2B4/SLAMF4, KIR2DS4, CD155/PVR, KIR3DL1, CD94, LMIR1/CD300A, CD69, LMIR2/CD300c, CRACC/SLAMF7, LMIR3/CD300LF, DNAM-1, LMIR5/CD300LB, Fc-epsilon RII, LMIR6/CD300LE, Fc-γ RI/CD64, MICA, Fc-γ RIIB/CD32b, MICB, Fc-γ RIIC/CD32c, MULT-1, Fc-γ RIIA/CD32a, Nectin-2/CD112, Fc-γ RIII/CD16, NKG2A, FcRH1/IRTA5, NKG2C, FcRH2/IRTA4, NKG2D, FcRH4/IRTA1, NKp30, FcRH5/IRTA2, NKp44, Fc-Receptor-like 3/CD16-2, NKp46/NCR1, NKp80/KLRF1, NTB-A/SLAMF6, Rae-1, Rae-1 α, Rae-1 β, Rae-1 delta, H60, Rae-1 epsilon, ILT2/CD85j, Rae-1 γ, ILT3/CD85k, TREM-1, ILT4/CD85d, TREM-2, ILT5/CD85a, TREM-3, KIR/CD158, TREML1/TLT-1, KIR2DL1, ULBP-1, KIR2DL3, ULBP-2, KIR2DL4/CD158d and ULBP-3. In various embodiments, a targeting moiety of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds one or more of these illustrative NK cell antigens.

Also, in some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) associated with macrophages/monocytes. In some embodiments, the recognition domains directly or indirectly recruit macrophages/monocytes, *e.g.*, in some embodiments, to a therapeutic site (*e.g.* a locus with one or more disease cell or cell to be modulated for a therapeutic effect). Illustrative macrophages/monocyte antigens of interest include, for example SIRP1a, B7-1/CD80, ILT4/CD85d, B7-H1, ILT5/CD85a, Common β Chain, Integrin α 4/CD49d, BLAME/SLAMF8, Integrin α X/CDIIc, CCL6/C10, Integrin β 2/CD18, CD155/PVR, Integrin β 3/CD61,

CD31/PECAM-1, Latexin, CD36/SR-B3, Leukotriene B4 R1, CD40/TNFRSF5, LIMPIIISR-B2, CD43, LMIR1/CD300A, CD45, LMIR2/CD300c, CD68, LMIR3/CD300LF, CD84/SLAMF5, LMIR5/CD300LB, CD97, LMIR6/CD300LE, CD163, LRP-1, CD2F-10/SLAMF9, MARCO, CRACC/SLAMF7, MD-1, ECF-L, MD-2, EMMPRIN/CD147, MGL2, Endoglin/CD105, Osteoactivin/GPNMB, Fc-γ RI/CD64, Osteopontin, Fc-γ RIIB/CD32b, PD-L2, Fc-γ RIIC/CD32c, Siglec-3/CD33, Fc-γ RIIA/CD32a, SIGNR1/CD209, Fc-γ RIII/CD16, SLAM, GM-CSF R α, TCCR/WSX-1, ICAM-2/CD102, TLR3, IFN-γ RI, TLR4, IFN- γ R2, TREM-I, IL-I RII, TREM-2, ILT2/CD85j, TREM-3, ILT3/CD85k, TREML1/TLT-1, 2B4/SLAMF 4, IL-10 R α, ALCAM, IL-10 R β, AminopeptidaseN/ANPEP, ILT2/CD85j, Common β Chain, ILT3/CD85k, Clq R1/CD93, ILT4/CD85d, CCR1, ILT5/CD85a, CCR2, Integrin α 4/CD49d, CCR5, Integrin α M/CDll b, CCR8, Integrin α X/CDllc, CD155/PVR, Integrin β 2/CD18, CD14, Integrin β 3/CD61, CD36/SR-B3, LAIR1, CD43, LAIR2, CD45, Leukotriene B4-R1, CD68, LIMPIIISR-B2, CD84/SLAMF5, LMIR1/CD300A, CD97, LMIR2/CD300c, LMIR3/CD300LF, Coagulation Factor III/Tissue Factor, LMIR5/CD300LB, CX3CR1, CX3CL1, LMIR6/CD300LE, CXCR4, LRP-1, CXCR6, M-CSF R, DEP-1/CD148, MD-1, DNAM-1, MD-2, EMMPRIN/CD147, MMR, Endoglin/CD105, NCAM-L1, Fc-γ RI/CD64, PSGL-1, Fc-γ RIIIICD16, RP105, G-CSF R, L-Selectin, GM-CSF R α, Siglec-3/CD33, HVEM/TNFRSF14, SLAM, ICAM-1/CD54, TCCR/WSX-1, ICAM-2/CD102, TREM-I, IL-6 R, TREM-2, CXCRI/IL-8 RA, TREM-3 and TREMLI/TLT-1. In various embodiments, a targeting moiety of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds one or more of these illustrative macrophage/monocyte antigens.

Also, in some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) associated with dendritic cells. In some embodiments, the recognition domains directly or indirectly recruit dendritic cells, *e.g.*, in some embodiments, to a therapeutic site (*e.g.* a locus with one or more disease cell or cell to be modulated for a therapeutic effect). Illustrative dendritic cell antigens of interest include, for example, CLEC9A, XCR1, RANK, CD36/SRB3, LOX-1/SR-E1, CD68, MARCO, CD163, SR-A1/MSR, CD5L, SREC-1, CL-PI/COLEC12, SREC-II, LIMPIIISRB2, RP105, TLR4, TLR1, TLR5, TLR2, TLR6, TLR3, TLR9, 4-IBB Ligand/TNFSF9, IL-12/IL-23 p40, 4-Amino-1,8-naphthalimide, ILT2/CD85j, CCL21/6Ckine, ILT3/CD85k, 8-oxo-dG, ILT4/CD85d, 8D6A, ILT5/CD85a, A2B5, lutegrin α 4/CD49d, Aag, Integrin β 2/CD18, AMICA, Langerin, B7-2/CD86, Leukotriene B4 RI, B7-H3, LMIR1/CD300A, BLAME/SLAMF8, LMIR2/CD300c, Clq R1/CD93, LMIR3/CD300LF, CCR6, LMIR5/CD300LB CCR7, LMIR6/CD300LE, CD40/TNFRSF5, MAG/Siglec-4-a, CD43, MCAM, CD45, MD-1, CD68, MD-2, CD83, MDL-1/CLEC5A, CD84/SLAMF5, MMR, CD97, NCAMLI, CD2F-10/SLAMF9, Osteoactivin GPNMB, Chern 23, PD-L2, CLEC-1, RP105, CLEC-2, CLEC-8, Siglec-2/CD22, CRACC/SLAMF7, Siglec-3/CD33, DC-SIGN, Siglec-5, DC-SIGNR/CD299, Siglec-6, DCAR, Siglec-7, DCIR/CLEC4A, Siglec-9, DEC-205, Siglec-10, Dectin-1/CLEC7A, Siglec-F, Dectin-2/CLEC6A, SIGNR1/CD209, DEP-1/CD148, SIGNR4, DLEC/CLEC4C, SLAM, EMMPRIN/CD147, TCCR/WSX-1, Fc-γ R1/CD64, TLR3, Fc-γ RIIB/CD32b, TREM-1, Fc-γ RIIC/CD32c, TREM-2, Fc-γ RIIA/CD32a, TREM-3, Fc-γ RIII/CD16, TREML1/TLT-1, ICAM-2/CD102 and Vanilloid R1. In various embodiments, a targeting moiety of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds one or more of these illustrative DC antigens.

In some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) on immune cells selected from, but not limited to, megakaryocytes, thrombocytes, erythrocytes, mast cells, basophils, neutrophils, myeloid cells, monocytes, eosinophils, or subsets thereof. In some embodiments, the recognition domains directly or indirectly recruit megakaryocytes, thrombocytes, erythrocytes, mast cells, basophils, neutrophils, myeloid cells, monocytes, eosinophils, or subsets thereof, *e.g.*, in some embodiments, to a therapeutic site (*e.g.* a locus with one or more disease cell or cell to be modulated for a therapeutic effect). In some embodiments, the immune cell is selected from a T cell, a B cell, a dendritic cell, a macrophage, a neutrophil, a mast cell, a monocyte, a red blood cell, myeloid cell, myeloid derived suppressor cell, a NKT cell, and a NK cell, or derivatives thereof.

In some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) associated with megakaryocytes and/or thrombocytes. Illustrative megakaryocyte and/or thrombocyte antigens of interest include, for example, GP IIb/IIIa, GPIb, vWF, PF4, and TSP. In various embodiments, a targeting moiety of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds one or more of these illustrative megakaryocyte and/or thrombocyte antigens.

In some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) associated with erythrocytes. Illustrative erythrocyte antigens of interest include, for example, CD34, CD36, CD38, CD41a (platelet glycoprotein IIb/IIIa), CD41b (GPIIb), CD71 (transferrin receptor), CD105, glycophorin A, glycophorin C, c-kit, HLA-DR, H2 (MHC-II), and Rhesus antigens. In various embodiments, a targeting moiety of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds one or more of these illustrative erythrocyte antigens.

In some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) associated with mast cells. Illustrative mast cells antigens of interest include, for example, SCFR/CD117, $Fc_{\varepsilon}RI$, CD2, CD25, CD35, CD88, CD203c, C5R1, CMAI, FCERIA, FCER2, TPSABI. In various embodiments, a targeting moiety of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds one or more of these mast cell antigens.

In some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) associated with basophils. Illustrative basophils antigens of interest include, for example, $Fc_{\varepsilon}RI$, CD203c, CD123, CD13, CD107a, CD107b, and CD164. In various embodiments, a targeting moiety of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds one or more of these basophil antigens.

In some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) associated with neutrophils. Illustrative neutrophils antigens of interest include, for example, 7D5, CD10/CALLA, CD13, CD16 (FcRIII), CD18 proteins (LFA-1, CR3, and p150, 95), CD45, CD67, and CD177. In various embodiments, a targeting moiety of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds one or more of these neutrophil antigens.

In some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) associated with eosinophils. Illustrative eosinophils antigens of interest include, for example, CD35, CD44 and CD69. In various

embodiments, a targeting moiety of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds one or more of these eosinophil antigens.

In various embodiments, the recognition domain may bind to any appropriate target, antigen, receptor, or cell surface markers known by the skilled artisan. In some embodiments, the antigen or cell surface marker is a tissue-specific marker. Illustrative tissue-specific markers include, but are not limited to, endothelial cell surface markers such as ACE, CD14, CD34, CDH5, ENG, ICAM2, MCAM, NOS3, PECAMI, PROCR, SELE, SELP, TEK, THBD, VCAMI, VWF; smooth muscle cell surface markers such as ACTA2, MYHIO, MYHI 1, MYH9, MYOCD; fibroblast (stromal) cell surface markers such as ALCAM, CD34, COLIAI, COL1A2, COL3A1, FAP, PH-4; epithelial cell surface markers such as CDID, K6IRS2, KRTIO, KRT13, KRT17, KRT18, KRT19, KRT4, KRT5, KRT8, MUCI, TACSTDI; neovasculature markers such as CD13, TFNA, Alpha-v beta-3 ($\alpha_V\beta_3$), E-selectin; and adipocyte surface markers such as ADIPOQ, FABP4, and RETN. In various embodiments, a targeting moiety of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds one or more of these antigens. In various embodiments, a targeting moiety of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds one or more of cells having these antigens.

In some embodiments, the recognition domains specifically bind to a target (*e.g.* antigen, receptor) associated with tumor cells. In some embodiments, the recognition domains directly or indirectly recruit tumor cells. For instance, in some embodiments, the direct or indirect recruitment of the tumor cell is to one or more effector cell (*e.g.* an immune cell as described herein) that can kill and/or suppress the tumor cell.

Tumor cells, or cancer cells refer to an uncontrolled growth of cells or tissues and/or an abnormal increase in cell survival and/or inhibition of apoptosis which interferes with the normal functioning of bodily organs and systems. For example, tumor cells include benign and malignant cancers, polyps, hyperplasia, as well as dormant tumors or micrometastases. Illustrative tumor cells include, but are not limited to cells of: basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.*, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic

leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (*e.g.* that associated with brain tumors), and Meigs' syndrome.

Tumor cells, or cancer cells also include, but are not limited to, carcinomas, *e.g.* various subtypes, including, for example, adenocarcinoma, basal cell carcinoma, squamous cell carcinoma, and transitional cell carcinoma), sarcomas (including, for example, bone and soft tissue), leukemias (including, for example, acute myeloid, acute lymphoblastic, chronic myeloid, chronic lymphocytic, and hairy cell), lymphomas and myelomas (including, for example, Hodgkin and non-Hodgkin lymphomas, light chain, non-secretory, MGUS, and plasmacytomas), and central nervous system cancers (including, for example, brain (*e.g.* gliomas (*e.g.* astrocytoma, oligodendroglioma, and ependymoma), meningioma, pituitary adenoma, and neuromas, and spinal cord tumors (*e.g.* meningiomas and neurofibroma).

Illustrative tumor antigens include, but are not limited to, MART-1/Melan-A, gp100, Dipeptidyl peptidase IV (DPPIV), adenosine deaminase-binding protein (ADAbp), cyclophilin b, Colorectal associated antigen (CRC)-0017-1A/GA733, Carcinoembryonic Antigen (CEA) and its immunogenic epitopes CAP-1 and CAP-2, etv6, aml1, Prostate Specific Antigen (PSA) and its immunogenic epitopes PSA-1, PSA-2, and PSA-3, prostate-specific membrane antigen (PSMA), T-cell receptor/CD3-zeta chain, MAGE-family of tumor antigens (*e.g.*, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, MAGE-C3, MAGE-C4, MAGE-C5), GAGE-family of tumor antigens (*e.g.*, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9), BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1, CDK4, tyrosinase, p53, MUC family, HER2/neu, p21ras, RCAS1, α-fetoprotein, E-cadherin, α-catenin, β-catenin and γ-catenin, p120ctn, gp100 Pmel117, PRAME, NY-ESO-1, cdc27, adenomatous polyposis coli protein (APC), fodrin, Connexin 37, Ig-idiotype, p15, gp75, GM2 and GD2 gangliosides, viral products such as human papilloma virus proteins, Smad family of tumor antigens, Imp-1, NA, EBV-encoded nuclear antigen (EBNA)-1, brain glycogen phosphorylase, SSX-1, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-1 CT-7, c-erbB-2, CD19, CD20, CD22, CD30, CD33, CD37, CD47, CS1, CD38, ASGPR, CD56, CD70, CD74, CD138, AGS16, MUC1 , GPNMB, Ep-CAM, PD-L1, PD-L2, PMSA, and BCMA (TNFRSF17). In various embodiments, a targeting moiety of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds one or more of these tumor antigens. In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds to HER2. In another embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes binds to PD-L2.

In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) one or more of the targeting moieties which is directed against an immune cell selected from a T cell, a B cell, a dendritic cell, a macrophage, a NK cell, or subsets thereof and (ii) one or more of the targeting moieties which is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a

variant thereof) described herein. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a T cell (including, without limitation an effector T cell) and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents described herein. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a B cell and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents described herein. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a dendritic cell and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents described herein. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a macrophage and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents described herein. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a NK cell and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents described herein.

By way of non-limiting example, in various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a T cell, for example, mediated by targeting to CD8, SLAMF4, IL-2 R α, 4-1BB/TNFRSF9, IL-2 R β, ALCAM, B7-1, IL-4 R, B7-H3, BLAME/SLAMFS, CEACAM1, IL-6 R, CCR3, IL-7 Rα, CCR4, CXCRI/IL-S RA, CCR5, CCR6, IL-10R α, CCR 7, IL-l 0 R β, CCRS, IL-12 R β 1, CCR9, IL-12 R β 2, CD2, IL-13 R α 1, IL-13, CD3, CD4, ILT2/CDS5j, ILT3/CDS5k, ILT4/CDS5d, ILT5/CDS5a, lutegrin α 4/CD49d, CDS, Integrin α E/CD103, CD6, Integrin α M/CD 11 b, CDS, Integrin α X/CD11c, Integrin β 2/CDIS, KIR/CD15S, CD27/TNFRSF7, KIR2DL1, CD2S, KIR2DL3, CD30/TNFRSFS, KIR2DL4/CD15Sd, CD31/PECAM-1, KIR2DS4, CD40 Ligand/TNFSF5, LAG-3, CD43, LAIR1, CD45, LAIR2, CDS3, Leukotriene B4-R1, CDS4/SLAMF5, NCAM-L1, CD94, NKG2A, CD97, NKG2C, CD229/SLAMF3, NKG2D, CD2F-10/SLAMF9, NT-4, CD69, NTB-A/SLAMF6, Common γ Chain/IL-2 R γ, Osteopontin, CRACC/SLAMF7, PD-1, CRTAM, PSGL-1, CTLA-4, RANK/TNFRSF11A, CX3CR1, CX3CL1, L-Selectin, CXCR3, SIRP β 1, CXCR4, SLAM, CXCR6, TCCR/WSX-1, DNAM-1, Thymopoietin, EMMPRIN/CD147, TIM-1, EphB6, TIM-2, Fas/TNFRSF6, TIM-3, Fas Ligand/TNFSF6, TIM-4, Fcγ RIII/CD16, TIM-6, TNFR1/TNFRSF1A, Granulysin, TNF RIII/TNFRSF1B, TRAIL RI/TNFRSFIOA, ICAM-1/CD54, TRAIL R2/TNFRSF10B, ICAM-2/CD102, TRAILR3/TNFRSF10C,IFN-γR1, TRAILR4/TNFRSF10D, IFN-γ R2, TSLP, IL-1 R1, or TSLP R; and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein.

By way of non-limiting example, in various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have a targeting moiety directed against (i) a checkpoint marker expressed on a T cell, *e.g.* one or more of PD-1, CD28, CTLA4, ICOS, BTLA, KIR, LAG3, CD137, OX40, CD27, CD40L, TIM3, and A2aR and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents described herein.

In various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have one or more targeting moieties directed against PD-1. In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have one or more targeting moieties which selectively bind a PD-1 polypeptide. In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes comprise one or more antibodies, antibody derivatives or formats, peptides or polypeptides, or fusion proteins that selectively bind a PD-1 polypeptide.

In an embodiment, the targeting moiety comprises the anti-PD-1 antibody pembrolizumab (aka MK-3475, KEYTRUDA), or fragments thereof. Pembrolizumab and other humanized anti-PD-1 antibodies are disclosed in Hamid, *et al.* (2013) New England Journal of Medicine 369 (2): 134-44, US 8,354,509, and WO 2009/114335, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, pembrolizumab or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising the amino acid sequence of (SEQ ID NO: 7) and/or a light chain comprising the amino acid sequence of (SEQ ID NO: 8).

In an embodiment, the targeting moiety comprises the anti-PD-1 antibody, nivolumab (aka BMS-936558, MDX-1106, ONO-4538, OPDIVO), or fragments thereof. Nivolumab (clone 5C4) and other human monoclonal antibodies that specifically bind to PD-1 are disclosed in US 8,008,449 and WO 2006/121168, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, nivolumab or an antigen-binding fragment thereof comprises a heavy chain comprising the amino acid sequence of (SEQ ID NO: 9) and/or a light chain comprising the amino acid sequence of (SEQ ID NO: 10).

In an embodiment, the targeting moiety comprises the anti-PD-1 antibody pidilizumab (aka CT-011, hBAT or hBAT-1), or fragments thereof. Pidilizumab and other humanized anti-PD-l monoclonal antibodies are disclosed in US 2008/0025980 and WO 2009/101611, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, the anti-PD-1 antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a light chain variable regions comprising an amino acid sequence selected from SEQ ID NOS: 15-18 of US 2008/0025980: SEQ ID No: 15 of US 2008/0025980 (SEQ ID NO: 11); SEQ ID No: 16 of US 2008/0025980(SEQ ID NO: 12);SEQ ID No: 17 of US 2008/0025980 (SEQ ID NO: 13); and SEQ ID No: 18 of US 2008/0025980 (SEQ ID NO: 14) ;and/or a heavy chain comprising an amino acid sequence selected from SEQ ID NOS: 20-24 of US 2008/0025980: SEQ ID No: 20 of US 2008/0025980 (SEQ ID NO: 15); SEQ ID No: 21 of US 2008/0025980 (SEQ ID NO: 16); SEQ ID No: 22 of US 2008/0025980 (SEQ ID NO: 17); SEQ ID No: 23 of US 2008/0025980 (SEQ ID NO: 18); and SEQ ID No: 24 of US 2008/0025980 (SEQ ID NO: 19).

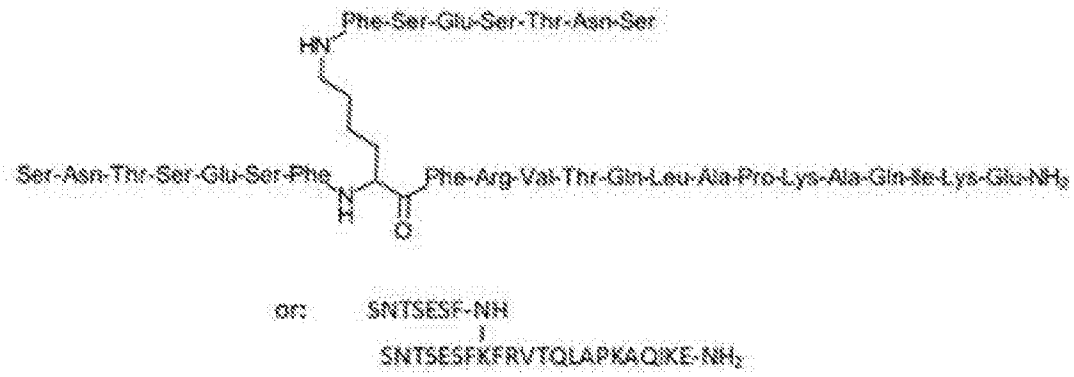
In an embodiment, the targeting moiety comprises a light chain comprising SEQ ID NO: 18 of US 2008/0025980 (SEQ ID NO: 14) and a heavy chain comprising SEQ ID NO: 22 of US 2008/0025980 (SEQ ID NO: 17).

In an embodiment, the targeting moiety comprises AMP-514 (aka MEDI-0680).

In an embodiment, the targeting moiety comprises the PD-L2-Fc fusion protein AMP-224, which is disclosed in WO2010/027827 and WO 2011/066342, the entire disclosures of which are hereby incorporated by reference. In such an embodiment, the targeting moiety may include a targeting domain which comprises SEQ ID NO:4 of

WO2010/027827 (SEQ ID NO :20) and/or the B7-DC fusion protein which comprises SEQ ID NO:83 of WO2010/027827 (SEQ ID NO: 21).

In an embodiment, the targeting moiety comprises the peptide AUNP 12 or any of the other peptides disclosed in US 2011/0318373 or 8,907,053. For example, the targeting moiety may comprise AUNP 12 (*i.e.*, Compound 8 or SEQ ID NO:49 of US 2011/0318373) which has the sequence of:



SEQ ID NO: 22).

In an embodiment, the targeting moiety comprises the anti-PD-1 antibody 1E3, or fragments thereof, as disclosed in US 2014/0044738, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 1E3 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 23); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 24).

In an embodiment, the targeting moiety comprises the anti-PD-1 antibody 1E8, or fragments thereof, as disclosed in US 2014/0044738, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 1E8 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 25) and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26.

In an embodiment, the targeting moiety comprises the anti-PD-1 antibody 1H3, or fragments thereof, as disclosed in US 2014/0044738, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 1H3 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 27) and/or light chain variable region comprising the amino acid sequence of (SEQ ID NO: 28).

In an embodiment, the targeting moiety comprises a VHH directed against PD-1 as disclosed, for example, in US 8,907,065 and WO 2008/071447, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, the VHHs against PD-1 comprise SEQ ID NOS: 347-351 of US 8,907,065 (SEQ ID No: 347 of US 8,907,065 (SEQ ID NO: 29); SEQ ID No: 348 of US 8,907,065 (SEQ ID NO:30); SEQ ID No: 349 of US 8,907,065 (SEQ ID NO:31); SEQ ID No: 350 of US 8,907,065 (SEQ ID NO:32); and SEQ ID No: 351 of US 8,907,065 (SEQ ID NO:33)).

In an embodiment, the targeting moiety comprises any one of the anti-PD-1 antibodies, or fragments thereof, as disclosed in US2011/0271358 and WO2010/036959, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NOS: 25-29 of US2011/0271358 (SEQ ID No: 25 of US2011/0271358 (SEQ ID NO:34); SEQ ID No: 26 of US2011/0271358 (SEQ ID NO:35); SEQ ID No: 27 of US2011/0271358 (SEQ ID NO:36); SEQ ID No: 28 of US2011/0271358 (SEQ ID NO:37);and SEQ ID No: 29 of US2011/0271358 (SEQ ID NO:38));and/or a light chain comprising an amino acid sequence selected from SEQ ID NOS: 30-33 of US2011/0271358 (SEQ ID No: 30 of US2011/0271358(SEQ ID NO:39); SEQ ID No: 31 of US2011/0271358 (SEQ ID NO:40); SEQ ID No: 32 of US2011/0271358 (SEQ ID NO:41); and SEQ ID No: 33 of US2011/0271358 (SEQ ID NO:42)).

In various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes comprise one or more antibodies directed against PD-1, or antibody fragments thereof, selected from TSR-042 (Tesaro, Inc.), REGN2810 (Regeneron Pharmaceuticals, Inc.), PDR001 (Novartis Pharmaceuticals), and BGB-A317 (BeiGene Ltd.)

In various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have one or more targeting moieties directed against PD-L1. In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have one or more targeting moieties which selectively bind a PD-L1 polypeptide. In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes comprise one or more antibodies, antibody derivatives or formats, peptides or polypeptides, or fusion proteins that selectively bind a PD-L1 polypeptide.

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody MEDI4736 (aka durvalumab), or fragments thereof. MEDI4736 is selective for PD-L1 and blocks the binding of PD-L1 to the PD-1 and CD80 receptors. MEDI4736 and antigen-binding fragments thereof for use in the methods provided herein comprises a heavy chain and a light chain or a heavy chain variable region and a light chain variable region. The sequence of MEDI4736 is disclosed in WO/2016/06272, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, MEDI4736 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising the amino acid sequence of (SEQ ID NO:43); and/or a light chain comprising the amino acid sequence of (SEQ ID NO:44).

In illustrative embodiments, the MEDI4736 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:4 of WO/2016/06272 (SEQ ID NO:45); and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:3 of WO/2016/06272 (SEQ ID NO:46).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody atezolizumab (aka MPDL3280A, RG7446), or fragments thereof. In illustrative embodiments, atezolizumab or an antigen-binding fragment thereof

for use in the methods provided herein comprises a heavy chain comprising the amino acid sequence of (SEQ ID NO:47); and/or a light chain comprising the amino acid sequence of (SEQ ID NO:48).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody avelumab (aka MSB0010718C), or fragments thereof. In illustrative embodiments, avelumab or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising the amino acid sequence of (SEQ ID NO:49); and/or a light chain comprising the amino acid sequence of (SEQ ID NO:50).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody BMS-936559 (aka 12A4, MDX-1105), or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, BMS-936559 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of: (SEQ ID NO:51); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO:52).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 3G10, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 3G10 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 53); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 54).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 10A5, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 10A5 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 55); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 56).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 5F8, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 5F8 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 57); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 58).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 10H10, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 10H10 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 59); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 60).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 1B12, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 1B12 or an antigen-binding fragment thereof for use in the methods

provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 61); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 62).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 7H1, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 7H1 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 63); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 64).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 11E6, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 11E6 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 65); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 66).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 12B7, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 12B7 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 67); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 68).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 13G4, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 13G4 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 69); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 70).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 1E12, or fragments thereof, as disclosed in US 2014/0044738, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 1E12 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 71); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 72).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 1F4, or fragments thereof, as disclosed in US 2014/0044738, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 1F4 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 73); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 74).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 2G11, or fragments thereof, as disclosed in US 2014/0044738, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 2G11 or an antigen-binding fragment thereof for use in the methods provided herein comprises a

heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 75); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 76).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 3B6, or fragments thereof, as disclosed in US 2014/0044738, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 3B6 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 77); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 78).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 3D10, or fragments thereof, as disclosed in US 2014/0044738 and WO2012/145493, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 3D10 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of (SEQ ID NO: 79); and/or a light chain variable region comprising the amino acid sequence of (SEQ ID NO: 80).

In an embodiment, the targeting moiety comprises any one of the anti-PD-L1 antibodies disclosed in US2011/0271358 and WO2010/036959, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID Nos: 34-38 of US2011/0271358 (SEQ ID No: 34 of US2011/0271358 (SEQ ID NO: 81); SEQ ID No: 35 of US2011/0271358 (SEQ ID NO: 82); SEQ ID No: 36 of US2011/0271358 (SEQ ID NO: 83); SEQ ID No: 37 of US2011/0271358 (SEQ ID NO: 84); and SEQ ID No: 38 of US2011/0271358 (SEQ ID NO: 85)); and/or a light chain comprising an amino acid sequence selected from SEQ ID Nos: 39-42 of US2011/0271358 (SEQ ID No: 39 of US2011/0271358 (SEQ ID NO: 86); SEQ ID No: 40 of US2011/0271358 (SEQ ID NO: 87); SEQ ID No: 41 of US2011/0271358 (SEQ ID NO: 88); and SEQ ID No: 42 of US2011/0271358(SEQ ID NO: 89)).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 2.7A4, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 2.7A4 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID No: 2 of WO 2011/066389 (SEQ ID NO: 90); and/or a light chain variable region comprising the amino acid sequence of SEQ ID No: 7 of WO 2011/066389 (SEQ ID NO: 91).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 2.9D10, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 2.9D10 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID No: 12 of WO 2011/066389 (SEQ ID NO: 92); and/or a light chain variable region comprising the amino acid sequence of SEQ ID No: 17 of WO 2011/066389 (SEQ ID NO: 93).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 2.14H9, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, the entire disclosures of which are hereby

incorporated by reference. In illustrative embodiments, 2.14H9 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID No: 22 of WO 2011/066389 (SEQ ID NO: 94); and/or a light chain variable region comprising the amino acid sequence of SEQ ID No: 27 of WO 2011/066389 (SEQ ID NO: 95).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 2.20A8, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 2.20A8 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID No: 32 of WO 2011/066389 (SEQ ID NO: 96); and/or a light chain variable region comprising the amino acid sequence of SEQ ID No: 37 of WO 2011/066389 (SEQ ID NO: 97).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 3.15G8, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 3.15G8 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID No: 42 of WO 2011/066389 (SEQ ID NO: 98); and/or a light chain variable region comprising the amino acid sequence of SEQ ID No: 47 of WO 2011/066389 (SEQ ID NO: 99).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 3.18G1, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 3.18G1 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID No: 52 of WO 2011/066389 (SEQ ID NO:100); and/or a light chain variable region comprising the amino acid sequence of SEQ ID No: 57 of WO 2011/066389 (SEQ ID NO: 101).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 2.7A4OPT, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, and US2014/0356353, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 2.7A4OPT or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID No: 62 of WO 2011/066389 (SEQ ID NO:102); and/or a light chain variable region comprising the amino acid sequence of SEQ ID No: 67 of WO 2011/066389 (SEQ ID NO:103).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 2.14H9OPT, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 2.14H9OPT or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID No: 72 of WO 2011/066389 (SEQ ID NO:104); and/or a light chain variable region comprising the amino acid sequence of SEQ ID No: 77 of WO 2011/066389 (SEQ ID NO:105).

In an embodiment, the targeting moiety comprises any one of the anti-PD-L1 antibodies disclosed in WO2016/061142, the entire contents of which are hereby incorporated by reference. In illustrative embodiments,

the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID Nos: 18, 30, 38, 46, 50, 54, 62, 70, and 78 of WO2016/061142 (SEQ ID No: 18 of WO2016/061142 (SEQ ID NO:106); SEQ ID No: 30 of WO2016/061142 (SEQ ID NO:107); SEQ ID No: 38 of WO2016/061142 (SEQ ID NO:108); SEQ ID No: 46 of WO2016/061142 (SEQ ID NO:109); SEQ ID No: 50 of WO2016/061142 (SEQ ID NO:110); SEQ ID No: 54 of WO2016/061142 (SEQ ID NO:111); SEQ ID No: 62 of WO2016/061142 (SEQ ID NO:112); SEQ ID No: 70 of WO2016/061142 (SEQ ID NO:113); and SEQ ID No: 78 of WO2016/061142 (SEQ ID NO:114)); and/or a light chain comprising an amino acid sequence selected from SEQ ID Nos: 22, 26, 34, 42, 58, 66, 74, 82, and 86 of WO2016/061142 (SEQ ID No: 22 of WO2016/061142 (SEQ ID NO:115); SEQ ID No: 26 of WO2016/061142 (SEQ ID NO:116); SEQ ID No: 34 of WO2016/061142 (SEQ ID NO:117); SEQ ID No: 42 of WO2016/061142 (SEQ ID NO:118);SEQ ID No: 58 of WO2016/061142 (SEQ ID NO:119); SEQ ID No: 66 of WO2016/061142(SEQ ID NO:120); SEQ ID No: 74 of WO2016/061142 (SEQ ID NO:121); SEQ ID No: 82 of WO2016/061142 (SEQ ID NO:122); and SEQ ID No: 86 of WO2016/061142 (SEQ ID NO:123)).

In an embodiment, the targeting moiety comprises any one of the anti-PD-L1 antibodies disclosed in WO2016/022630, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID Nos: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, and 46 of WO2016/022630 (SEQ ID No: 2 of WO2016/022630 (SEQ ID NO:124); SEQ ID No: 6 of WO2016/022630 (SEQ ID NO:125); SEQ ID No: 10 of WO2016/022630 (SEQ ID NO:126); SEQ ID No: 14 of WO2016/022630 (SEQ ID NO:127); SEQ ID No: 18 of WO2016/022630 (SEQ ID NO:128); SEQ ID No: 22 of WO2016/022630 (SEQ ID NO:129); SEQ ID No: 26 of WO2016/022630 (SEQ ID NO:130); SEQ ID No: 30 of WO2016/022630 (SEQ ID NO:131); SEQ ID No: 34 of WO2016/022630 (SEQ ID NO:132); SEQ ID No: 38 of WO2016/022630 (SEQ ID NO:133); SEQ ID No: 42 of WO2016/022630 (SEQ ID NO:134); and SEQ ID No: 46 of WO2016/022630 (SEQ ID NO:135)); and/or a light chain comprising an amino acid sequence selected from SEQ ID Nos: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 of WO2016/022630 (SEQ ID No: 4 of WO2016/022630 (SEQ ID NO:136); SEQ ID No: 8 of WO2016/022630 (SEQ ID NO:137); SEQ ID No: 12 of WO2016/022630 (SEQ ID NO:138); SEQ ID No: 16 of WO2016/022630 (SEQ ID NO:139); SEQ ID No: 20 of WO2016/022630 (SEQ ID NO:140); SEQ ID No: 24 of WO2016/022630 (SEQ ID NO:141); SEQ ID No: 28 of WO2016/022630 (SEQ ID NO:142); SEQ ID No: 32 of WO2016/022630 (SEQ ID NO:143); SEQ ID No: 36 of WO2016/022630 (SEQ ID NO:144); SEQ ID No: 40 of WO2016/022630 (SEQ ID NO:145); SEQ ID No: 44 of WO2016/022630 (SEQ ID NO:146); and SEQ ID No: 48 of WO2016/022630 (SEQ ID NO:147)).

In an embodiment, the targeting moiety comprises any one of the anti-PD-L1 antibodies disclosed in WO2015/112900, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID Nos: 38, 50, 82, and 86 of WO 2015/112900 (SEQ ID No: 38 of WO2015/112900 (SEQ ID NO:148); SEQ ID No: 50 of WO 2015/112900 (SEQ ID NO:149);

SEQ ID No: 82 of WO 2015/112900 (SEQ ID NO:150); and SEQ ID No: 86 of WO 2015/112900 (SEQ ID NO:151)); and/or a light chain comprising an amino acid sequence selected from SEQ ID Nos: 42, 46, 54, 58, 62, 66, 70, 74, and 78 of WO 2015/112900 (SEQ ID No: 42 of WO2015/112900 (SEQ ID NO:152); SEQ ID No: 46 of WO 2015/112900: (SEQ ID NO:153); SEQ ID No: 54 of WO 2015/112900 (SEQ ID NO:154); SEQ ID No: 58 of WO 2015/112900 (SEQ ID NO:155); SEQ ID No: 62 of WO 2015/112900 (SEQ ID NO:156); SEQ ID No: 66 of WO 2015/112900 (SEQ ID NO:157); SEQ ID No: 70 of WO 2015/112900 (SEQ ID NO:158); SEQ ID No: 74 of WO 2015/112900 (SEQ ID NO:159); and SEQ ID No: 78 of WO 2015/112900 (SEQ ID NO:160)).

In an embodiment, the targeting moiety comprises any one of the anti-PD-L1 antibodies disclosed in WO 2010/077634 and US 8,217,149, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, the anti-PD-L1 antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain region comprising the amino acid sequence of SEQ ID No: 20 of WO 2010/077634 (SEQ ID NO: 161); and/or a light chain variable region comprising the amino acid sequence of SEQ ID No: 21 of WO 2010/077634 (SEQ ID NO: 162).

In an embodiment, the targeting moiety comprises any one of the anti-PD-L1 antibodies obtainable from the hybridoma accessible under CNCM deposit numbers CNCM I-4122, CNCM I-4080 and CNCM I-4081 as disclosed in US 20120039906, the entire disclosures of which are hereby incorporated by reference.

In an embodiment, the targeting moiety comprises a VHH directed against PD-L1 as disclosed, for example, in US 8,907,065 and WO 2008/071447, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, the VHHs against PD-L1 comprise SEQ ID NOS: 394-399 of US 8,907,065 (SEQ ID No: 394 of US 8,907,065 (SEQ ID NO:163); SEQ ID No: 395 of US 8,907,065 (SEQ ID NO:164); SEQ ID No: 396 of US 8,907,065 (SEQ ID NO:165); SEQ ID No: 397 of US 8,907,065 (SEQ ID NO:166); SEQ ID No: 398 of US 8,907,065 (SEQ ID NO:167); and SEQ ID No: 399 of US 8,907,065 (SEQ ID NO:168)).

In various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have one or more targeting moieties directed against PD-L2. In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have one or more targeting moieties which selectively bind a PD-L2 polypeptide. In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes comprise one or more antibodies, antibody derivatives or formats, peptides or polypeptides, or fusion proteins that selectively bind a PD-L2 polypeptide.

In an embodiment, the targeting moiety comprises a VHH directed against PD-L2 as disclosed, for example, in US 8,907,065 and WO 2008/071447, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, the VHHs against PD-L2 comprise SEQ ID Nos: 449-455 of US 8,907,065 (SEQ ID No: 449 of US 8,907,065 (SEQ ID NO:169); SEQ ID No: 450 of US 8,907,065 (SEQ ID NO:170); SEQ ID No: 451 of US 8,907,065 (SEQ ID NO:171); SEQ ID No: 452 of US 8,907,065 (SEQ ID NO:172); SEQ ID No: 453 of US 8,907,065 (SEQ ID NO:173); SEQ ID No: 454 of US 8,907,065 (SEQ ID NO:174); and SEQ ID No: 455 of US 8,907,065 (SEQ ID NO:175)).

In an embodiment, the targeting moiety comprises any one of the anti-PD-L2 antibodies disclosed in US2011/0271358 and WO2010/036959, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID Nos: 43-47 of US2011/0271358 (SEQ ID No: 43 of US2011/0271358 (SEQ ID NO:176); SEQ ID No: 44 of US2011/0271358 (SEQ ID NO:177); SEQ ID No: 45 of US2011/0271358 (SEQ ID NO:178); SEQ ID No: 46 of US2011/0271358 (SEQ ID NO:179); and SEQ ID No: 47 of US2011/0271358 (SEQ ID NO:180)); and/or a light chain comprising an amino acid sequence selected from SEQ ID Nos: 48-51 of US2011/0271358 (SEQ ID No: 48 of US2011/0271358 (SEQ ID NO:181); SEQ ID No: 49 of US2011/0271358 (SEQ ID NO:182); SEQ ID No: 50 of US2011/0271358 (SEQ ID NO:183); and SEQ ID No: 51 of US2011/0271358 (SEQ ID NO:184)).

In various embodiments, the targeting moieties of the invention may comprise a sequence that targets PD-1, PD-L1, and/or PD-L2 which is at least about 60%, at least about 61%, at least about 62%, at least about 63%, at least about 64%, at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to any of the sequences disclosed herein (*e.g.* about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, about 99% or about 100% sequence identity with any of the sequences disclosed herein).

In various embodiments, the targeting moieties of the invention may comprise any combination of heavy chain, light chain, heavy chain variable region, light chain variable region, complementarity determining region (CDR), and framework region sequences that target PD-1, PD-L1, and/or PD-L2 as disclosed herein.

Additional antibodies, antibody derivatives or formats, peptides or polypeptides, or fusion proteins that selectively bind or target PD-1, PD-L1 and/or PD-L2 are disclosed in WO 2011/066389, US 2008/0025980, US 2013/0034559, US 8,779,108, US 2014/0356353, US 8,609,089, US 2010/028330, US 2012/0114649, WO 2010/027827, WO 2011,/066342, US 8,907,065, WO 2016/062722, WO 2009/101611, WO2010/027827, WO 2011/066342, WO 2007/005874 , WO 2001/014556, US2011/0271358, WO 2010/036959, WO 2010/077634, US 8,217,149, US 2012/0039906, WO 2012/145493, US 2011/0318373, U.S. Patent No. 8,779,108, US 20140044738, WO 2009/089149, WO 2007/00587, WO 2016061142, WO 2016,02263, WO 2010/077634, and WO 2015/112900, the entire disclosures of which are hereby incorporated by reference.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a T cell, for example, mediated by targeting to CD8 and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein. In an embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have a targeting moiety directed against CD8 on T cells and a second targeting moiety directed against PD-L1 or PD-L2 on tumor cells.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a T cell, for example, mediated by targeting to CD4 and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein. In an embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have a targeting moiety directed against CD4 on T cells and a second targeting moiety directed against PD-L1 or PD-L2 on tumor cells.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a T cell, for example, mediated by targeting to CD3, CXCR3, CCR4, CCR9, CD70, CD103, or one or more immune checkpoint markers and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 interferon or a variant thereof) described herein. In an embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have a targeting moiety directed against CD3 on T cells and a second targeting moiety directed against PD-L1 or PD-L2 on tumor cells.

In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have one or more targeting moieties directed against CD3 expressed on T cells. In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have one or more targeting moieties which selectively bind a CD3 polypeptide. In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes comprise one or more antibodies, antibody derivatives or formats, peptides or polypeptides, or fusion proteins that selectively bind a CD3 polypeptide.

In an embodiment, the targeting moiety comprises the anti-CD3 antibody muromonab-CD3 (aka Orthoclone OKT3), or fragments thereof. Muromonab-CD3 is disclosed in U.S. Patent No. 4,361,549 and Wilde *et al.* (1996) 51:865-894, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, muromonab-CD3 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising the amino acid sequence of (SEQ ID NO:185); and/or a light chain comprising the amino acid sequence of (SEQ ID NO:186).

In an embodiment, the targeting moiety comprises the anti-CD3 antibody otelixizumab, or fragments thereof. Otelixizumab is disclosed in U.S. Patent Publication No. 20160000916 and Chatenoud *et al.* (2012) 9:372-381, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, otelixizumab or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising the

amino acid sequence of: SEQ ID NO:187; and/or a light chain comprising the amino acid sequence of SEQ ID NO:188.

In an embodiment, the targeting moiety comprises the anti-CD3 antibody teplizumab (AKA MGA031 and hOKT3γ1(Ala-Ala)), or fragments thereof. Teplizumab is disclosed in Chatenoud *et al.* (2012) 9:372-381, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, teplizumab or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:189; and/or a light chain comprising the amino acid sequence of SEQ ID NO:190.

In an embodiment, the targeting moiety comprises the anti-CD3 antibody visilizumab (AKA Nuvion®; HuM291), or fragments thereof. Visilizumab is disclosed in U.S. 5,834,597 and WO2004052397, and Cole *et al.*, Transplantation (1999) 68:563-571, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, visilizumab or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:191; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:192.

In an embodiment, the targeting moiety comprises the anti-CD3 antibody foralumab (aka NI-0401), or fragments thereof. In various embodiments, the targeting moiety comprises any one of the anti-CD3 antibodies disclosed in US20140193399, US 7,728,114, US20100183554, and US 8,551,478, the entire disclosures of which are hereby incorporated by reference.

In illustrative embodiments, the anti-CD3 antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID Nos: 2 and 6 of US 7,728,114 (SEQ ID No: 2 of US 7,728,114 (SEQ ID NO:193) and SEQ ID No: 6 of US 7,728,114 (SEQ ID NO:194)); and/or a light chain variable region comprising the amino acid sequence of SEQ ID NOs 4 and 8 of US 7,728,114 (SEQ ID No: 4 of US 7,728,114 (SEQ ID NO:195) and SEQ ID No: 8 of US 7,728,114 (SEQ ID NO:196)).

In an embodiment, the targeting moiety comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:2 of US 7,728,114 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4 of US 7,728,114. In an embodiment, the targeting moiety comprises any one of the anti-CD3 antibodies disclosed in US2016/0168247, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID Nos: 6-9 of US2016/0168247 (SEQ ID No: 6 of US2016/0168247 (SEQ ID NO:197); SEQ ID No: 7 of US2016/0168247 (SEQ ID NO:198); SEQ ID No: 8 of US2016/0168247 (SEQ ID NO:199); and SEQ ID No: 9 of US2016/0168247 (SEQ ID NO:200)); and/or a light chain comprising an amino acid sequence selected from SEQ ID Nos: 10-12 of US2016/0168247 (SEQ ID No: 10 of US2016/0168247 (SEQ ID NO:201); SEQ ID No: 11 of US2016/0168247 (SEQ ID NO:202); and SEQ ID No: 12 of US2016/0168247 (SEQ ID NO:203)).

In an embodiment, the targeting moiety comprises any one of the anti-CD3 antibodies disclosed in US2015/0175699, the entire contents of which are hereby incorporated by reference. In illustrative embodiments,

the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID No: 9 of US2015/0175699 (SEQ ID NO:204); and/or a light chain comprising an amino acid sequence selected from SEQ ID No: 10 of US2015/0175699 (SEQ ID NO:205).

In an embodiment, the targeting moiety comprises any one of the anti-CD3 antibodies disclosed in US 8,784,821, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID Nos: 2, 18, 34, 50, 66, 82, 98 and 114 of US 8,784,821 (SEQ ID No: 2 of US 8,784,821 (SEQ ID NO:206); SEQ ID No: 18 of US 8,784,821 (SEQ ID NO:207); SEQ ID No: 34 of US 8,784,821 (SEQ ID NO:208); SEQ ID No: 50 of US 8,784,821 (SEQ ID NO:209); SEQ ID No: 66 of US 8,784,821 (SEQ ID NO:210); SEQ ID No: 82 of US 8,784,821 (SEQ ID NO:211); SEQ ID No: 98 of US 8,784,821 (SEQ ID NO:212); and SEQ ID No: 114 of US 8,784,821 (SEQ ID NO:213)); and/or a light chain comprising an amino acid sequence selected from SEQ ID Nos: 10, 26, 42, 58, 74, 90, 106 and 122 of US 8,784,821 (SEQ ID No: 10 of US 8,784,821 (SEQ ID NO:214); SEQ ID No: 26 of US 8,784,821 (SEQ ID NO:215); SEQ ID No: 42 of US 8,784,821 (SEQ ID NO:216); SEQ ID No: 58 of US 8,784,821 (SEQ ID NO:217); SEQ ID No: 74 of US 8,784,821 (SEQ ID NO:218); SEQ ID No: 90 of US 8,784,821 (SEQ ID NO:219); SEQ ID No: 106 of US 8,784,821 (SEQ ID NO:220); and SEQ ID No: 122 of US 8,784,821 (SEQ ID NO:221).

In an embodiment, the targeting moiety comprises any one of the anti-CD3 binding constructs disclosed in US20150118252, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID Nos: 6 and 86 of US20150118252 (SEQ ID No: 6 of US20150118252 (SEQ ID NO:222) and SEQ ID No: 86 of US20150118252 (SEQ ID NO:223)) and/or a light chain comprising an amino acid sequence selected from SEQ ID No: 3 of US2015/0175699 (SEQ ID No: 3 of US20150118252 (SEQ ID NO:224)).

In an embodiment, the targeting moiety comprises any one of the anti-CD3 binding proteins disclosed in US2016/0039934, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID Nos: 6-9 of US2016/0039934 (SEQ ID No: 6 of US2016/0039934 (SEQ ID NO:225); SEQ ID No: 7 of US2016/0039934 (SEQ ID NO:226); SEQ ID No: 8 of US2016/0039934 (SEQ ID NO:227); and SEQ ID No: 9 of US2016/0039934 (SEQ ID NO:228)); and/or a light chain comprising an amino acid sequence selected from SEQ ID Nos: 1-4 of US2016/0039934 (SEQ ID No: 1 of US2016/0039934 (SEQ ID NO:229); SEQ ID No: 2 of US2016/0039934 (SEQ ID NO:230); SEQ ID No: 3 of US2016/0039934 (SEQ ID NO:231); and SEQ ID No: 4 of US2016/0039934 (SEQ ID NO:232)).

In various embodiments, the targeting moieties of the invention may comprise a sequence that targets CD3 which is at least about 60%, at least about 61%, at least about 62%, at least about 63%, at least about 64%, at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at

least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to any of the sequences disclosed herein (*e.g.* about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, about 99% or about 100% sequence identity with any of the sequences disclosed herein).

In various embodiments, the targeting moieties of the invention may comprise any combination of heavy chain, light chain, heavy chain variable region, light chain variable region, complementarity determining region (CDR), and framework region sequences that target CD3 as disclosed herein. In various embodiments, the targeting moieties of the invention may comprise any heavy chain, light chain, heavy chain variable region, light chain variable region, complementarity determining region (CDR), and framework region sequences of the CD3-specific antibodies including, but not limited to, X35-3, VIT3, BMA030 (BW264/56), CLB-T3/3, CRIS7, YTH12.5, FI 11-409, CLB-T3.4.2, TR-66, WT32, SPv-T3b, 11D8, XIII-141, XIII-46, XIII-87, 12F6, T3/RW2-8C8, T3/RW2-4B6, OKT3D, M-T301, SMC2, WT31 and F101.01. These CD3-specific antibodies are well known in the art and, *inter alia*, described in Tunnacliffe (1989), Int. Immunol. 1, 546-550, the entire disclosures of which are hereby incorporated by reference.

Additional antibodies, antibody derivatives or formats, peptides or polypeptides, or fusion proteins that selectively bind or target CD3 are disclosed in US Patent Publication No. 2016/0000916, US Patent Nos. 4,361,549, 5,834,597, 6,491,916, 6,406,696, 6,143,297, 6,750,325 and International Publication No. WO 2004/052397, the entire disclosures of which are hereby incorporated by reference.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a T cell, for example, mediated by targeting to PD-1 and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein.

By way of non-limiting example, in various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a B cell, for example, mediated by targeting to CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD38, CD39, CD40, CD70, CD72, CD73, CD74, CDw75, CDw76, CD77, CD78, CD79a/b, CD80, CD81, CD82, CD83, CD84, CD85, CD86, CD89, CD98, CD126, CD127, CDw130, CD138, or CDw150; and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein. In an

embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have a targeting moiety directed against CD20.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a B cell, for example, mediated by targeting to CD19, CD20 or CD70 and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a B cell, for example, mediated by targeting to CD20 and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein. In an embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have a targeting moiety directed against CD20 on B cells and a second targeting moiety directed against PD-L1 or PD-L2 on tumor cells. By way of example, in some embodiments, the CD20 targeting moiety is a recombinant heavy-chain-only antibody (VHH) having the sequence of:

QVQLQESGGGLAQAGGSLRLSCAASGRTFSMGWFRQAPGKEREFVAAITYSGGSPYYASSVRGRFTISRDNAK NTVYLQMNSLKPEDTAVYYCAANPTYGSDWNAENWGQGTQVTVSS (SEQ ID NO: 288).

By way of non-limiting example, in various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a NK cell, for example, mediated by targeting to 2B4/SLAMF4, KIR2DS4, CD155/PVR, KIR3DL1, CD94, LMIR1/CD300A, CD69, LMIR2/CD300c, CRACC/SLAMF7, LMIR3/CD300LF, DNAM-1, LMIR5/CD300LB, Fc-epsilon RII, LMIR6/CD300LE, Fc-γ RI/CD64, MICA, Fc-γ RIIB/CD32b, MICB, Fc-γ RIIC/CD32c, MULT-1, Fc-γ RIIA/CD32a, Nectin-2/CD112, Fc-γ RIII/CD16, NKG2A, FcRH1/IRTA5, NKG2C, FcRH2/IRTA4, NKG2D, FcRH4/IRTA1, NKp30, FcRH5/IRTA2, NKp44, Fc-Receptor-like 3/CD16-2, NKp46/NCR1, NKp80/KLRF1, NTB-A/SLAMF6, Rae-1, Rae-1 α, Rae-1 β, Rae-1 delta, H60, Rae-1 epsilon, ILT2/CD85j, Rae-1 γ, ILT3/CD85k, TREM-1, ILT4/CD85d, TREM-2, ILT5/CD85a, TREM-3, KIR/CD158, TREML1/TLT-1, KIR2DL1, ULBP-1, KIR2DL3, ULBP-2, KIR2DL4/CD158d, or ULBP-3; and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a NK cell, for example, mediated by targeting to Kir1alpha, DNAM-1 or CD64 and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a NK cell, for example, mediated by targeting to KIR1 and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein. In an embodiment, the present chimeric proteins or chimeric protein complexes

such as Fc-based chimeric protein complexes have a targeting moiety directed against KIR1 on NK cells and a second targeting moiety directed against PD-L1 or PD-L2 on tumor cells.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a NK cell, for example, mediated by targeting to TIGIT or KIR1 and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein. In an embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have a targeting moiety directed against TIGIT on NK cells and a second targeting moiety directed against PD-L1 or PD-L2 on tumor cells.

By way of non-limiting example, in various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a dendritic cell, for example, mediated by targeting to CLEC-9A, XCR1, RANK, CD36/SRB3, LOX-1/SR-E1, CD68, MARCO, CD163, SR-A1/MSR, CD5L, SREC-1, CL-PI/COLEC12, SREC-II, LIMPIIISRB2, RP105, TLR4, TLR1, TLR5, TLR2, TLR6, TLR3, TLR9, 4-IBB Ligand/TNFSF9, IL-12/IL-23 p40, 4-Amino-1,8-naphthalimide, ILT2/CD85j, CCL21/6Ckine, ILT3/CD85k, 8-oxo-dG, ILT4/CD85d, 8D6A, ILT5/CD85a, A2B5, lutegrin α 4/CD49d, Aag, Integrin β 2/CD18, AMICA, Langerin, B7-2/CD86, Leukotriene B4 Rl, B7-H3, LMIR1/CD300A, BLAME/SLAMF8, LMIR2/CD300c, Clq R1/CD93, LMIR3/CD300LF, CCR6, LMIR5/CD300LB CCR7, LMIR6/CD300LE, CD40/TNFRSF5, MAG/Siglec-4-a, CD43, MCAM, CD45, MD-1, CD68, MD-2, CD83, MDL-1/CLEC5A, CD84/SLAMF5, MMR, CD97, NCAMLl, CD2F-10/SLAMF9, Osteoactivin GPNMB, Chern 23, PD-L2, CLEC-1, RP105, CLEC-2, Siglec-2/CD22, CRACC/SLAMF7, Siglec-3/CD33, DC-SIGN, Siglec-5, DC-SIGNR/CD299, Siglec-6, DCAR, Siglec-7, DCIR/CLEC4A, Siglec-9, DEC-205, Siglec-10, Dectin-1/CLEC7A, Siglec-F, Dectin-2/CLEC6A, SIGNR1/CD209, DEP-1/CD148, SIGNR4, DLEC, SLAM, EMMPRIN/CD147, TCCR/WSX-1, Fc-γ R1/CD64, TLR3, Fc-γ RIIB/CD32b, TREM-1, Fc-γ RIIC/CD32c, TREM-2, Fc-γ RIIA/CD32a, TREM-3, Fc-γ RIII/CD16, TREML1/TLT-1, ICAM-2/CD102, or Vanilloid R1; and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a dendritic cell, for example, mediated by targeting to CLEC-9A, DC-SIGN, CD64, CLEC4A, or DEC205 and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein. In an embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have a targeting moiety directed against CLEC9A on dendritic cells and a second targeting moiety directed against PD-L1 or PD-L2 on tumor cells.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a dendritic cell, for example, mediated by targeting to CLEC9A and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein. In an embodiment, the present chimeric proteins or chimeric protein

complexes such as Fc-based chimeric protein complexes have a targeting moiety directed against CLEC9A on dendritic cells and a second targeting moiety directed against PD-L1 or PD-L2 on tumor cells.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a dendritic cell, for example, mediated by targeting to XCR1 and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein. In an embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have a targeting moiety directed against XCR1 on dendritic cells and a second targeting moiety directed against PD-L1 or PD-L2 on tumor cells.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a dendritic cell, for example, mediated by targeting to RANK and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein. In an embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have a targeting moiety directed against RANK on dendritic cells and a second targeting moiety directed against PD-L1 or PD-L2 on tumor cells.

By way of non-limiting example, in various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a monocyte/macrophage, for example, mediated by targeting to SIRP1a, B7-1/CD80, ILT4/CD85d, B7-H1, ILT5/CD85a, Common β Chain, Integrin α 4/CD49d, BLAME/SLAMF8, Integrin α X/CDIIc, CCL6/C10, Integrin β 2/CD18, CD155/PVR, Integrin β 3/CD61, CD31/PECAM-1, Latexin, CD36/SR-B3, Leukotriene B4 R1, CD40/TNFRSF5, LIMPIIISR-B2, CD43, LMIR1/CD300A, CD45, LMIR2/CD300c, CD68, LMIR3/CD300LF, CD84/SLAMF5, LMIR5/CD300LB, CD97, LMIR6/CD300LE, CD163, LRP-1, CD2F-10/SLAMF9, MARCO, CRACC/SLAMF7, MD-1, ECF-L, MD-2, EMMPRIN/CD147, MGL2, Endoglin/CD105, Osteoactivin/GPNMB, Fc-γ RI/CD64, Osteopontin, Fc-γ RIIB/CD32b, PD-L2, Fc-γ RIIC/CD32c, Siglec-3/CD33, Fc-γ RIIA/CD32a, SIGNR1/CD209, Fc-γ RIII/CD16, SLAM, GM-CSF R α, TCCR/WSX-1, ICAM-2/CD102, TLR3, IFN-γ RI, TLR4, IFN-γ R2, TREM-I, IL-I RII, TREM-2, ILT2/CD85j, TREM-3, ILT3/CD85k, TREML1/TLT-1, 2B4/SLAMF 4, IL-10 R α, ALCAM, IL-10 R β, AminopeptidaseN/ANPEP, ILT2/CD85j, Common β Chain, ILT3/CD85k, Clq R1/CD93, ILT4/CD85d, CCR1, ILT5/CD85a, CCR2, CD206, Integrin α 4/CD49d, CCR5, Integrin α M/CDII b, CCR8, Integrin α X/CDIIc, CD155/PVR, Integrin β 2/CD18, CD14, Integrin β 3/CD61, CD36/SR-B3, LAIR1, CD43, LAIR2, CD45, Leukotriene B4-R1, CD68, LIMPIIISR-B2, CD84/SLAMF5, LMIR1/CD300A, CD97, LMIR2/CD300c, CD163, LMIR3/CD300LF, Coagulation Factor III/Tissue Factor, LMIR5/CD300LB, CX3CR1, CX3CL1, LMIR6/CD300LE, CXCR4, LRP-1, CXCR6, M-CSF R, DEP-1/CD148, MD-1, DNAM-1, MD-2, EMMPRIN/CD147, MMR, Endoglin/CD105, NCAM-L1, Fc-γ RI/CD64, PSGL-1, Fc-γ RIIIICD16, RP105, G-CSF R, L-Selectin, GM-CSF R α, Siglec-3/CD33, HVEM/TNFRSF14, SLAM, ICAM-1/CD54, TCCR/WSX-1, ICAM-2/CD102, TREM-I, IL-6 R, TREM-2, CXCRI/IL-8 RA, TREM-3, or TREMLI/TLT-1; and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have (i) a targeting moiety directed against a monocyte/macrophage, for example, mediated by targeting to B7-H1, CD31/PECAM-1, CD163, CCR2, or Macrophage Mannose Receptor CD206 and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex has (i) a targeting moiety directed against a monocyte/macrophage, for example, mediated by targeting to SIRP1a and (ii) a targeting moiety is directed against a tumor cell, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein. In an embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex has a targeting moiety directed against SIRP1a on macrophage cells and a second targeting moiety directed against PD-L1 or PD-L2 on tumor cells.

In various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex has one or more targeting moieties directed against a checkpoint marker, *e.g.* one or more of PD-1/PD-L1 or PD-L2, CD28/CD80 or CD86, CTLA4/ CD80 or CD86, ICOS/ICOSL or B7RP1, BTLA/HVEM, KIR, LAG3, CD137/CD137L, OX40/OX40L, CD27, CD40L, TIM3/Gal9, CD47, CD70, and A2aR. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex has (i) a targeting moiety directed against a checkpoint marker on a T cell, for example, PD-1 and (ii) a targeting moiety directed against a tumor cell, for example, PD-L1 or PD-L2, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein. In an embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex has a targeting moiety directed against PD-1 on T cells and a second targeting moiety directed against PD-L1 on tumor cells. In another embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex has a targeting moiety directed against PD-1 on T cells and a second targeting moiety directed against PD-L2 on tumor cells.

In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises two or more targeting moieties directed to the same or different immune cells. In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex has (i) one or more targeting moieties directed against an immune cell selected from a T cell, a B cell, a dendritic cell, a macrophage, a NK cell, or subsets thereof and (ii) one or more targeting moieties directed against either the same or another immune cell selected from a T cell, a B cell, a dendritic cell, a macrophage, a NK cell, or subsets thereof, along with any of the signaling agents (*e.g.*, IFNα1 or a variant thereof) described herein.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a T cell and one or more targeting moieties directed against the same or another T cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a T cell and one or more targeting moieties directed against a B cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more

targeting moieties directed against a T cell and one or more targeting moieties directed against a dendritic cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against a T cell and one or more targeting moieties directed against a macrophage. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against a T cell and one or more targeting moieties directed against a NK cell. For example, in an illustrative embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may include a targeting moiety against CD8 and a targeting moiety against Clec9A. In another illustrative embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may include a targeting moiety against CD8 and a targeting moiety against CD3. In another illustrative embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may include a targeting moiety against CD8 and a targeting moiety against PD-1.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a B cell and one or more targeting moieties directed against the same or another B cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a B cell and one or more targeting moieties directed against a T cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a B cell and one or more targeting moieties directed against a dendritic cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against a B cell and one or more targeting moieties directed against a macrophage. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against a B cell and one or more targeting moieties directed against a NK cell.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a dendritic cell and one or more targeting moieties directed against the same or another dendritic cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a dendritic cell and one or more targeting moieties directed against a T cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a dendritic cell and one or more targeting moieties directed against a B cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against a dendritic cell and one or more targeting moieties directed against a macrophage. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against a dendritic cell and one or more targeting moieties directed against a NK cell.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a macrophage and one or more targeting moieties directed against the same or another macrophage. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a macrophage and one or more targeting moieties directed against a T cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against a macrophage and one or more targeting moieties directed against a B cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against a macrophage and one or more targeting moieties directed against a dendritic cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against a macrophage and one or more targeting moieties directed against a NK cell.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against an NK cell and one or more targeting moieties directed against the same or another NK cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against an NK cell and one or more targeting moieties directed against a T cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties directed against an NK cell and one or more targeting moieties directed against a B cell. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against an NK cell and one or more targeting moieties directed against a macrophage. In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises one or more targeting moieties against an NK cell and one or more targeting moieties directed against a dendritic cell.

In one embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises a targeting moiety directed against a tumor cell and a second targeting moiety directed against the same or a different tumor cell. In such embodiments, the targeting moieties may bind to any of the tumor antigens described herein.

In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention comprises one or more targeting moieties having recognition domains that bind to a target (*e.g.* antigen, receptor) of interest including those found on one or more cells selected from adipocytes (*e.g.*, white fat cell, brown fat cell), liver lipocytes, hepatic cells, kidney cells (*e.g.*, kidney parietal cell, kidney salivary gland, mammary gland, *etc.*), duct cells (of seminal vesicle, prostate gland, *etc.*), intestinal brush border cells (with microvilli), exocrine gland striated duct cells, gall bladder epithelial cells, ductulus efferens nonciliated cells, epididymal principal cells, epididymal basal cells, endothelial cells, ameloblast epithelial cells (tooth enamel secretion), planum semilunatum epithelial cells of vestibular system of ear (proteoglycan secretion), organ of Corti

interdental epithelial cells (secreting tectorial membrane covering hair cells), loose connective tissue fibroblasts, corneal fibroblasts (corneal keratocytes), tendon fibroblasts, bone marrow reticular tissue fibroblasts, nonepithelial fibroblasts, pericytes, nucleus pulposus cells of intervertebral disc, cementoblasts/cementocytes (tooth root bonelike ewan cell secretion), odontoblasts/odontocytes (tooth dentin secretion), hyaline cartilage chondrocytes, fibrocartilage chondrocytes, elastic cartilage chondrocytes, osteoblasts/osteocytes, osteoprogenitor cells (stem cell of osteoblasts), hyalocytes of vitreous body of eye, stellate cells of perilymphatic space of ear, hepatic stellate cells (Ito cell), pancreatic stelle cells, skeletal muscle cells, satellite cells, heart muscle cells, smooth muscle cells, myoepithelial cells of iris, myoepithelial cells of exocrine glands, exocrine secretory epithelial cells (*e.g.*, salivary gland cells, mammary gland cells, lacrimal gland cells, sweat gland cells, sebaceious gland cells, prostate gland cells, gastric glad cells, pancreatic acinar cells, pneumocytes), a hormone secreting cells (*e.g.*, pituitary cells, neurosecretory cells, gut and respiratory tract cells, thyroid gland cells, parathyroid glad cells, adrenal gland cells, Leydig cells of testes, pancreatic islet cells), keratinizing epithelial cells, wet stratified barrier epithelial cells, neuronal cells (*e.g.*, sensory transducer cells, autonomic neuron cells, sense organ and peripheral neuron supporting cells, and central nervous system neurons and glial cells such as interneurons, principal cells, astrocytes, oligodendrocytes, and ependymal cells).

Targeting Moiety Formats

In various embodiments, the targeting moiety of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is a protein-based agent capable of specific binding, such as an antibody or derivatives thereof. In an embodiment, the targeting moiety comprises an antibody. In various embodiments, the antibody is a full-length multimeric protein that includes two heavy chains and two light chains. Each heavy chain includes one variable region (*e.g.*, V_H) and at least three constant regions (*e.g.*, CH_1, CH_2 and CH_3), and each light chain includes one variable region (V_L) and one constant region (C_L). The variable regions determine the specificity of the antibody. Each variable region comprises three hypervariable regions also known as complementarity determining regions (CDRs) flanked by four relatively conserved framework regions (FRs). The three CDRs, referred to as CDR1, CDR2, and CDR3, contribute to the antibody binding specificity. In some embodiments, the antibody is a chimeric antibody. In some embodiments, the antibody is a humanized antibody.

In some embodiments, the targeting moiety comprises antibody derivatives or formats. In some embodiments, the targeting moiety of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a shark heavy-chain-only antibody (VNAR), a microprotein (cysteine knot protein, knottin), a DARPin; a Tetranectin; an Affibody; a Transbody; an Anticalin; an AdNectin; an Affilin; a Microbody; a peptide aptamer; an alterases; a plastic antibodies; a phylomer; a stradobodies; a maxibodies; an evibody; a fynomer, an armadillo repeat protein, a Kunitz domain, an avimer, an atrimer, a probody, an immunobody, a triomab, a troybody; a pepbody; a vaccibody, a UniBody; affimers, a DuoBody, a Fv, a Fab, a Fab', a $F(ab')_2$, a peptide mimetic molecule, or a synthetic molecule, as described in US Patent Nos. or Patent Publication Nos. US 7,417,130, US 2004/132094, US 5,831,012, US 2004/023334, US 7,250,297, US 6,818,418, US 2004/209243, US 7,838,629, US

7,186,524, US 6,004,746, US 5,475,096, US 2004/146938, US 2004/157209, US 6,994,982, US 6,794,144, US 2010/239633, US 7,803,907, US 2010/119446, and/or US 7,166,697, the contents of which are hereby incorporated by reference in their entireties. *See* also, Storz MAbs. 2011 May-Jun; 3(3): 310–317.

In one embodiment, the targeting moiety comprises a single-domain antibody, such as VHH from, for example, an organism that produces VHH antibody such as a camelid, a shark, or a designed VHH. VHHs are antibody-derived therapeutic proteins that contain the unique structural and functional properties of naturally-occurring heavy-chain antibodies. VHH technology is based on fully functional antibodies from camelids that lack light chains. These heavy-chain antibodies contain a single variable domain (VHH) and two constant domains (CH2 and CH3). VHHs are commercially available under the trademark of NANOBODY or NANOBODIES.

In an embodiment, the targeting moiety comprises a VHH. In some embodiments, the VHH is a humanized VHH or camelized VHH.

In some embodiments, the VHH comprises a fully human VH domain, *e.g.* a HUMABODY (Crescendo Biologics, Cambridge, UK). In some embodiments, fully human VH domain, *e.g.* a HUMABODY is monovalent, bivalent, or trivalent. In some embodiments, the fully human VH domain, *e.g.* a HUMABODY is mono- or multi-specific such as monospecific, bispecific, or trispecific. Illustrative fully human VH domains, *e.g.* a HUMABODIES are described in, for example, WO 2016/113555 and WO2016/113557, the entire disclosure of which is incorporated by reference.

In various embodiments, the targeting moiety of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is a protein-based agent capable of specific binding to a cell receptor, such as a natural ligand for the cell receptor. In various embodiments, the cell receptor is found on one or more immune cells, which can include, without limitation, T cells, cytotoxic T lymphocytes, T helper cells, natural killer (NK) cells, natural killer T (NKT) cells, anti-tumor macrophages (*e.g.* M1 macrophages), B cells, dendritic cells, or subsets thereof. In some embodiments, the cell receptor is found on megakaryocytes, thrombocytes, erythrocytes, mast cells, basophils, neutrophils, eosinophils, or subsets thereof.

In some embodiments, the targeting moiety is a natural ligand such as a chemokine. Illustrative chemokines that may be included in the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention include, but are not limited to, CCL1, CCL2, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9, CCL10, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CLL25, CCL26, CCL27, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL17, XCL1, XCL2, CX3CL1, HCC-4, and LDGF-PBP. In an illustrative embodiment, the targeting moiety may be XCL1 which is a chemokine that recognizes and binds to the dendritic cell receptor XCR1. In another illustrative embodiment, the targeting moiety is CCL1, which is a chemokine that recognizes and binds to CCR8. In another illustrative embodiment, the targeting moiety is CCL2, which is a chemokine that recognizes and binds to CCR2 or CCR9. In another illustrative embodiment, the targeting moiety is CCL3, which is a chemokine that recognizes and binds to CCR1, CCR5, or CCR9. In another illustrative embodiment, the targeting moiety is CCL4, which is a chemokine that recognizes and binds to CCR1 or

CCR5 or CCR9. In another illustrative embodiment, the targeting moiety is CCL5, which is a chemokine that recognizes and binds to CCR1 or CCR3 or CCR4 or CCR5. In another illustrative embodiment, the targeting moiety is CCL6, which is a chemokine that recognizes and binds to CCR1. In another illustrative embodiment, the targeting moiety is CCL7, which is a chemokine that recognizes and binds to CCR2 or CCR9. In another illustrative embodiment, the targeting moiety is CCL8, which is a chemokine that recognizes and binds to CCR1 or CCR2 or CCR2B or CCR5 or CCR9. In another illustrative embodiment, the targeting moiety is CCL9, which is a chemokine that recognizes and binds to CCR1. In another illustrative embodiment, the targeting moiety is CCL10, which is a chemokine that recognizes and binds to CCR1. In another illustrative embodiment, the targeting moiety is CCL11, which is a chemokine that recognizes and binds to CCR2 or CCR3 or CCR5 or CCR9. In another illustrative embodiment, the targeting moiety is CCL13, which is a chemokine that recognizes and binds to CCR2 or CCR3 or CCR5 or CCR9. In another illustrative embodiment, the targeting moiety is CCL14, which is a chemokine that recognizes and binds to CCR1 or CCR9. In another illustrative embodiment, the targeting moiety is CCL15, which is a chemokine that recognizes and binds to CCR1 or CCR3. In another illustrative embodiment, the targeting moiety is CCL16, which is a chemokine that recognizes and binds to CCR1, CCR2, CCR5, or CCR8. In another illustrative embodiment, the targeting moiety is CCL17, which is a chemokine that recognizes and binds to CCR4. In another illustrative embodiment, the targeting moiety is CCL19, which is a chemokine that recognizes and binds to CCR7. In another illustrative embodiment, the targeting moiety is CCL20, which is a chemokine that recognizes and binds to CCR6. In another illustrative embodiment, the targeting moiety is CCL21, which is a chemokine that recognizes and binds to CCR7. In another illustrative embodiment, the targeting moiety is CCL22, which is a chemokine that recognizes and binds to CCR4. In another illustrative embodiment, the targeting moiety is CCL23, which is a chemokine that recognizes and binds to CCR1. In another illustrative embodiment, the targeting moiety is CCL24, which is a chemokine that recognizes and binds to CCR3. In another illustrative embodiment, the targeting moiety is CCL25, which is a chemokine that recognizes and binds to CCR9. In another illustrative embodiment, the targeting moiety is CCL26, which is a chemokine that recognizes and binds to CCR3. In another illustrative embodiment, the targeting moiety is CCL27, which is a chemokine that recognizes and binds to CCR10. In another illustrative embodiment, the targeting moiety is CCL28, which is a chemokine that recognizes and binds to CCR3 or CCR10. In another illustrative embodiment, the targeting moiety is CXCL1, which is a chemokine that recognizes and binds to CXCR1 or CXCR2. In another illustrative embodiment, the targeting moiety is CXCL2, which is a chemokine that recognizes and binds to CXCR2. In another illustrative embodiment, the targeting moiety is CXCL3, which is a chemokine that recognizes and binds to CXCR2. In another illustrative embodiment, the targeting moiety is CXCL4, which is a chemokine that recognizes and binds to CXCR3B. In another illustrative embodiment, the targeting moiety is CXCL5, which is a chemokine that recognizes and binds to CXCR2. In another illustrative embodiment, the targeting moiety is CXCL6, which is a chemokine that recognizes and binds to CXCR1 or CXCR2. In another illustrative embodiment, the targeting moiety is CXCL8, which is a chemokine that recognizes and binds to CXCR1 or CXCR2. In another illustrative embodiment, the targeting moiety is CXCL9, which is a chemokine that recognizes and binds to CXCR3. In another illustrative embodiment, the targeting moiety is CXCL10, which is a chemokine that recognizes and binds to CXCR3. In another illustrative embodiment, the

targeting moiety is CXCL11, which is a chemokine that recognizes and binds to CXCR3 or CXCR7. In another illustrative embodiment, the targeting moiety is CXCL12, which is a chemokine that recognizes and binds to CXCR4 or CXCR7. In another illustrative embodiment, the targeting moiety is CXCL13, which is a chemokine that recognizes and binds to CXCR5. In another illustrative embodiment, the targeting moiety is CXCL16, which is a chemokine that recognizes and binds to CXCR6. In another illustrative embodiment, the targeting moiety is LDGF-PBP, which is a chemokine that recognizes and binds to CXCR2. In another illustrative embodiment, the targeting moiety is XCL2, which is a chemokine that recognizes and binds to XCR1. In another illustrative embodiment, the targeting moiety is CX3CL1, which is a chemokine that recognizes and binds to CX3CR1.

In some embodiments, the targeting moiety is a natural ligand such as FMS-like tyrosine kinase 3 ligand (Flt3L) or a truncated region thereof (e.g., which is able to bind Flt3). In some embodiments, the targeting moiety is an extracellular domain of Flt3L. In some embodiments, the targeting moiety comprising a Flt3L domain, wherein the Flt3L domain is a single chain dimer, optionally where one Flt3L domain is conncted to the other Flt3L domain via one or more linkers, wherein the linker is a flexible linker. In some embodiments, the targeting moiety of the present invention comprises Flt3L domain, wherein the Flt3L domain is a single chain dimer and an Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain. In some embodiments, the targeting moiety recognizes CD20. In some embodiments, the targeting moiety recognizes PD-L1. In some embodiments, the targeting moiety recognizes Clec9A.

In various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises targeting moieties in various combinations. In an illustrative embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may comprise two targeting moieties, wherein both targeting moieties are antibodies or derivatives thereof. In another illustrative embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may comprise two targeting moieties, wherein both targeting moieties are natural ligands for cell receptors. In a further illustrative embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may comprise two targeting moieties, wherein one of the targeting moieties is an antibody or derivative thereof, and the other targeting moiety is a natural ligand for a cell receptor.

In various embodiments, the recognition domain of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex functionally modulates (by way of non-limitation, partially or completely neutralizes) the target (*e.g.* antigen, receptor) of interest, *e.g.* substantially inhibiting, reducing, or neutralizing a biological effect that the antigen has. For example, various recognition domains may be directed against one or more tumor antigens that are actively suppressing, or have the capacity to suppress, the immune system of, for example, a patient bearing a tumor. For example, in some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex functionally modulates immune inhibitory signals (*e.g.* checkpoint inhibitors), for example, one or more of TIM-3, BTLA, PD-1, CTLA-4, B7-H4, GITR, galectin-9, HVEM, PD-L1, PD-L2, B7-H3, CD244, CD160, TIGIT, SIRPα, ICOS, CD172a, and TMIGD2. For example, in some

embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is engineered to disrupt, block, reduce, and/or inhibit the transmission of an immune inhibitory signal, by way of non-limiting example, the binding of PD-1 with PD-L1 or PD-L2 and/or the binding of CTLA-4 with one or more of AP2M1, CD80, CD86, SHP-2, and PPP2R5A.

In various embodiments, the recognition domain of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex binds but does not functionally modulate the target (*e.g.* antigen, receptor) of interest, *e.g.* the recognition domain is, or is akin to, a binding antibody. For instance, in various embodiments, the recognition domain simply targets the antigen or receptor but does not substantially inhibit, reduce or functionally modulate a biological effect that the antigen or receptor has. For example, some of the smaller antibody formats described above (*e.g.* as compared to, for example, full antibodies) have the ability to target hard to access epitopes and provide a larger spectrum of specific binding locales. In various embodiments, the recognition domain binds an epitope that is physically separate from an antigen or receptor site that is important for its biological activity (*e.g.* the antigen's active site).

Such non-neutralizing binding finds use in various embodiments of the present invention, including methods in which the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is used to directly or indirectly recruit active immune cells to a site of need via an effector antigen, such as any of those described herein. For example, in various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be used to directly or indirectly recruit cytotoxic T cells via CD8 to a tumor cell in a method of reducing or eliminating a tumor (*e.g.* the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may comprise an anti-CD8 recognition domain and a recognition domain directed against a tumor antigen). In such embodiments, it is desirable to directly or indirectly recruit CD8-expressing cytotoxic T cells but not to functionally modulate the CD8 activity. On the contrary, in these embodiments, CD8 signaling is an important piece of the tumor reducing or eliminating effect. By way of further example, in various methods of reducing or eliminating tumors, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is used to directly or indirectly recruit dendritic cells (DCs) via CLEC9A (*e.g.* the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may comprise an anti-CLEC9A recognition domain and a recognition domain directed against a tumor antigen). In such embodiments, it is desirable to directly or indirectly recruit CLEC9A-expressing DCs but not to functionally modulate the CLEC9A activity. On the contrary, in these embodiments, CLEC9A signaling is an important piece of the tumor reducing or eliminating effect.

In various embodiments, the recognition domain of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex binds to XCR1 *e.g.* on dendritic cells. For instance, the recognition domain, in some embodiments comprises all or part of XCL1 or a non-neutralizing anti-XCR1 agent.

In various embodiments, the recognition domain of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex binds to an immune modulatory antigen (*e.g.* immune stimulatory or immune inhibitory). In various embodiments, the immune modulatory antigen is one or more of 4-1BB, OX-40,

HVEM, GITR, CD27, CD28, CD30, CD40, ICOS ligand; OX-40 ligand, LIGHT (CD258), GITR ligand, CD70, B7-1, B7-2, CD30 ligand, CD40 ligand, ICOS, ICOS ligand, CD137 ligand and TL1A. In various embodiments, such immune stimulatory antigens are expressed on a tumor cell. In various embodiments, the recognition domain of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex binds but does not functionally modulate such immune stimulatory antigens and therefore allows recruitment of cells expressing these antigens without the reduction or loss of their potential tumor reducing or eliminating capacity.

In various embodiments, the recognition domain of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be in the context of chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex that comprises two recognition domains that have neutralizing activity, or comprises two recognition domains that have non-neutralizing (*e.g.* binding) activity, or comprises one recognition domain that has neutralizing activity and one recognition domain that has non-neutralizing (*e.g.* binding) activity.

Fc Domains

The fragment crystallizable domain (Fc domain) is the tail region of an antibody that interacts with Fc receptors located on the cell surface of cells that are involved in the immune system, *e.g.*, B lymphocytes, dendritic cells, natural killer cells, macrophages, neutrophils, eosinophils, basophils, and mast cells. In IgG, IgA and IgD antibody isotypes, the Fc domain is composed of two identical protein fragments, derived from the second and third constant domains of the antibody's two heavy chains. In IgM and IgE antibody isotypes, the Fc domain contains three heavy chain constant domains (C_H domains 2–4) in each polypeptide chain.

In some embodiments, the Fc-based chimeric protein of complex the present technology includes a Fc domain. In some embodiments, the Fc domains are from selected from IgG, IgA, IgD, IgM or IgE. In some embodiments, the Fc domains are from selected from IgG1, IgG2, IgG3, or IgG4.

In some embodiments, the Fc domains are from selected from human IgG, IgA, IgD, IgM or IgE. In some embodiments, the Fc domains are from selected from human IgG1, IgG2, IgG3, or IgG4.

In some embodiments, the Fc domains of the Fc-based chimeric protein complex comprise the CH2 and CH3 regions of IgG. In some embodiments, the IgG is human IgG. In some embodiments, the human IgG is selected from IgG1, IgG2, IgG3, or IgG4.

In some embodiments, the Fc domains comprise one or more mutations. In some embodiments, the mutation(s) to the Fc domains reduces or eliminates the effector function the Fc domains. In some embodiments, the mutated Fc domain has reduced affinity or binding to a target receptor. By way of example, in some embodiments, the mutation to the Fc domains reduces or eliminates the binding of the Fc domains to FcγR. In some embodiments, the FcγR is selected from FcγRI; FcγRIIa, 131 R/R; FcγRIIa, 131 H/H, FcγRIIb; and FcγRIII. In some embodiments, the mutation to the Fc domains reduces or eliminated binding to complement proteins, such as, *e.g.*, C1q. In some embodiments, the mutation to the Fc domains reduces or eliminated binding to both FcγR and complement proteins, such as, *e.g.*, C1q.

In some embodiments, the Fc domains comprise the LALA mutation to reduce or eliminate the effector function of the Fc domains. By way of example, in some embodiments, the LALA mutation comprises L234A and L235A substitutions in human IgG (*e.g.*, IgG1) (wherein the numbering is based on the commonly used numbering of the CH2 residues for human IgG1 according to EU convention (PNAS, Edelman et al., 1969; 63 (1) 78-85)).

In some embodiments, the Fc domains of human IgG comprise a mutation at 46. to reduce or eliminate the effector function of the Fc domains. By way of example, in some embodiments, the mutations are selected from L234A, L234F, L235A, L235E, L235Q, K322A, K322Q, D265A, P329G, P329A, P331G, and P331S.

In some embodiments, the Fc domains comprise the FALA mutation to reduce or eliminate the effector function of the Fc domains. By way of example, in some embodiments, the FALA mutation comprises F234A and L235A substitutions in human IgG4.

In some embodiments, the Fc domains of human IgG4 comprise a mutation at one or more of F234, L235, K322, D265, and P329 to reduce or eliminate the effector function of the Fc domains. By way of example, in some embodiments, the mutations are selected from F234A, L235A, L235E, L235Q, K322A, K322Q, D265A, P329G, and P329A.

In some embodiments, the mutation(s) to the Fc domain stabilize a hinge region in the Fc domain. By way of example, in some embodiments, the Fc domain comprises a mutation at S228 of human IgG to stabilize a hinge region. In some embodiments, the mutation is S228P.

In some embodiments, the mutation(s) to the Fc domain promote chain pairing in the Fc domain. In some embodiments, chain pairing is promoted by ionic pairing (a/k/a charged pairs, ionic bond, or charged residue pair).

In some embodiments, the Fc domain comprises a mutation at one more of the following amino acid residues of IgG to promote of ionic pairing: D356, E357, L368, K370, K392, D399, and K409.

By way of example, in some embodiments, the human IgG Fc domain comprise one of the mutation combinations in **Table 1** to promote of ionic pairing.

Table 1

Substitution(s) on one Fc Chain	Substitution(s) on other Fc Chain
D356K D399K	K392D K409D
E357R L368R	K370D K409D
E357R L368K	K370D K409D
E357R D399K	K370D K409D
E357R	K370D
L368R D399K	K392D K409D
L368K D399K	K392D K409D
L368R D399K	K409D
L368K D399K	K409D
L368R	K409D
L368K	K409D
K370D K409D	E357R D399K
K370D K409D	E357R L368R
K370D K409D	E357R L368K
K370D K409D	E357R D399K
K370D K409D	E357R L368R

Table 1	
Substitution(s) on one Fc Chain	**Substitution(s) on other Fc Chain**
K370D K409D	E357R L368K
K370D	E357R
K370D	E357R
K392D K409D	D356K D399K
K392D K409D	L368R D399K
K392D K409D	L368K D399K
K392D K409D	D399K
D399K	K392D K409D
D399K	K409D
K409D	L368R
K409D	L368K
K409D	L368R D399K
K409D	L368K D399K
K409D	L368R
K409D	L368K
K409D	L368R D399K
K409D	L368K D399K
K409D	D399K

In some embodiments, chain pairing is promoted by a knob-in-hole mutations. In some embodiments, the Fc domain comprises one or more mutations to allow for a knob-in-hole interaction in the Fc domain. In some embodiments, a first Fc chain is engineered to express the "knob" and a second Fc chain is engineered to express the complementary "hole." By way of example, in some embodiments, human IgG Fc domain comprises the mutations of **Table 2** to allow for a knob-in-hole interaction.

Table 2	
Substitution(s) on one Fc Chain	**Substitution(s) on other Fc Chain**
T366Y	Y407T
T366Y/F405A	T394W/Y407T
T366W	Y407A
T366W	Y407V
T366Y	Y407A
T366Y	Y407V
T366Y	Y407T

In some embodiments, the Fc domains in the Fc-based chimeric protein complexes of the present technology comprise any combination of the above disclosed mutations. By way of example, in some embodiments, the Fc domain comprises mutations that promote ionic pairing and/or a knob-in-hole interaction. By way of example, in some embodiments, the Fc domain comprises mutations that have one or more of the following properties: promote ionic pairing, induce a knob-in-hole interaction, reduce or eliminate the effector function of the Fc domain, and cause Fc stabilization (*e.g.* at hinge).

By way of example, in some embodiments, a human IgG Fc domains comprise mutations disclosed in **Table 3**, which promote ionic pairing and/or promote a knob-in-hole interaction in the Fc domain.

Table 3	
Substitution(s) on one Fc Chain	**Substitution(s) on other Fc Chain**
T366W K370D	E357R Y407A
T366W K370D	E357R Y407V
T366W K409D	L368R Y407A
T366W K409D	L368R Y407V
T366W K409D	L368K Y407A
T366W K409D	L368K Y407V
T366W K409D	L368R D399K Y407A
T366W K409D	L368R D399K Y407V
T366W K409D	L368K D399K Y407A
T366W K409D	L368K D399K Y407V
T366W K409D	D399K Y407A
T366W K409D	D399K Y407V
T366W K392D K409D	D399K Y407A
T366W K392D K409D	D399K Y407V
T366W K392D K409D	D356K D399K Y407A
T366W K392D K409D	D356K D399K Y407V
T366W K370D K409D	E357R D399K Y407A
T366W K370D K409D	E357R D399K Y407V
T366W K370D K409D	E357R L368R Y407A
T366W K370D K409D	E357R L368R Y407V
T366W K370D K409D	E357R L368K Y407A
T366W K370D K409D	E357R L368K Y407V
T366W K392D K409D	L368R D399K Y407A
T366W K392D K409D	L368R D399K Y407V
T366W K392D K409D	L368K D399K Y407A
T366W K392D K409D	L368K D399K Y407V
E357R T366W	K370D Y407A
E357R T366W	K370D Y407V
T366W L368R	Y407A K409D
T366W L368R	Y407V K409D
T366W L368K	Y407A K409D
T366W L368K	Y407V K409D
T366W L368R D399K	Y407A K409D
T366W L368R D399K	Y407V K409D
T366W L368K D399K	Y407A K409D
T366W L368K D399K	Y407V K409D
T366W D399K	Y407A K409D
T366W D399K	Y407V K409D
1366W D399K	K392D Y407A K409D
T366W D399K	K392D Y407V K409D
T366W D356K D399K	K392D Y407A K409D
T366W D356K D399K	K392D Y407V K409D
E357R T366W D399K	K370D Y407A K409D
E357R T366W D399K	K370D Y407V K409D
E357R T366W L368R	K370D Y407A K409D
E357R T366W L368R	K370D Y407V K409D
E357R T366W L368K	K370D Y407A K409D
E357R T366W L368K	K370D Y407V K409D
T366W L368R D399K	K392D Y407A K409D
T366W L368R D399K	K392D Y407V K409D
T366W L368K D399K	K392D Y407A K409D

By way of example, in some embodiments, a human IgG Fc domains comprise mutations disclosed in **Table 4**, which promote ionic pairing, promote a knob-in-hole interaction, or a combination thereof in the Fc domain. In embodiments, the "Chain 1" and "Chain 2" of **Table 4** can be interchanged (*e.g.* Chain 1 can have Y407T and Chain 2 can have T366Y).

Table 4

Chain 1 mutation	Chain 2 mutation	Reference	IgG
T366Y	Y407T	Ridgway *et al.*, 1996 Protein Engineering, Design and Selection, Volume 9, Issue 7, 1 July 1996, Pages 617–62	IgG1
T366Y/F405A	T394W/Y407T	Ridgway *et al.*, 1996 Protein Engineering, Design and Selection, Volume 9, Issue 7, 1 July 1996, Pages 617–62	IgG1
T366W	Y407A	Atwell *et al.*, 1997 JMB Volume 270, Issue 1, 4 July 1997, Pages 26-35	IgG1
T366W	T366S/L368V/Y407A	Atwell *et al.*, 1997 JMB Volume 270, Issue 1, 4 July 1997, Pages 26-35	IgG1
T366W	L368A/Y407A	Atwell *et al.*, 1997 JMB Volume 270, Issue 1, 4 July 1997, Pages 26-35	IgG1
T366W	T366S/L368A/Y407A	Atwell *et al.*, 1997 JMB Volume 270, Issue 1, 4 July 1997, Pages 26-35	IgG1
T366W	T366S/L368G/Y407V	Atwell *et al.*, 1997 JMB Volume 270, Issue 1, 4 July 1997, Pages 26-35	IgG1
T366W/D399C	T366S/L368A/K392C/Y407V	Merchant *et al.*, 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1
T366W/K392C	T366S/L368A/D399C/Y407V	Merchant *et al.*, 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1
S354C/T366W	Y349C/T366S/L368A/Y407V	Merchant *et al.*, 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1
Y349C/T366W	S354C/T366S/L368A/Y407V	Merchant *et al.*, 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1
E356C/T366W	Y349C/T366S/L368A/Y407V	Merchant *et al.*, 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1
Y349C/T366W	E356C/T366S/L368A/Y407V	Merchant *et al.*, 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1
E357C/T366W	Y349C/T366S/L368A/Y407V	Merchant *et al.*, 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1

Y349C/T366W	E357C/T366S/L368A/Y407V	Merchant *et al.*, 1998 Nature Biotechnology volume 16, pages 677–681 (1998)	IgG1
D339R	K409E	Gunasekaran *et al.*, 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K	K409E	Gunasekaran *et al.*, 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339R	K409D	Gunasekaran *et al.*, 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K	K409D	Gunasekaran *et al.*, 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K	K360D/K409E	Gunasekaran *et al.*, 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K	K392D/K409E	Gunasekaran *et al.*, 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K/E356K	K392D/K409E	Gunasekaran *et al.*, 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K/E357K	K392D/K409E	Gunasekaran *et al.*, 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K/E356K	K409E/K439D	Gunasekaran *et al.*, 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K/E357K	K370D/K409E	Gunasekaran *et al.*, 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
D339K/E356K/E357K	K370D/K392D/K409E	Gunasekaran *et al.*, 2010 The Journal of Biological Chemistry 285, 19637-19646.	IgG1
S364H/F405A	Y349T/T394F	Moore *et al.*, 2011 mAbs, 3:6, 546-557	IgG1
S364H/T394F	Y349T/F405A	Moore *et al.*, 2011 mAbs, 3:6, 546-557	IgG1
D221R/P228R/K409R	D221E/P228E/L368E	Strop *et al.*, 2012 JMB Volume 420, Issue 3, 13 July 2012, Pages 204-219	IgG1
C223R/E225R/P228R/K409R	C223E/P228E/L368E	Strop *et al.*, 2012 JMB Volume 420, Issue 3, 13 July 2012, Pages 204-219	IgG2
F405L	K409R	Labrijn *et al.*, 2013 PNAS March 26, 2013. 110 (13) 5145-5150	IgG1
F405A/Y407V	T394W	Von Kreudenstein *et al.*, 2013 mAbs Volume 5, 2013 - Issue 5, pp.644-654	IgG1
F405A/Y407V	T366I/T394W	Von Kreudenstein *et al.*, 2013 mAbs Volume 5, 2013 - Issue 5, pp.644-654	IgG1
F405A/Y407V	T366L/T394W	Von Kreudenstein *et al.*, 2013 mAbs Volume 5, 2013 - Issue 5, pp.644-654	IgG1
F405A/Y407V	T366L/K392M/T394W	Von Kreudenstein *et al.*, 2013 mAbs Volume 5, 2013 - Issue 5, pp.644-654	IgG1
L351Y/F405A/Y407V	T366L/K392M/T394W	Von Kreudenstein *et al.*, 2013 mAbs Volume 5, 2013 - Issue 5, pp.644-654	IgG1
T350V/L351Y/F405A/Y407V	T350V/T366L/K392M/T394W	Von Kreudenstein *et al.*, 2013 mAbs Volume 5, 2013 - Issue 5, pp.644-654	IgG1
T350V/L351Y/F405A/Y407V	T350V/T366L/K392L/T394W	Von Kreudenstein *et al.*, 2013 mAbs Volume 5, 2013 - Issue 5, pp.644-654	IgG1
K409W	D339V/F405T	Choi *et al.*, 2013 PNAS January 2, 2013. 110 (1) 270-275	IgG1
K360E	Q347R	Choi *et al.*, 2013 PNAS January 2, 2013. 110 (1) 270-275	IgG1
K360E/K409W	D339V/Q347R/F405T	Choi *et al.*, 2013 PNAS January 2, 2013. 110 (1) 270-275	IgG1

Y349C/K360E/K409W	D339V/Q347R/S354C/F405T	Choi *et al.*, 2013 PNAS January 2, 2013. 110 (1) 270-275	IgG1
K392A/K409D	E356K/D399K	Leaver-Fey *et al.*, 2016 Structure Volume 24, Issue 4, 5 April 2016, Pages 641-651	IgG1
T366W	T366S/L358A/Y407A	Leaver-Fey *et al.*, 2016 Structure Volume 24, Issue 4, 5 April 2016, Pages 641-651	IgG1
D339M/Y407A	T336V/K409V	Leaver-Fey *et al.*, 2016 Structure Volume 24, Issue 4, 5 April 2016, Pages 641-651	IgG1
D339M/K360D/Y407A	T336V/E345R/Q347R/K409V	Leaver-Fey *et al.*, 2016 Structure Volume 24, Issue 4, 5 April 2016, Pages 641-651	IgG1
Y349S/T366V/K370Y/K409V	E357D/S364Q/Y407A	Leaver-Fey *et al.*, 2016 Structure Volume 24, Issue 4, 5 April 2016, Pages 641-651	IgG1
Y349S/T366M/K370Y/K409V	E356G/E357D/S364Q/Y407A	Leaver-Fey *et al.*, 2016 Structure Volume 24, Issue 4, 5 April 2016, Pages 641-651	IgG1
Y349S/T366M/K370Y/K409V	E357D/S364R/Y407A	Leaver-Fey *et al.*, 2016 Structure Volume 24, Issue 4, 5 April 2016, Pages 641-651	IgG1
And any combination as described in Tables 1-3 of US20150284475A1			

By way of example, in some embodiments, a human IgG Fc domains comprise mutations disclosed in **Table 5**, which reduce or eliminate FcγR and/or complement binding in the Fc domain. In embodiments, the **Table 5** mutations are in both chains.

Table 5

Chain 1 mutation	Reference	IgG
L234A/L235A	Alegre *et al.*, 1994 Transplantation 57:1537–1543	IgG1
F234A/L235A	Alegre *et al.*, 1994 Transplantation 57:1537–1543	IgG4
L235E	Morgan *et al.*, 1995 Immunology. 1995 Oct; 86(2): 319–324.	IgG1
L235E	Morgan *et al.*, 1995 Immunology. 1995 Oct; 86(2): 319–324.	IgG4
L235A	Morgan *et al.*, 1995 Immunology. 1995 Oct; 86(2): 319–324.	IgG1
G237A	Morgan *et al.*, 1995 Immunology. 1995 Oct; 86(2): 319–324.	IgG1
N297H	Tao and Morrison, J. Immunol. 1989; 143:2595-2601	IgG1

N297Q	Tao and Morrison, J. Immunol. 1989; 143:2595-2601	IgG1
N297K	Tao and Morrison, J. Immunol. 1989; 143:2595-2601	IgG3
N297Q	Tao and Morrison, J. Immunol. 1989; 143:2595-2601	IgG3
D265A	Idusogie *et al.*, 2000 J Immunol April 15, 2000, 164 (8) 4178-4184	IgG1
D270A, V, K	Idusogie *et al.*, 2000 J Immunol April 15, 2000, 164 (8) 4178-4184	IgG1
K322A, L, M, D, E	Idusogie *et al.*, 2000 J Immunol April 15, 2000, 164 (8) 4178-4184	IgG1
P329A, X	Idusogie *et al.*, 2000 J Immunol April 15, 2000, 164 (8) 4178-4184	IgG1
P331A, S, G, X	Idusogie *et al.*, 2000 J Immunol April 15, 2000, 164 (8) 4178-4184	IgG1
D265A	Idusogie *et al.*, 2000 J Immunol April 15, 2000, 164 (8) 4178-4184	IgG1
L234A	Hezareh *et al.*, 2001 J. Virol. December 2001 vol. 75 no. 24 12161-12168	IgG1
L234A/L235A	Hezareh *et al.*, 2001 J. Virol. December 2001 vol. 75 no. 24 12161-12168	IgG1
L234F/L235E/P331S	Oganesyan *et al.*, 2008 Acta Cryst. (2008). D64, 700-704	IgG1
H268Q/V309L/A330S/P331S	An *et al.*, 2009 mAbs Volume 1, 2009 - Issue 6, pp. 572-579	IgG1
G236R/L328R	Moore *et al.*, 2011 mAbs Volume 3, 2011 - Issue 6, pp. 546-557	IgG1
N297G	Couch *et al.*, 2013 Sci. Transl. Med., 5 (2013) 183ra57, 1–12	IgG1
N297G/D265A	Couch *et al.*, 2013 Sci. Transl. Med., 5 (2013) 183ra57, 1–12	IgG1
V234A/G237A/P328S/H268A/V309L/A330S/P331S	Vafa *et al.*, 2014 Methods Volume 65, Issue 1, 1 January 2014, Pages 114-126	IgG2
L234A/L235A/P329G	Lo *et al.*, 2016 The Journal of Biological Chemistry 292, 3900-3908	IgG1

N297D	Schlothauer *et al.*, 2016 Protein Engineering, Design and Selection, Volume 29, Issue 10, 1 October 2016, Pages 457–466	IgG1
S228P/L235E	Schlothauer *et al.*, 2016 Protein Engineering, Design and Selection, Volume 29, Issue 10, 1 October 2016, Pages 457–466	IgG4
S228P/L235E/P329G	Schlothauer *et al.*, 2016 Protein Engineering, Design and Selection, Volume 29, Issue 10, 1 October 2016, Pages 457–466	IgG4
L234F/L235A/K322Q	Borrok *et al.*, 2017 J Pharm Sci April 2017 Volume 106, Issue 4, Pages 1008–1017	IgG1
L234F/L235Q/P331G	Borrok *et al.*, 2017 J Pharm Sci April 2017 Volume 106, Issue 4, Pages 1008–1017	IgG1
L234F/L235Q/K322Q	Borrok *et al.*, 2017 J Pharm Sci April 2017 Volume 106, Issue 4, Pages 1008–1017	IgG1
L234A/L235A/G237A/P328S/H268A/A330S/P331S	Tam *et al.*, 2017 Open Access Antibodies 2017, 6(3), 12; doi:10.3390/antib6030012	IgG1
S228P/F234A/L235A	Tam *et al.*, 2017 Open Access Antibodies 2017, 6(3), 12; doi:10.3390/antib6030012	IgG4
S228P/F234A/L235A/G237A/P238S	Tam *et al.*, 2017 Open Access Antibodies 2017, 6(3), 12; doi:10.3390/antib6030012	IgG4
S228P/F234A/L235A/G236□/G237A/P238S	Tam *et al.*, 2017 Open Access Antibodies 2017, 6(3), 12; doi:10.3390/antib6030012	IgG4

In some embodiments, the Fc domains in the Fc-based chimeric protein complexes of the present technology are homodimeric, *i.e.*, the Fc region in the chimeric protein complex comprises two identical protein fragments.

In some embodiments, the Fc domains in the Fc-based chimeric protein complexes of the present technology are heterodimeric, *i.e.*, the Fc domain comprises two non-identical protein fragments.

In some embodiments, heterodimeric Fc domains are engineered using ionic pairing and/or knob-in-hole mutations described herein. In some embodiments, the heterodimeric Fc-based chimeric protein complexes have a trans orientation/configuration. In a trans orientation/configuration, the targeting moiety and signaling agent, *e.g.* IFNα1 are, in embodiments, not found on the same polypeptide chain in the present Fc-based chimeric protein complexes.

In some embodiments, the Fc domains includes or starts with the core hinge region of wild-type human IgG1, which contains the sequence Cys-Pro-Pro-Cys. In some embodiments, the Fc domains also include the upper hinge, or parts thereof (*e.g.*, DKTHTCPPC; see WO 2009053368), EPKSCDKTHTCPPC, or EPKSSDKTHTCPPC; see Lo et al., Protein Engineering vol.11 no.6 pp.495–500, 1998)).

Fc-based Chimeric Protein Complexes

The Fc-based chimeric protein complexes of the present technology comprise at least one Fc domain disclosed herein, at least one signaling agent, *e.g.* IFNα1 (SA) disclosed herein, *e.g.* IFNα1, and at least one targeting moiety (TM) disclosed herein.

It is understood that, the present Fc-based chimeric protein complexes may encompass a complex of two fusion proteins, each comprising an Fc domain.

In some embodiments, the Fc-based chimeric protein complex is heterodimeric. In some embodiments, the heterodimeric Fc-based chimeric protein complex has a trans orientation/configuration. In some embodiments, the heterodimeric Fc-based chimeric protein complex has a cis orientation/configuration.

In some embodiments, heterodimeric Fc domains are engineered using ionic pairing and/or knob-in-hole mutations described herein. In some embodiments, the heterodimeric Fc-based chimeric protein complexes have a trans orientation.

In a trans orientation, the targeting moiety and signaling agent are, in embodiments, not found on the same polypeptide chain in the present Fc-based chimeric protein complexes. In a trans orientation, the targeting moiety and signaling agent are, in embodiments, found on separate polypeptide chains in the Fc-based chimeric protein complexes. In a cis orientation, the targeting moiety and signaling agent are, in embodiments, found on the same polypeptide chain in the Fc-based chimeric protein complexes.

In some embodiments, where more than one targeting moiety is present in the heterodimeric protein complexes described herein, one targeting moiety may be in trans orientation (relative to the signaling agent), whereas another targeting moiety may be in cis orientation (relative to the signaling agent). In some embodiments, the signaling agent and target moiety are on the same ends/sides (N-terminal or C-terminal ends) of an Fc domain. In some embodiments, the signaling agent and targeting moiety are on different sides/ends of a Fc domain (N-terminal and C-terminal ends).

In some embodiments, where more than one targeting moiety is present in the heterodimeric protein complexes described herein, the targeting moieties may be found on the same Fc chain or on two different Fc chains in the heterodimeric protein complex (in the latter case the targeting moieties would be in trans relative to each other, as they are on different Fc chains). In some embodiments, where more than one targeting moiety is present on the same Fc chain, the targeting moieties may be on the same or different sides/ends of a Fc chain (N-terminal or/and C-terminal ends).

In some embodiments, where more than one signaling agent is present in the heterodimeric protein complexes described herein, the signaling agents may be found on the same Fc chain or on two different Fc chains in the

heterodimeric protein complex (in the latter case the signaling agents would be in trans relative to each other, as they are on different Fc chains). In some embodiments, where more than one signaling agent is present on the same Fc chain, the signaling agents may be on the same or different sides/ends of a Fc chain (N-terminal or/and C-terminal ends).

In some embodiments, where more than one signaling agent is present in the heterodimeric protein complexes described herein, one signaling agent may be in trans orientation (as relates to the targeting moiety), whereas another signaling agent may be in cis orientation (as relates to the targeting moiety).

In some embodiments, the heterodimeric Fc-based chimeric protein complex does not comprise the signaling agent, *e.g.* IFNα1 and targeting moiety on a single polypeptide.

In some embodiments, the Fc-based chimeric protein has an improved in vivo half-life relative to a chimeric protein lacking an Fc or a chimeric protein which is not a heterodimeric complex. In some embodiments, the Fc-based chimeric protein has an improved solubility, stability and other pharmacological properties relative to a chimeric protein lacking an Fc or a chimeric protein which is not a heterodimeric complex.

Heterodimeric Fc-based chimeric protein complexes are composed of two different polypeptides. In embodiments described herein, the targeting domain is on a different polypeptide than the signaling agent, *e.g.* IFNα1, and accordingly, proteins that contain only one targeting domain copy, and also only one signaling agent, *e.g.* IFNα1 copy can be made (this provides a configuration in which potential interference with desired properties can be controlled). Further, in embodiments, one targeting domain (*e.g.* VHH) only can avoid cross-linking of the antigen on the cell surface (which could elicit undesired effects in some cases). Further, in embodiments, one signaling agent, *e.g.* IFNα1 may alleviate molecular "crowding" and potential interference with avidity mediated induction or restoration of effector function in dependence of the targeting domain. Further, in embodiments, heterodimeric Fc-based chimeric protein complexes can have two targeting moieties and these can be placed on the two different polypeptides. For instance, in embodiments, the C-terminus of both targeting moieties (*e.g.* VHHs) can be masked to avoid potential autoantibodies or pre-existing antibodies (*e.g.* VHH autoantibodies or pre-existing antibodies). Further, in embodiments, heterodimeric Fc-based chimeric protein complexes, *e.g.* with the targeting domain on a different polypeptide than the signaling agent, *e.g.* IFNα1 (*e.g.* wild type signaling agent, *e.g.* wild type IFNα1), may favor "cross-linking" of two cell types (*e.g.* a tumor cell and an immune cell). Further, in embodiments, heterodimeric Fc-based chimeric protein complexes can have two signaling agent, each on different polypeptides to allow more complex effector responses.

Further, in embodiments, heterodimeric Fc-based chimeric protein complexes, *e.g.* with the targeting domain on a different polypeptide than the signaling agent, *e.g.* IFNα1, combinatorial diversity of targeting moiety and signaling agent, *e.g.* IFNα1 is provided in a practical manner. For instance, in embodiments, polypeptides with any of the targeting moieties described herein can be combined "off the shelf" with polypeptides with any of the signaling agents described herein to allow rapid generation of various combinations of targeting moieties and signaling agents in single Fc-based chimeric protein complexes.

In some embodiments, the Fc-based chimeric protein complex comprises one or more linkers. In some embodiments, the Fc-based chimeric protein complex includes a linker that connects the Fc domain, signaling agent, *e.g.* IFNα1(s) and targeting moiety(ies). In some embodiments, the Fc-based chimeric protein complex includes a linker that connects each signaling agent, *e.g.* IFNα1 and targeting moiety (or, if more than one targeting moiety, a signaling agent, *e.g.* IFNα1 to one of the targeting moieties). In some embodiments, the Fc-based chimeric protein complex includes a linker that connects each signaling agent, *e.g.* IFNα1 to the Fc domain. In some embodiments, the Fc-based chimeric protein complex includes a linker that connects each targeting moiety to the Fc domain. In some embodiments, the Fc-based chimeric protein complex includes a linker that connects a targeting moiety to another targeting moiety. In some embodiments, the Fc-based chimeric protein complex includes a linker that connects a signaling agent, *e.g.* IFNα1 to another signaling agent.

In some embodiments, a Fc-based chimeric protein complex comprises two or more targeting moieties. In such embodiments, the targeting moieties can be the same targeting moiety or they can be different targeting moieties.

In some embodiments, a Fc-based chimeric protein complex comprises two or more signaling agents. In such embodiments, the signaling agents can be the same targeting moiety or they can be different targeting moieties.

By way of example, in some embodiments, the Fc-based chimeric protein complex comprise a Fc domain, at least two signaling agents (SA), and at least two targeting moieties (TM), wherein the Fc domain, signaling agents, and targeting moieties are selected from any of the Fc domains, signaling agents, and targeting moieties disclosed herein. In some embodiments, the Fc domain is homodimeric.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 1A-F.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 2A-H.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 3A-H.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 4A-D.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 5A-F.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 6A-J.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 7A-D.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 8A-F.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 9A-J.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 10A-F.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 11A-L.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 12A-L.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 13A-F.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 14A-L.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 15A-L.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 16A-J.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 17A-J.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 18A-F.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 19A-F.

In various embodiments, the Fc-based chimeric protein complex takes the form of any of the schematics of Figs. 20A-E.

In some embodiments, the signaling agents are linked to the targeting moieties and the targeting moieties are linked to the Fc domain on the same terminus (see FIGs. 1A-F). In some embodiments, the Fc domain is homodimeric.

In some embodiments, the signaling agents and targeting moieties are linked to the Fc domain, wherein the targeting moieties and signaling agents are linked on the same terminus (see FIGs. 1A-F). In some embodiments, the Fc domain is homodimeric.

In some embodiments, the targeting moieties are linked to signaling agents and the signaling agents are linked to the Fc domain on the same terminus (see FIGs. 1A-F). In some embodiments, the Fc domain is homodimeric.

In some embodiments, the homodimeric Fc-based chimeric protein complex has two or more targeting moieties. In some embodiments, there are four targeting moieties and two signaling agents, the targeting moieties are linked

to the Fc domain and the signaling agents are linked to targeting moieties on the same terminus (see FIGS. 2A-H). In some embodiments, the Fc domain is homodimeric. In some embodiments, where there are four targeting moieties and two signaling agents, two targeting moieties are linked to the Fc domain and two targeting moieties are linked to the signaling agents, which are linked to the Fc domain on the same terminus (see FIGS. 2A-H). In some embodiments, the Fc domain is homodimeric. In some embodiments, where there are four targeting moieties and two signaling agents, two targeting moieties are linked to each other and one of the targeting moieties of from each pair is linked to the Fc domain on the same terminus and the signaling agents are linked to the Fc domain on the same terminus (see FIGS. 2A-H). In some embodiments, the Fc domain is homodimeric. In some embodiments, where there are four targeting moieties and two signaling agents, two targeting moieties are linked to each other, wherein one of the targeting moieties of from each pair is linked to a signaling agent, *e.g.* IFNα1 and the other targeting moiety of the pair is linked the Fc domain, wherein the targeting moieties linked to the Fc domain are linked on the same terminus (see FIGS. 2A-H). In some embodiments, the Fc domain is homodimeric.

In some embodiments, the homodimeric Fc-based chimeric protein complex has two or more signaling agents. In some embodiments, where there are four signaling agents and two targeting moieties, two signaling agents are linked to each other and one of the signaling agents of from pair is linked to the Fc domain on the same terminus and the targeting moieties are linked to the Fc domain on the same terminus (see FIGs. 3A-H). In some embodiments, the Fc domain is homodimeric. In some embodiments, where there are four signaling agents and two targeting moieties, two signaling agents are linked to the Fc domain one the same terminus and two of the signaling agents are each linked to a targeting moiety, wherein the targeting moieties are linked to the Fc domain at the same terminus (see FIGs. 3A-H). In some embodiments, the Fc domain is homodimeric. In some embodiments, where there are four signaling agents and two targeting moieties, two signaling agents are linked to each other and one of the signaling agents of from pair is linked to a targeting moiety and the targeting moieties are linked to the Fc domain on the same terminus (see FIGs. 3A-H). In some embodiments, the Fc domain is homodimeric.

By way of example, in some embodiments, the Fc-based chimeric protein complex comprise a Fc domain, wherein the Fc domain comprises ionic pairing mutation(s) and/or knob-in-hole mutation(s), at least one signaling agent, *e.g.* IFNα1, and at least one targeting moiety, wherein the ionic pairing motif and/or a knob-in-hole motif, signaling agent, *e.g.* IFNα1, and targeting moiety are selected from any of the ionic pairing motif and/or a knob-in-hole motif, signaling agents, and targeting moieties disclosed herein. In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, the signaling agent, *e.g.* IFNα1 is linked to the targeting moiety, which is linked to the Fc domain (see FIGs. 10A-F and 13A-F). In some embodiments, the targeting moiety is linked to the signaling agent, *e.g.* IFNα1, which is linked to the Fc domain (see FIGs. 10A-F and 13A-F). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, the signaling agent, *e.g.* IFNα1 and targeting moiety are linked to the Fc domain (see FIGs. 4A-D, 7A-D, 10A-F, and 13A-F). In some embodiments, the targeting moiety and the signaling agent, *e.g.* IFNα1 are linked to different Fc chains on the same terminus (see FIGs. 4A-D and 7A-D). In some embodiments, the targeting moiety and the signaling agent, *e.g.* IFNα1 are linked to different Fc chains on different termini (see FIGs. 4A-D and 7A-D). In some embodiments, the targeting moiety and the signaling agent, *e.g.* IFNα1 are linked to the same Fc chain (see FIGs. 10A-F and 13A-F). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are one signaling agent, *e.g.* IFNα1 and two targeting moieties, the signaling agent, *e.g.* IFNα1 is linked to the Fc domain and two targeting moieties can be: 1) linked to each other with one of the targeting moieties linked to the Fc domain; or 2) each linked to the Fc domain (see FIGs. 5A-F, 8A-F, 11A-L, 14A-L, 16A-J, and 17A-J). In some embodiments, the targeting moieties are linked on one Fc chain and the signaling agent, *e.g.* IFNα1 is on the other Fc chain (see FIGs. 5A-F and 8A-F). In some embodiments, the paired targeting moieties and the signaling agent, *e.g.* IFNα1 are linked to the same Fc chain (see FIGs. 11A-L and 14A-L). In some embodiments, a targeting moiety is linked to the Fc domain and the other targeting moiety is linked to the signaling agent, *e.g.* IFNα1, and the paired targeting moiety is linked to the Fc domain (see FIGs. 11A-L, 14A-L, 16A-J, and 17A-J). In some embodiments, the unpaired targeting moiety and paired targeting moiety are linked to the same Fc chain (see FIGs. 11A-L and 14A-L). In some embodiments, the unpaired targeting moiety and paired targeting moiety are linked to different Fc chains (see FIGs. 16A-J and 17A-J). In some embodiments, the unpaired targeting moiety and paired targeting moiety are linked on the same terminus (see FIGs. 16A-J and 17A-J). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are one signaling agent, *e.g.* IFNα1 and two targeting moieties, a targeting moiety is linked to the signaling agent, *e.g.* IFNα1, which is linked to the Fc domain, and the unpaired targeting moiety is linked the Fc domain (see FIGs. 11A-L, 14A-L, 16A-J, and 17A-J). In some embodiments, the paired signaling agent, *e.g.* IFNα1 and unpaired targeting moiety are linked to the same Fc chain (see FIGs. 11A-L and 14A-L). In some embodiments, the paired signaling agent, *e.g.* IFNα1 and unpaired targeting moiety are linked to different Fc chains (see FIGs. 16A-J and 17A-J). In some embodiments, the paired signaling agent, *e.g.* IFNα1 and unpaired targeting moiety are linked on the same terminus (see FIGs. 16A-J and 17A-J). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are one signaling agent, *e.g.* IFNα1 and two targeting moieties, the targeting moieties are linked together and the signaling agent, *e.g.* IFNα1 is linked to one of the paired targeting moieties, wherein the targeting moiety not linked to the signaling agent, *e.g.* IFNα1 is linked to the Fc domain (see FIGs. 11A-L and 14A-L). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are one signaling agent, *e.g.* IFNα1 and two targeting moieties, the targeting moieties are linked together and the signaling agent, *e.g.* IFNα1 is linked to one of the paired targeting moieties, wherein the signaling agent, *e.g.* IFNα1 is linked to the Fc domain (see FIGs. 11A-L and 14A-L). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are one signaling agent, *e.g.* IFNα1 and two targeting moieties, the targeting moieties are both linked to the signaling agent, *e.g.* IFNα1, wherein one of the targeting moieties is linked to the Fc domain (see FIGs. 11A-L and 14A-L). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are one signaling agent, *e.g.* IFNα1 and two targeting moieties, the targeting moieties and the signaling agent, *e.g.* IFNα1 are linked to the Fc domain (see FIGs. 16A-J and 17A-J). In some embodiments, the targeting moieties are linked on the terminus (see FIGs. 16A-J and 17A-J). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are two signaling agents and one targeting moiety, the signaling agents are linked to the Fc domain on the same terminus and the targeting moiety is linked to the Fc domain (see FIGs. 6A-J and 9A-J). In some embodiments, the signaling agents are linked to the Fc domain on the same Fc chain and the targeting moiety is linked on the other Fc chain (see FIGs. 18A-F and 19A-F). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are two signaling agents and one targeting moiety, a signaling agent, *e.g.* IFNα1 is linked to the targeting moiety, which is linked to the Fc domain and the other signaling agent, *e.g.* IFNα1 is linked to the Fc domain (see FIGs. 6A-J, 9A-J, 12A-L, and 15A-L). In some embodiments, the targeting moiety and the unpaired signaling agent, *e.g.* IFNα1 are linked to different Fc chains (see FIGs. 6A-J and 9A-J). In some embodiments, the targeting moiety and the unpaired signaling agent, *e.g.* IFNα1 are linked to different Fc chains on the same terminus (see FIGs. 6A-J and 9A-J). In some embodiments, the targeting moiety and the unpaired signaling agent, *e.g.* IFNα1 are linked to different Fc chains on different termini (see FIGs. 6A-J and 9A-J). In some embodiments, the targeting moiety and the unpaired signaling agent, *e.g.* IFNα1 are linked to the same Fc chains (see FIGs. 12A-L and 15A-L). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are two signaling agents and one targeting moiety, the targeting moiety is linked to a signaling agent, *e.g.* IFNα1, which is linked to the Fc domain and the other signaling agent, *e.g.* IFNα1 is linked to the Fc domain (see FIGs. 6A-J and 9A-J). In some embodiments, the paired signaling agent, *e.g.* IFNα1 and the unpaired signaling agent, *e.g.* IFNα1 are linked to different Fc chains (see FIGs. 6A-J and 9A-J). In some embodiments, the paired signaling agent, *e.g.* IFNα1 and the unpaired signaling agent, *e.g.* IFNα1 are linked to different Fc chains on the same terminus (see FIGs. 6A-J and 9A-J). In some embodiments, the paired signaling

agent, *e.g.* IFNα1 and the unpaired signaling agent, *e.g.* IFNα1 are linked to different Fc chains on different termini (see FIGs. 6A-J and 9A-J). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are two signaling agents and one targeting moiety, the signaling agents are linked together and the targeting moiety is linked to one of the paired signaling agents, wherein the targeting moiety is linked to the Fc domain (see FIGs. 12A-L and 15A-L). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are two signaling agents and one targeting moiety, the signaling agents are linked together and one of the signaling agents is linked to the Fc domain and the targeting moiety is linked to the Fc domain (see FIGs. 12A-L, 15A-L, 18A-F, and 19A-F). In some embodiments, the paired signaling agents and targeting moiety are linked to the same Fc chain (see FIGs. 12A-L and 15A-L). In some embodiments, the paired signaling agents and targeting moiety are linked to different Fc chains (see FIGs. 18A-F and 19A-F). In some embodiments, the paired signaling agents and targeting moiety are linked to different Fc chains on the same terminus (see FIGs. 18A-F and 19A-F). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are two signaling agents and one targeting moiety, the signaling agents are both linked to the targeting moiety, wherein one of the signaling agents is linked to the Fc domain (see FIGs. 12A-L and 15A-L). In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises a mutation that reduces or eliminates its effector function.

In some embodiments, where there are two signaling agents and one targeting moiety, the signaling agents are linked together and one of the signaling agents is linked to the targeting moiety and the other signaling agent, *e.g.* IFNα1 is linked to the Fc domain (see FIGs. 12A-L and 15A-L).

In some embodiments, where there are two signaling agents and one targeting moiety, each signaling agent, *e.g.* IFNα1 is linked to the Fc domain and the targeting moiety is linked to one of the signaling agents (see FIGs. 12A-L and 15A-L). In some embodiments, the signaling agents are linked to the same Fc chain (see FIGs. 12A-L and 15A-L).

In some embodiments, a targeting moiety or signaling agent, *e.g.* IFNα1 is linked to the Fc domain, comprising one or both of C_H2 and C_H3 domains, and optionally a hinge region. For example, vectors encoding the targeting moiety, signaling agent, *e.g.* IFNα1, or combination thereof, linked as a single nucleotide sequence to an Fc domain can be used to prepare such polypeptides.

In some embodiments, the Fc-based chimeric protein complex comprises a polypeptide having an amino acid sequence having at least 95%, or at least 98%, or at least 99% identity with any one of SEQ ID NOs: 290, 291, 293-303. In embodiments, the Fc-based chimeric protein complex comprises a polypeptide having an amino acid sequence selected from SEQ ID NOs: 290, 291, 293-303 and less than 10 mutations to the amino acid sequence. In embodiments, the Fc-based chimeric protein complex comprises a polypeptide having an amino acid sequence selected from SEQ ID NOs: 290, 291, 293-303, and less than 5 mutations to the amino acid sequence. In some

embodiments, the Fc-based chimeric protein complex comprises a polypeptide having an amino acid sequence selected from SEQ ID NOs: 290, 291, 293-303.

In some embodiments, the Fc-based chimeric protein complex comprises a first amino acid sequence having at least 95%, or at least 98%, or at least 99% identity to SEQ ID NO: 290 and a second amino acid sequence having at least 95%, or at least 98%, or at least 99% identity to SEQ ID NO: 291. In some embodiments, the Fc-based chimeric protein complex comprises a first amino acid sequence having at least 95%, or at least 98%, or at least 99% identity to SEQ ID NO: 293 and a second amino acid sequence having at least 95%, or at least 98%, or at least 99% identity to any one of SEQ ID NO: 294, 295, 296, 297, 298, or 299. In embodiments, the Fc-based chimeric protein complex comprises a first amino acid sequence having at least 95%, or at least 98%, or at least 99% identity to any one of SEQ ID NO: 300, 301, 302, 303 and a second amino acid sequence having at least 95%, or at least 98%, or at least 99% identity to SEQ ID NO: 294.

Additional Signaling Agents

In one aspect, the present invention provides a chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprising one or more signaling agents (for instance, an immune-modulating agent) in addition to the IFNα1 or a variant thereof described herein. In illustrative embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may comprise two, three, four, five, six, seven, eight, nine, ten or more signaling agents in addition to the IFNα1 or a variant thereof described herein. In various embodiments, the additional signaling agent is modified to have reduced affinity or activity for one or more of its receptors, which allows for attenuation of activity (inclusive of agonism or antagonism) and/or prevents non-specific signaling or undesirable sequestration of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex.

In various embodiments, the additional signaling agent is antagonistic in its wild type form and bears one or more mutations that attenuate its antagonistic activity. In various embodiments, the additional signaling agent is antagonistic due to one or more mutations, *e.g.* an agonistic signaling agent is converted to an antagonistic signaling agent and, such a converted signaling agent, optionally, also bears one or more mutations that attenuate its antagonistic activity (*e.g.* as described in WO 2015/007520, the entire contents of which are hereby incorporated by reference).

In various embodiments, the additional signaling agent is selected from modified versions of cytokines, growth factors, and hormones. Illustrative examples of such cytokines, growth factors, and hormones include, but are not limited to, lymphokines, monokines, traditional polypeptide hormones, such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-α and tumor necrosis factor-β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin

(TPO); nerve growth factors such as NGF-α; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and –II ; osteo inductive factors; interferons such as, for example, interferon-α, interferon-β and interferon-γ (and interferon type I, II, and III), colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as, for example, IL-1β, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, and IL-18; a tumor necrosis factor such as, for example, TNF-α or TNF-β; and other polypeptide factors including, for example, LIF and kit ligand (KL). As used herein, cytokines, growth factors, and hormones include proteins obtained from natural sources or produced from recombinant bacterial, eukaryotic or mammalian cell culture systems and biologically active equivalents of the native sequence cytokines.

In some embodiments, the additional signaling agent is a modified version of a growth factor selected from, but not limited to, transforming growth factors (TGFs) such as TGF-α and TGF-β, epidermal growth factor (EGF), insulin-like growth factor such as insulin-like growth factor-I and -II, fibroblast growth factor (FGF), heregulin, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF).

In an embodiment, the growth factor is a modified version of a fibroblast growth factor (FGF). Illustrative FGFs include, but are not limited to, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, murine FGF15, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22, and FGF23.

In an embodiment, the growth factor is a modified version of a vascular endothelial growth factor (VEGF). Illustrative VEGFs include, but are not limited to, VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PGF and isoforms thereof including the various isoforms of VEGF-A such as $VEGF_{121}$, $VEGF_{121}b$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{165}b$, $VEGF_{189}$, and $VEGF_{206}$.

In an embodiment, the growth factor is a modified version of a transforming growth factor (TGF). Illustrative TGFs include, but are not limited to, TGF-α and TGF-β and subtypes thereof including the various subtypes of TGF-β including TGFβ1, TGFβ2, and TGFβ3.

In some embodiments, the additional signaling agent is a modified version of a hormone selected from, but not limited to, human chorionic gonadotropin, gonadotropin releasing hormone, an androgen, an estrogen, thyroid-stimulating hormone, follicle-stimulating hormone, luteinizing hormone, prolactin, growth hormone, adrenocorticotropic hormone, antidiuretic hormone, oxytocin, thyrotropin-releasing hormone, growth hormone releasing hormone, corticotropin-releasing hormone, somatostatin, dopamine, melatonin, thyroxine, calcitonin, parathyroid hormone, glucocorticoids, mineralocorticoids, adrenaline, noradrenaline, progesterone, insulin, glucagon, amylin, calcitriol, calciferol, atrial-natriuretic peptide, gastrin, secretin, cholecystokinin, neuropeptide Y, ghrelin, PYY3-36, insulin-like growth factor (IGF), leptin, thrombopoietin, erythropoietin (EPO), and angiotensinogen.

In some embodiments, the additional signaling agent is an immune-modulating agent, *e.g.* one or more of an interleukin, interferon, and tumor necrosis factor.

In some embodiments, the additional signaling agent is an interleukin, including for example IL-1β; IL-2; IL-3; IL-4; IL-5; IL-6; IL-7; IL-8; IL-9; IL-10; IL-11; IL-12; IL-13; IL-14; IL-15; IL-16; IL-17; IL-18; IL-19; IL-20; IL-21; IL-22; IL-

23; IL-24; IL-25; IL-26; IL-27; IL-28; IL-29; IL-30; IL-31; IL-32; IL-33; IL-35; IL-36 or a fragment, variant, analogue, or family-member thereof. Interleukins are a group of multi- functional cytokines synthesized by lymphocytes, monocytes, and macrophages. Known functions include stimulating proliferation of immune cells (*e.g.*, T helper cells, B cells, eosinophils, and lymphocytes), chemotaxis of neutrophils and T lymphocytes, and/or inhibition of interferons. Interleukin activity can be determined using assays known in the art: Matthews *et al.*, in *Lymphokines and Interferens: A Practical Approach*, Clemens *et al.*, eds, IRL Press, Washington, D.C. 1987, pp. 221-225; and Orencole & Dinarello (1989) Cytokine 1, 14-20.

In some embodiments, the signaling agent is a modified version of an interferon such as interferon types I, II, and III. Illustrative interferons, including for example, interferon-α-1, 2, 4, 5, 6, 7, 8, 10, 13, 14, 16, 17, and 21, interferon-β and interferon-γ, interferon κ, interferon ε, interferon τ, and interferon ϖ.

In embodiments, the additional signaling agent is a type I interferon. In embodiments, the type I interferon is selected from IFN-α2, IFNα1, IFN-β, IFN-γ, Consensus IFN, IFN-ε, IFN-κ, IFN-τ, IFN-δ, and IFN-v.

In some embodiments, the additional signaling agent is a modified version of a tumor necrosis factor (TNF) or a protein in the TNF family, including but not limited to, TNF-α, TNF-β, LT-β, CD40L, CD27L, CD30L, FASL, 4-1BBL, OX40L, and TRAIL.

In various embodiments, the additional signaling agent is a modified (*e.g.* mutant) form of the signaling agent having one or more mutations. In various embodiments, the mutations allow for the modified signaling agent to have one or more of attenuated activity such as one or more of reduced binding affinity, reduced endogenous activity, and reduced specific bioactivity relative to unmodified or unmutated, *i.e.* the wild type form of the signaling agent (*e.g.* comparing the same signaling agent in a wild type form versus a modified (*e.g.* mutant) form). In various embodiments, the mutations allow for the modified signaling agent to have one or more of attenuated activity such as one or more of reduced binding affinity, reduced endogenous activity, and reduced specific bioactivity relative to unmodified or unmutated, *i.e.* the unmutated IFNα1. In some embodiments, the mutations which attenuate or reduce binding or affinity include those mutations which substantially reduce or ablate binding or activity. In some embodiments, the mutations which attenuate or reduce binding or affinity are different than those mutations which substantially reduce or ablate binding or activity. Consequentially, in various embodiments, the mutations allow for the signaling agent to be more safe, *e.g.* have reduced systemic toxicity, reduced side effects, and reduced off-target effects relative to unmutated, *i.e.* wild type, signaling agent (*e.g.* comparing the same signaling agent in a wild type form versus a modified (*e.g.* mutant) form). In various embodiments, the mutations allow for the signaling agent to be safer, *e.g.* have reduced systemic toxicity, reduced side effects, and reduced off-target effects relative to unmutated interferon, *e.g.* the unmutated sequence of IFNα1.

In various embodiments, the additional signaling agent is modified to have one or more mutations that reduce its binding affinity or activity for one or more of its receptors. In some embodiments, the signaling agent is modified to have one or more mutations that substantially reduce or ablate binding affinity or activity for the receptors. In some embodiments, the activity provided by the wild type signaling agent is agonism at the receptor (*e.g.* activation of a cellular effect at a site of therapy). For example, the wild type signaling agent may activate its receptor. In such

embodiments, the mutations result in the modified signaling agent to have reduced or ablated activating activity at the receptor. For example, the mutations may result in the modified signaling agent to deliver a reduced activating signal to a target cell or the activating signal could be ablated. In some embodiments, the activity provided by the wild type signaling agent is antagonism at the receptor (*e.g.* blocking or dampening of a cellular effect at a site of therapy). For example, the wild type signaling agent may antagonize or inhibit the receptor. In these embodiments, the mutations result in the modified signaling agent to have a reduced or ablated antagonizing activity at the receptor. For example, the mutations may result in the modified signaling agent to deliver a reduced inhibitory signal to a target cell or the inhibitory signal could be ablated. In various embodiments, the signaling agent is antagonistic due to one or more mutations, *e.g.* an agonistic signaling agent is converted to an antagonistic signaling agent (*e.g.* as described in WO 2015/007520, the entire contents of which are hereby incorporated by reference) and, such a converted signaling agent, optionally, also bears one or more mutations that reduce its binding affinity or activity for one or more of its receptors or that substantially reduce or ablate binding affinity or activity for one or more of its receptors.

In some embodiments, the reduced affinity or activity at the receptor is inducible or restorable by attachment with one or more of the targeting moieties or upon inclusion in the Fc-based chimeric protein complex disclosed herein. In other embodiments, the reduced affinity or activity at the receptor is not substantially inducible or restorable by the activity of one or more of the targeting moieties or upon inclusion in the Fc-based chimeric protein complex disclosed herein.

In various embodiments, the additional signaling agent is active on target cells because the targeting moiety(ies) compensates for the missing/insufficient binding (*e.g.*, without limitation and/or avidity) required for substantial activation. In various embodiments, the modified signaling agent is substantially inactive *en route* to the site of therapeutic activity and has its effect substantially on specifically targeted cell types which greatly reduces undesired side effects.

In some embodiments, the additional signaling agent may include one or more mutations that attenuate or reduce binding or affinity for one receptor (*i.e.*, a therapeutic receptor) and one or more mutations that substantially reduce or ablate binding or activity at a second receptor. In such embodiments, these mutations may be at the same or at different positions (*i.e.*, the same mutation or multiple mutations). In some embodiments, the mutation(s) that reduce binding and/or activity at one receptor is different than the mutation(s) that substantially reduce or ablate at another receptor. In some embodiments, the mutation(s) that reduce binding and/or activity at one receptor is the same as the mutation(s) that substantially reduce or ablate at another receptor. In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes have a modified signaling agent that has both mutations that attenuate binding and/or activity at a therapeutic receptor and therefore allow for a more controlled, on-target therapeutic effect (*e.g.* relative wild type signaling agent) and mutations that substantially reduce or ablate binding and/or activity at another receptor and therefore reduce side effects (*e.g.* relative to wild type signaling agent).

In some embodiments, the substantial reduction or ablation of binding or activity is not substantially inducible or restorable with a targeting moiety or upon inclusion in the Fc-based chimeric protein complex disclosed herein. In some embodiments, the substantial reduction or ablation of binding or activity is inducible or restorable with a targeting moiety or upon inclusion in the Fc-based chimeric protein complex disclosed herein. In various embodiments, substantially reducing or ablating binding or activity at a second receptor also may prevent deleterious effects that are mediated by the other receptor. Alternatively, or in addition, substantially reducing or ablating binding or activity at the other receptor causes the therapeutic effect to improve as there is a reduced or eliminated sequestration of the therapeutic chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes away from the site of therapeutic action. For instance, in some embodiments, this obviates the need of high doses of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes that compensate for loss at the other receptor. Such ability to reduce dose further provides a lower likelihood of side effects.

In various embodiments, the additional modified signaling agent comprises one or more mutations that cause the signaling agent to have reduced, substantially reduced, or ablated affinity, *e.g.* binding (*e.g.* K_D) and/or activation (for instance, when the modified signaling agent is an agonist of its receptor, measurable as, for example, K_A and/or EC_{50}) and/or inhibition (for instance, when the modified signaling agent is an antagonist of its receptor, measurable as, for example, K_I and/or IC_{50}), for one or more of its receptors. In various embodiments, the reduced affinity at the signaling agent's receptor allows for attenuation of activity (inclusive of agonism or antagonism). In such embodiments, the modified signaling agent has about 1%, or about 3%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 10%-20%, about 20%-40%, about 50%, about 40%-60%, about 60%-80%, about 80%-100% of the affinity for the receptor relative to the wild type signaling agent. In some embodiments, the binding affinity is at least about 2-fold lower, about 3-fold lower, about 4-fold lower, about 5-fold lower, about 6-fold lower, about 7-fold lower, about 8-fold lower, about 9-fold lower, at least about 10-fold lower, at least about 15-fold lower, at least about 20-fold lower, at least about 25-fold lower, at least about 30-fold lower, at least about 35-fold lower, at least about 40-fold lower, at least about 45-fold lower, at least about 50-fold lower, at least about 100-fold lower, at least about 150-fold lower, or about 10-50-fold lower, about 50-100-fold lower, about 100-150-fold lower, about 150-200-fold lower, or more than 200-fold lower relative to the wild type signaling agent (including, by way of non-limitation, relative to the unmutated IFNα1).

In embodiments wherein the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex has mutations that reduce binding at one receptor and substantially reduce or ablate binding at a second receptor, the attenuation or reduction in binding affinity of a modified signaling agent for one receptor is less than the substantial reduction or ablation in affinity for the other receptor. In some embodiments, the attenuation or reduction in binding affinity of a modified signaling agent for one receptor is less than the substantial reduction or ablation in affinity for the other receptor by about 1%, or about 3%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 60%, about 65%, about 70%, about

75%, about 80%, about 85%, about 90%, or about 95%. In various embodiments, substantial reduction or ablation refers to a greater reduction in binding affinity and/or activity than attenuation or reduction.

In various embodiments, the additional modified signaling agent comprises one or more mutations that reduce the endogenous activity of the signaling agent to about 75%, or about 70%, or about 60%, or about 50%, or about 40%, or about 30%, or about 25%, or about 20%, or about 10%, or about 5%, or about 3%, or about 1%, *e.g.*, relative to the wild type signaling agent (including, by way of non-limitation, relative to the unmutated IFNα1).

In various embodiments, the additional modified signaling agent comprises one or more mutations that cause the signaling agent to have reduced affinity and/or activity for a receptor of any one of the cytokines, growth factors, and hormones as described herein.

In some embodiments, the additional modified signaling agent comprises one or more mutations that cause the signaling agent to have reduced affinity for its receptor that is lower than the binding affinity of the targeting moiety(ies) for its(their) receptor(s). In some embodiments, this binding affinity differential is between signaling agent/receptor and targeting moiety/receptor on the same cell. In some embodiments, this binding affinity differential allows for the signaling agent, *e.g.* mutated signaling agent, to have localized, on-target effects and to minimize off-target effects that underlie side effects that are observed with wild type signaling agent. In some embodiments, this binding affinity is at least about 2-fold, or at least about 5-fold, or at least about 10-fold, or at least about 15-fold lower, or at least about 25-fold, or at least about 50-fold lower, or at least about 100-fold, or at least about 150-fold.

Receptor binding activity may be measured using methods known in the art. For example, affinity and/or binding activity may be assessed by Scatchard plot analysis and computer-fitting of binding data (*e.g.* Scatchard, 1949) or by reflectometric interference spectroscopy under flow through conditions, as described by Brecht *et al.* (1993), the entire contents of all of which are hereby incorporated by reference.

The amino acid sequences of the wild type signaling agents described herein are well known in the art. Accordingly, in various embodiments the additional modified signaling agent comprises an amino acid sequence that has at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with the known wild type amino acid sequences of the signaling agents described herein (*e.g.* about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or

about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% sequence identity).

In various embodiments the additional modified signaling agent comprises an amino acid sequence that has at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with any of the sequences disclosed herein (*e.g.* about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% sequence identity).

In various embodiments, the additional modified signaling agent comprises an amino acid sequence having one or more amino acid mutations. In some embodiments, the one or more amino acid mutations may be independently selected from substitutions, insertions, deletions, and truncations.

In some embodiments, the amino acid mutations are amino acid substitutions, and may include conservative and/or non-conservative substitutions as described herein.

As described herein, the additional modified signaling agents bear mutations that affect affinity and/or activity at one or more receptors. In various embodiments, there is reduced affinity and/or activity at a therapeutic receptor, *e.g.* a receptor through which a desired therapeutic effect is mediated (*e.g.* agonism or antagonism). In various embodiments, the modified signaling agents bear mutations that substantially reduce or ablate affinity and/or activity at a receptor, *e.g.* a receptor through which a desired therapeutic effect is not mediated (*e.g.* as the result of promiscuity of binding). The receptors of any modified signaling agents, *e.g.* one of the cytokines, growth factors, and hormones as described herein, are known in the art.

Illustrative mutations which provide reduced affinity and/or activity (*e.g.* agonistic) at a receptor are found in WO 2013/107791 (*e.g.* with regard to interferons), WO 2015/007542 (*e.g.* with regard to interleukins), and WO 2015/007903 (*e.g.* with regard to TNF), the entire contents of each of which are hereby incorporated by reference. Illustrative mutations which provide reduced affinity and/or activity (*e.g.* antagonistic) at a therapeutic receptor are found in WO 2015/007520, the entire contents of which are hereby incorporated by reference.

In some embodiments, the additional modified signaling agent comprises one or more mutations that cause the signaling agent to have reduced affinity and/or activity for a type I cytokine receptor, a type II cytokine receptor, a chemokine receptor, a receptor in the Tumor Necrosis Factor Receptor (TNFR) superfamily, TGF-beta Receptors, a receptor in the immunoglobulin (Ig) superfamily, and/or a receptor in the tyrosine kinase superfamily.

In various embodiments, the receptor for the additional signaling agent is a Type I cytokine receptor. Type I cytokine receptors are known in the art and include, but are not limited to receptors for IL2 (beta-subunit), IL3, IL4, IL5, IL6, IL7, IL9, IL11, IL12, GM-CSF, G-CSF, LIF, CNTF, and also the receptors for Thrombopoietin (TPO), Prolactin, and Growth hormone. Illustrative type I cytokine receptors include, but are not limited to, GM-CSF receptor, G-CSF receptor, LIF receptor, CNTF receptor, TPO receptor, and type I IL receptors.

In various embodiments, the receptor for the additional signaling agent is a Type II cytokine receptor. Type II cytokine receptors are multimeric receptors composed of heterologous subunits, and are receptors mainly for interferons. This family of receptors includes, but is not limited to, receptors for interferon-α, interferon-β and interferon-γ, IL10, IL22, and tissue factor. Illustrative type II cytokine receptors include, but are not limited to, IFN-α receptor (*e.g.* IFNAR1 and IFNAR2), IFN-β receptor, IFN-γ receptor (*e.g.* IFNGR1 and IFNGR2), and type II IL receptors.

In various embodiments, the receptor for the additional signaling agent is a G protein-coupled receptor. Chemokine receptors are G protein-coupled receptors with seven transmembrane structure and coupled to G-protein for signal transduction. Chemokine receptors include, but are not limited to, CC chemokine receptors, CXC chemokine receptors, CX3C chemokine receptors, and XC chemokine receptor (XCR1). Illustrative chemokine receptors include, but are not limited to, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, CXCR1, CXCR2, CXCR3, CXCR3B, CXCR4, CXCR5, CSCR6, CXCR7, XCR1, and CX3CR1.

In various embodiments, the receptor for the additional signaling agent is a TNFR family member. Tumor necrosis factor receptor (TNFR) family members share a cysteine-rich domain (CRD) formed of three disulfide bonds surrounding a core motif of CXXCXXC creating an elongated molecule. Illustrative tumor necrosis factor receptor family members include: CDI 20a (TNFRSFIA), CD 120b (TNFRSFIB), Lymphotoxin beta receptor (LTBR, TNFRSF3), CD 134 (TNFRSF4), CD40 (CD40, TNFRSF5), FAS (FAS, TNFRSF6), TNFRSF6B (TNFRSF6B), CD27 (CD27, TNFRSF7), CD30 (TNFRSF8), CD137 (TNFRSF9), TNFRSFIOA (TNFRSFIOA), TNFRSFIOB, (TNFRSFIOB), TNFRSFIOC (TNFRSFIOC), TNFRSFIOD (TNFRSFIOD), RANK (TNFRSFI IA), Osteoprotegerin (TNFRSFI IB), TNFRSF12A (TNFRSF12A), TNFRSF13B (TNFRSF13B), TNFRSF13C (TNFRSF13C), TNFRSF14 (TNFRSF14), Nerve growth factor receptor (NGFR, TNFRSF16), TNFRSF17 (TNFRSF17), TNFRSF18 (TNFRSF18), TNFRSF19 (TNFRSF19), TNFRSF21 (TNFRSF21), and TNFRSF25 (TNFRSF25).

In various embodiments, the receptor for the additional signaling agent is a TGF-beta receptor. TGF-beta receptors are single pass serine/threonine kinase receptors. TGF-beta receptors include, but are not limited to, TGFBR1, TGFBR2, and TGFBR3.

In various embodiments, the receptor for the additional signaling agent is an Ig superfamily receptor. Receptors in the immunoglobulin (Ig) superfamily share structural homology with immunoglobulins. Receptors in the Ig

superfamily include, but are not limited to, interleukin-1 receptors, CSF-1R, PDGFR (*e.g.* PDGFRA and PDGFRB), and SCFR.

In various embodiments, the receptor for the additional signaling agent is a tyrosine kinase superfamily receptor. Receptors in the tyrosine kinase superfamily are well known in the art. There are about 58 known receptor tyrosine kinases (RTKs), grouped into 20 subfamilies. Receptors in the tyrosine kinase superfamily include, but are not limited to, FGF receptors and their various isoforms such as FGFR1, FGFR2, FGFR3, FGFR4, and FGFR5.

In an embodiment, the additional modified signaling agent is interferon α. In such embodiments, the modified IFN-α agent has reduced affinity and/or activity for the IFN-α/β receptor (IFNAR), *i.e.*, IFNAR1 and/or IFNAR2 chains. In some embodiments, the modified IFN-α agent has substantially reduced or ablated affinity and/or activity for the IFN-α/β receptor (IFNAR), *i.e.*, IFNAR1 and/or IFNAR2 chains.

Mutant forms of interferon α are known to the person skilled in the art. In an illustrative embodiment, the modified signaling agent is the allelic form IFN-α2a having the amino acid sequence of SEQ ID NO:233.

In an illustrative embodiment, the modified signaling agent is the allelic form IFN-α2b having the amino acid sequence of SEQ ID NO:234 (which differs from IFN-α2a at amino acid position 23).

In some embodiments, said IFN-α2 mutant (IFN-α2a or IFN-α2b) is mutated at one or more amino acids at positions 144-154, such as amino acid positions 148, 149 and/or 153. In some embodiments, the IFN-α2 mutant comprises one or more mutations selected from L153A, R149A, and M148A. Such mutants are described, for example, in WO2013/107791 and Piehler *et al.*, (2000) J. Biol. Chem, 275:40425-33, the entire contents of all of which are hereby incorporated by reference.

In some embodiments, the IFN-α2 mutants have reduced affinity and/or activity for IFNAR1. In some embodiments, the IFN-α2 mutant comprises one or more mutations selected from F64A, N65A, T69A, L80A, Y85A, and Y89A, as described in WO2010/030671, the entire contents of which is hereby incorporated by reference.

In some embodiments, the IFN-α2 mutant comprises one or more mutations selected from K133A, R144A, R149A, and L153A as described in WO2008/124086, the entire contents of which is hereby incorporated by reference.

In some embodiments, the IFN-α2 mutant comprises one or more mutations selected from R120E and R120E/K121E, as described in WO2015/007520 and WO2010/030671, the entire contents of which are hereby incorporated by reference. In such embodiments, said IFN-α2 mutant antagonizes wildtype IFN-α2 activity. In such embodiments, said mutant IFN-α2 has reduced affinity and/or activity for IFNAR1 while affinity and/or activity of IFNR2 is retained.

In some embodiments, the human IFN-α2 mutant comprises (1) one or more mutations selected from R120E and R120E/K121E, which, without wishing to be bound by theory, create an antagonistic effect and (2) one or more mutations selected from K133A, R144A, R149A, and L153A, which, without wishing to be bound by theory, allow for an attenuated effect at, for example, IFNAR2. In an embodiment, the human IFN-α2 mutant comprises R120E and L153A.

In some embodiments, the human IFN-α2 mutant comprises one or more mutations selected from, L15A, A19W, R22A, R23A, L26A, F27A, L30A, L30V, K31A, D32A, R33K, R33A, R33Q, H34A, D35A, Q40A, D114R, L117A, R120A, R125A, K134A, R144A, A145G, A145M, M148A, R149A, S152A, L153A, and N156A as disclosed in WO 2013/059885, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the human IFN-α2 mutant comprises the mutations H57Y, E58N, Q61S, and/or L30A as disclosed in WO 2013/059885. In some embodiments, the human IFN-α2 mutant comprises the mutations H57Y, E58N, Q61S, and/or R33A as disclosed in WO 2013/059885. In some embodiments, the human IFN-α2 mutant comprises the mutations H57Y, E58N, Q61S, and/or M148A as disclosed in WO 2013/059885. In some embodiments, the human IFN-α2 mutant comprises the mutations H57Y, E58N, Q61S, and/or L153A as disclosed in WO 2013/059885. In some embodiments, the human IFN-α2 mutant comprises the mutations N65A, L80A, Y85A, and/or Y89A as disclosed in WO 2013/059885. In some embodiments, the human IFN-α2 mutant comprises the mutations N65A, L80A, Y85A, Y89A, and/or D114A as disclosed in WO 2013/059885. In some embodiments, the human IFN-α2 mutant comprises one or more mutations selected from $R144X_1$, $A145X_2$, and R33A, wherein X_1 is selected from A, S, T, Y, L, and I, and wherein X_2 is selected from G, H, Y, K, and D. In some embodiments, the human IFN-α2 mutant comprises one or more mutations selected from R33A, $T106X_3$, R120E, $R144X_1$ $A145X_2$, M148A, R149A, and L153A with respect to amino acid sequence of SEQ ID NO: 233 or 234, wherein X_1 is selected from A, S, T, Y, L, and I, wherein X_2 is selected from G, H, Y, K, and D, and wherein X_3 is selected from A and E.

In an embodiment, the additional modified signaling agent is interferon β. In such embodiments, the modified interferon β agent also has reduced affinity and/or activity for the IFN-α/β receptor (IFNAR), *i.e.*, IFNAR1 and/or IFNAR2 chains. In some embodiments, the modified interferon β agent has substantially reduced or ablated affinity and/or activity for the IFN-α/β receptor (IFNAR), *i.e.*, IFNAR1 and/or IFNAR2 chains.

In an illustrative embodiment, the modified additional signaling agent is IFN-β. In various embodiments, the IFN-β encompasses functional derivatives, analogs, precursors, isoforms, splice variants, or fragments of IFN-β. In various embodiments, the IFN-β encompasses IFN-β derived from any species. In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises a modified version of mouse IFN-β. In another embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises a modified version of human IFN-β. Human IFN-β is a polypeptide with a molecular weight of about 22 kDa comprising 166 amino acid residues. The amino acid sequence of human IFN-β is SEQ ID NO: 277.

In some embodiments, the human IFN-β is IFN-β-1a which is a glycosylated form of human IFN-β. In some embodiments, the human IFN-β is IFN-β-1b which is a non-glycosylated form of human IFN-β that has a Met-1 deletion and a Cys-17 to Ser mutation.

In various embodiments, the modified IFN-β has one or more mutations that reduce its binding to or its affinity for the IFNAR1 subunit of IFNAR. In one embodiment, the modified IFN-β has reduced affinity and/or activity at IFNAR1. In various embodiments, the modified IFN-β is human IFN-β and has one or more mutations at positions F67, R71, L88, Y92, I95, N96, K123, and R124. In some embodiments, the one or more mutations are substitutions

selected from F67G, F67S, R71A, L88G, L88S, Y92G, Y92S, I95A, N96G, K123G, and R124G. In an embodiment, the modified IFN-β comprises the F67G mutation. In an embodiment, the modified IFN-β comprises the K123G mutation. In an embodiment, the modified IFN-β comprises the F67G and R71A mutations. In an embodiment, the modified IFN-β comprises the L88G and Y92G mutations. In an embodiment, the modified IFN-β comprises the Y92G, I95A, and N96G mutations. In an embodiment, the modified IFN-β comprises the K123G and R124G mutations. In an embodiment, the modified IFN-β comprises the F67G, L88G, and Y92G mutations. In an embodiment, the modified IFN-β comprises the F67S, L88S, and Y92S mutations.

In some embodiments, the modified IFN-β has one or more mutations that reduce its binding to or its affinity for the IFNAR2 subunit of IFNAR. In one embodiment, the modified IFN-β has reduced affinity and/or activity at IFNAR2. In various embodiments, the modified IFN-β is human IFN-β and has one or more mutations at positions W22, R27, L32, R35, V148, L151, R152, and Y155. In some embodiments, the one or more mutations are substitutions selected from W22G, R27G, L32A, L32G, R35A, R35G, V148G, L151G, R152A, R152G, and Y155G. In an embodiment, the modified IFN-β comprises the W22G mutation. In an embodiment, the modified IFN-β comprises the L32A mutation. In an embodiment, the modified IFN-β comprises the L32G mutation. In an embodiment, the modified IFN-β comprises the R35A mutation. In an embodiment, the modified IFN-β comprises the R35G mutation. In an embodiment, the modified IFN-β comprises the V148G mutation. In an embodiment, the modified IFN-β comprises the R152A mutation. In an embodiment, the modified IFN-β comprises the R152G mutation. In an embodiment, the modified IFN-β comprises the Y155G mutation. In an embodiment, the modified IFN-β comprises the W22G and R27G mutations. In an embodiment, the modified IFN-β comprises the L32A and R35A mutation. In an embodiment, the modified IFN-β comprises the L151G and R152A mutations. In an embodiment, the modified IFN-β comprises the V148G and R152A mutations.

In some embodiments, the modified IFN-β has one or more of the following mutations: R35A, R35T, E42K, M62I, G78S, A141Y, A142T, E149K, and R152H. In some embodiments, the modified IFN-β has one or more of the following mutations: R35A, R35T, E42K, M62I, G78S, A141Y, A142T, E149K, and R152H in combination with C17S or C17A.

In some embodiments, the modified IFN-β has one or more of the following mutations: R35A, R35T, E42K, M62I, G78S, A141Y, A142T, E149K, and R152H in combination with any of the other IFN-β mutations described herein.

The crystal structure of human IFN-β is known and is described in Karpusas *et al.*, (1998) PNAS, 94(22): 11813–11818. Specifically, the structure of human IFN-β has been shown to include five α-helices (*i.e.*, A, B, C, D, and E) and four loop regions that connect these helices (*i.e.*, AB, BC, CD, and DE loops). In various embodiments, the modified IFN-β has one or more mutations in the A, B, C, D, E helices and/or the AB, BC, CD, and DE loops which reduce its binding affinity or activity at a therapeutic receptor such as IFNAR. Illustrative mutations are described in WO2000/023114 and US20150011732, the entire contents of which are hereby incorporated by reference. In an illustrative embodiment, the modified IFN-β is human IFN-β comprising alanine substitutions at amino acid positions 15, 16, 18, 19, 22, and/or 23. In an illustrative embodiment, the modified IFN-β is human IFN-β comprising alanine substitutions at amino acid positions 28-30, 32, and 33. In an illustrative embodiment, the modified IFN-β

is human IFN-β comprising alanine substitutions at amino acid positions 36, 37, 39, and 42. In an illustrative embodiment, the modified IFN-β is human IFN-β comprising alanine substitutions at amino acid positions 64 and 67 and a serine substitution at position 68. In an illustrative embodiment, the modified IFN-β is human IFN-β comprising alanine substitutions at amino acid positions 71-73. In an illustrative embodiment, the modified IFN-β is human IFN-β comprising alanine substitutions at amino acid positions 92, 96, 99, and 100. In an illustrative embodiment, the modified IFN-β is human IFN-β comprising alanine substitutions at amino acid positions 128, 130, 131, and 134. In an illustrative embodiment, the modified IFN-β is human IFN-β comprising alanine substitutions at amino acid positions 149, 153, 156, and 159. In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at W22, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at R27, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at W22, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V) and a mutation at R27, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at L32, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at R35, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at L32, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), isoleucine (I), methionine (M), and valine (V) and a mutation at R35, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at F67, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at R71, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at F67, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and

valine (V) and a mutation at R71, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at L88, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at Y92, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at F67, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V) and a mutation at L88, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), isoleucine (I), methionine (M), and valine (V) and a mutation at Y92, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at L88, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), isoleucine (I), methionine (M), and valine (V) and a mutation at Y92, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at I95, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), methionine (M), and valine (V) and a mutation at Y92, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO:277 and a mutation at N96, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V) and a mutation at Y92, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO: 277 and a mutation at Y92, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V) and a mutation at I95, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), methionine (M), and valine (V) and a mutation at N96, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO: 277 and a mutation at K123, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO: 277 and a mutation at R124, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO: 277 and a mutation at K123, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V) and a mutation at R124, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO: 277 and a mutation at L151, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO: 277 and a mutation at R152, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO: 277 and a mutation at L151, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), isoleucine (I), methionine (M), and valine (V) and a mutation at R152, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO: 277 and a mutation at V148, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), and methionine (M).

In some embodiments, the mutant IFNβ comprises SEQ ID NO: 277 and a mutation at V148, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V) and a mutation at R152, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the mutant IFNβ comprises SEQ ID NO: 277 and a mutation at Y155, the mutation being an aliphatic hydrophobic residue selected from glycine (G), alanine (A), leucine (L), isoleucine (I), methionine (M), and valine (V).

In some embodiments, the present invention relates to a chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprising: (a) a modified IFN-β, having the amino acid sequence of SEQ ID NO: 277 and a mutation at position W22, wherein the mutation is an aliphatic hydrophobic residue and a modified IL-2 or modified IL-2 variant disclosed here; and (b) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to antigens or receptors of interest, the modified IFN-β and the one or more targeting moieties are optionally connected with one or more linkers. In various embodiments the mutation at position W22 is aliphatic hydrophobic residue is selected from G, A, L, I, M, and V. In various embodiments the mutation at position W22 is G.

Additional illustrative IFNβ mutants are provided in PCT/EP2017/061544, the entire disclosure of which is incorporated by reference herein.

In some embodiments, the modified additional signaling agent is interferon γ. In such embodiments, the modified interferon γ agent has reduced affinity and/or activity for the interferon-gamma receptor (IFNGR), *i.e.*, IFNGR1 and IFNGR2 chains. In some embodiments, the modified interferon γ agent has substantially reduced or ablated affinity and/or activity for the interferon-gamma receptor (IFNGR), *i.e.*, IFNGR1 and/or IFNGR2 chains.

In some embodiments, the modified additional signaling agent is a consensus interferon. The consensus interferon is generated by scanning the sequences of several human non-allelic IFN-α subtypes and assigning the most frequently observed amino acid in each corresponding position. The consensus interferon differs from IFN-α2b at 20 out of 166 amino acids (88% homology), and comparison with IFN-β shows identity at over 30% of the amino acid positions. In various embodiments, the consensus interferon comprises the following amino acid sequence of SEQ ID NO: 278.

In some embodiments, the consensus interferon comprises the amino acid sequence of SEQ ID NO: 279, which differs from the amino acid sequence of SEQ ID NO: 278 by one amino acid, *i.e.*, SEQ ID NO: 279 lacks the initial methionine residue of SEQ ID NO: 278:

In various embodiments, the consensus interferon comprises a modified version of the consensus interferon, *i.e.*, a consensus interferon variant, as a signaling agent. In various embodiments, the consensus interferon variant encompasses functional derivatives, analogs, precursors, isoforms, splice variants, or fragments of the consensus interferon.

In an embodiment, the consensus interferon variants are selected form the consensus interferon variants disclosed in U.S. Patent Nos. 4,695,623, 4,897,471, 5,541,293, and 8,496,921, the entire contents of all of which are hereby incorporated by reference. For example, the consensus interferon variant may comprise the amino acid sequence of IFN-CON_2 or IFN-CON_3 as disclosed in U.S. Patent Nos. 4,695,623, 4,897,471, and 5,541,293. In an embodiment, the consensus interferon variant comprises the amino acid sequence of IFN-CON_2: SEQ ID NO: 280.

In an embodiment, the consensus interferon variant comprises the amino acid sequence of IFN-CON_3: SEQ ID NO: 281.

In an embodiment, the consensus interferon variant comprises the amino acid sequence of any one of the variants disclosed in U.S. Patent No. 8,496,921. For example, the consensus variant may comprise the amino acid sequence of: SEQ ID NO: 282.

In another embodiment, the consensus interferon variant may comprise the amino acid sequence of: SEQ ID NO: 283.

In some embodiments, the consensus interferon variant may be PEGylated, *i.e.*, comprises a PEG moiety. In an embodiment, the consensus interferon variant may comprise a PEG moiety attached at the S156C position of SEQ ID NO: 283.

In some embodiments, the engineered interferon is a variant of human IFN-α2a, with an insertion of Asp at approximately position 41 in the sequence Glu-Glu-Phe-Gly-Asn-Gln (SEQ ID NO: 284) to yield Glu-Glu-Phe-Asp-Gly-Asn-Gln (SEQ ID NO: 285) (which resulted in a renumbering of the sequence relative to IFN-α2a sequence) and the following mutations of Arg23Lys, Leu26Pro, Glu53Gln, Thr54Ala, Pro56Ser, Asp86Glu, Ile104Thr, Gly106Glu, Thr110Glu, Lys117Asn, Arg125Lys, and Lys136Thr. All embodiments herein that describe consensus interferons apply equally to this engineered interferon

In some embodiments, the additional modified signaling agent is vascular endothelial growth factor (VEGF). VEGF is a potent growth factor that plays major roles in physiological but also pathological angiogenesis, regulates vascular permeability and can act as a growth factor on cells expressing VEGF receptors. Additional functions include, among others, stimulation of cell migration in macrophage lineage and endothelial cells. Several members of the VEGF family of growth factors exist, as well as at least three receptors (VEGFR-1, VEGFR -2, and VEGFR -3). Members of the VEGF family can bind and activate more than one VEGFR type. For example, VEGF-A binds VEGFR-1 and -2, while VEGF-C can bind VEGFR-2 and -3. VEGFR-1 and VEGFR-2 activation regulate angiogenesis while VEGFR-3 activation is associated with lymphangiogenesis. The major pro-angiogenic signal is generated from activation of VEGFR-2. VEGFR-1 activation has been reported to be possibly associated with negative role in angiogenesis. It has also been reported that VEGFR-1 signaling is important for progression of tumors in vivo via bone marrow-derived VEGFR-1 positive cells (contributing to formation of premetastatic niche in the bone). Several therapies based on VEGF-A directed/neutralizing therapeutic antibodies have been developed, primarily for use in treatment of various human tumors relying on angiogenesis. These are not without side effects though. This may not be surprising considering that these operate as general, non-cell/tissue specific VEGF/VEGFR interaction inhibitors. Hence, it would be desirable to restrict VEGF (*e.g.* VEGF-A)/VEGFR-2 inhibition to specific target cells (*e.g.* tumor vasculature endothelial cells).

In some embodiments, the VEGF is VEGF-A, VEGF-B, VEFG-C, VEGF-D, or VEGF-E and isoforms thereof including the various isoforms of VEGF-A such as $VEGF_{121}$, $VEGF_{121}b$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{165}b$, $VEGF_{189}$, and $VEGF_{206}$. In some embodiments, the modified signaling agent has reduced affinity and/or activity for VEGFR-1 (Flt-1) and/or VEGFR-2 (KDR/Flk-1). In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for VEGFR-1 (Flt-1) and/or VEGFR-2 (KDR/Flk-1). In an embodiment, the modified signaling agent has reduced affinity and/or activity for VEGFR-2 (KDR/Flk-1) and/or substantially reduced or ablated affinity and/or activity for VEGFR-1 (Flt-1). Such an embodiment finds use, for example, in wound healing methods or treatment of ischmia-related diseases (without wishing to be bound by theory, mediated by VEGFR-2's effects on endothelial cell function and angiogenesis). In various embodiments, binding to VEGFR-1 (Flt-1), which is linked to cancers and pro-inflammatory activities, is avoided. In various embodiments, VEGFR-1 (Flt-1) acts a decoy receptor and therefore substantially reduces or ablates affinity at this receptor avoids sequestration of the therapeutic agent. In an embodiment, the modified signaling agent has substantially reduced or ablated affinity and/or activity for VEGFR-1 (Flt-1) and/or substantially reduced or ablated affinity and/or activity for VEGFR-2 (KDR/Flk-1). In some embodiments, the VEGF is VEGF-C or VEGF-D. In such embodiments, the

modified signaling agent has reduced affinity and/or activity for VEGFR-3. Alternatively, the modified signaling agent has substantially reduced or ablated affinity and/or activity for VEGFR-3.

Proangiogenic therapies are also important in various diseases (*e.g.* ischemic heart disease, bleeding *etc.*), and include VEGF-based therapeutics. Activation of VEGFR-2 is proangiogenic (acting on endothelial cells). Activation of VEFGR-1 can cause stimulation of migration of inflammatory cells (including, for example, macrophages) and lead to inflammation associated hypervascular permeability. Activation of VEFGR-1 can also promote bone marrow associated tumor niche formation. Thus, VEGF based therapeutic selective for VEGFR-2 activation would be desirable in this case. In addition, cell specific targeting, *e.g.* to endothelial cells, would be desirable.

In some embodiments, the additional modified signaling agent has reduced affinity and/or activity (*e.g.* antagonistic) for VEGFR-2 and/or has substantially reduced or ablated affinity and/or activity for VEGFR-1. When targeted to tumor vasculature endothelial cells via a targeting moiety that binds to a tumor endothelial cell marker (*e.g.* PSMA and others), such construct inhibits VEGFR-2 activation specifically on such marker-positive cells, while not activating VEGFR-1 *en route* and on target cells (if activity ablated), thus eliminating induction of inflammatory responses, for example. This would provide a more selective and safe anti-angiogenic therapy for many tumor types as compared to VEGF-A neutralizing therapies.

In some embodiments, the additional modified signaling agent has reduced affinity and/or activity (*e.g.* agonistic) for VEGFR-2 and/or has substantially reduced or ablated affinity and/or activity for VEGFR-1. Through targeting to vascular endothelial cells, such construct, in some embodiments, promotes angiogenesis without causing VEGFR-1 associated induction of inflammatory responses. Hence, such a construct would have targeted proangiogenic effects with substantially reduced risk of side effects caused by systemic activation of VEGFR-2 as well as VEGR-1.

In an illustrative embodiment, the modified signaling agent is $VEGF_{165}$, which has the amino acid sequence of SEQ ID NO:235.

In another illustrative embodiment, the additional modified signaling agent is $VEGF_{165b}$, which has the amino acid sequence of SEQ ID NO:236.

In these embodiments, the modified signaling agent has a mutation at amino acid I83 (*e.g.*, a substitution mutation at I83, *e.g.*, I83K, I83R, or I83H). Without wishing to be bound by theory, it is believed that such mutations may result in reduced receptor binding affinity. See, for example, U.S. Patent No. 9,078,860, the entire contents of which are hereby incorporated by reference.

In an embodiment, the additional modified signaling agent is TNF-α. TNF is a pleiotropic cytokine with many diverse functions, including regulation of cell growth, differentiation, apoptosis, tumorigenesis, viral replication, autoimmunity, immune cell functions and trafficking, inflammation, and septic shock. It binds to two distinct membrane receptors on target cells: TNFR1 (p55) and TNFR2 (p75). TNFR1 exhibits a very broad expression pattern whereas TNFR2 is expressed preferentially on certain populations of lymphocytes, Tregs, endothelial cells, certain neurons, microglia, cardiac myocytes and mesenchymal stem cells. Very distinct biological pathways are activated in response to receptor activation, although there is also some overlap. As a general rule, without wishing

to be bound by theory, TNFR1 signaling is associated with induction of apoptosis (cell death) and TNFR2 signaling is associated with activation of cell survival signals (*e.g.* activation of NFkB pathway). Administration of TNF is systemically toxic, and this is largely due to TNFR1 engagement. However, it should be noted that activation of TNFR2 is also associated with a broad range of activities and, as with TNFR1, in the context of developing TNF based therapeutics, control over TNF targeting and activity is important.

In some embodiments, the additional modified signaling agent has reduced affinity and/or activity for TNFR1 and/or TNFR2. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for TNFR1 and/or TNFR2. TNFR1 is expressed in most tissues, and is involved in cell death signaling while, by contrast, TNFR2 is involved in cell survival signaling. Accordingly, in embodiments directed to methods of treating cancer, the modified signaling agent has reduced affinity and/or activity for TNFR1 and/or substantially reduced or ablated affinity and/or activity for TNFR2. In these embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be targeted to a cell for which apoptosis is desired, *e.g.* a tumor cell or a tumor vasculature endothelial cell. In embodiments directed to methods of promoting cell survival, for example, in neurogenesis for the treatment of neurodegenerative disorders, the modified signaling agent has reduced affinity and/or activity for TNFR2 and/or substantially reduced or ablated affinity and/or activity for TNFR1. Stated another way, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex, in some embodiments, comprise modified TNF-α agent that allows of favoring either death or survival signals.

In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex has a modified TNF having reduced affinity and/or activity for TNFR1 and/or substantially reduced or ablated affinity and/or activity for TNFR2. Such a chimera, in some embodiments, is a more potent inducer of apoptosis as compared to a wild type TNF and/or a chimera bearing only mutation(s) causing reduced affinity and/or activity for TNFR1. Such a chimera, in some embodiments, finds use in inducing tumor cell death or a tumor vasculature endothelial cell death (*e.g.* in the treatment of cancers). Also, in some embodiments, these chimeras avoid or reduce activation of T_{reg} cells via TNFR2, for example, thus further supporting TNFR1-mediated antitumor activity *in vivo*.

In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex has a modified TNF having reduced affinity and/or activity for TNFR2 and/or substantially reduced or ablated affinity and/or activity for TNFR1. Such a chimera, in some embodiments, is a more potent activator of cell survival in some cell types, which may be a specific therapeutic objective in various disease settings, including without limitation, stimulation of neurogenesis. In addition, such a TNFR2-favoring chimeras also are useful in the treatment of autoimmune diseases (*e.g.* Crohn's, diabetes, MS, colitis *etc.* and many others described herein). In some embodiments, the chimera is targeted to auto-reactive T cells. In some embodiments, the chimera promotes T_{reg} cell activation and indirect suppression of cytotoxic T cells.

In some embodiments, the chimera causes the death of auto-reactive T cells, *e.g.* by activation of TNFR2 and/or avoidance of TNFR1 (*e.g.* a modified TNF having reduced affinity and/or activity for TNFR2 and/or substantially

reduced or ablated affinity and/or activity for TNFR1). Without wishing to be bound by theory these auto-reactive T cells, have their apoptosis/survival signals altered *e.g.* by NFkB pathway activity/signaling alterations.

In some embodiments, a TNFR2 based chimera has additional therapeutic applications in diseases, including various autoimmune diseases, heart disease, de-myelinating and neurodegenerative disorders, and infectious disease, among others.

In an embodiment, the wild type TNF-α has the amino acid sequence of SEQ ID NO:237.

In such embodiments, the modified TNF-α agent has mutations at one or more amino acid positions 29, 31, 32, 84, 85, 86, 87, 88, 89, 145, 146 and 147 which produces a modified TNF-α with reduced receptor binding affinity. *See*, for example, U.S. Patent No. 7,993,636, the entire contents of which are hereby incorporated by reference.

In some embodiments, the modified human TNF-α moiety has mutations at one or more amino acid positions R32, N34, Q67, H73, L75, T77, S86, Y87, V91, I97, T105, P106, A109, P113, Y115, E127, N137, D143, and A145, as described, for example, in WO/2015/007903, the entire contents of which is hereby incorporated by reference (numbering according to the human TNF sequence, Genbank accession number BAG70306, version BAG70306.1 GI: 197692685). In some embodiments, the modified human TNF-α moiety has substitution mutations selected from R32G, N34G, Q67G, H73G, L75G, L75A, L75S, T77A, S86G, Y87Q, Y87L, Y87A, Y87F, V91G, V91A, I97A, I97Q, I97S, T105G, P106G, A109Y, P113G, Y115G, Y115A, E127G, N137G, D143N, A145G and A145T. In an embodiment, the human TNF-α moiety has a mutation selected from Y87Q, Y87L, Y87A, and Y87F. In another embodiment, the human TNF-α moiety has a mutation selected from I97A, I97Q, and I97S. In a further embodiment, the human TNF-α moiety has a mutation selected from Y115A and Y115G.

In some embodiments, the modified TNF-α agent has one or more mutations selected from N39Y, S147Y, and Y87H, as described in WO2008/124086, the entire contents of which is hereby incorporated by reference.

In an embodiment, the additional modified signaling agent is TNF-β. TNF-β can form a homotrimer or a heterotrimer with LT-β (LT-α1β2). In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for TNFR1 and/or TNFR2 and/or herpes virus entry mediator (HEVM) and/or LT-βR.

In an embodiment, the wild type TNF-β has the amino acid sequence of SEQ ID NO:238.

In such embodiments, the modified TNF-β agent may comprise mutations at one or more amino acids at positions 106-113, which produce a modified TNF-β with reduced receptor binding affinity to TNFR2. In an embodiment, the modified signaling agent has one or more substitution mutations at amino acid positions 106-113. In illustrative embodiments, the substitution mutations are selected from Q107E, Q107D, S106E, S106D, Q107R, Q107N, Q107E/S106E, Q107E/S106D, Q107D/S106E, and Q107D/S106D. In another embodiment, the modified signaling agent has an insertion of about 1 to about 3 amino acids at positions 106-113.

In some embodiments, the additional modified agent is a TNF family member (*e.g.* TNF-alpha, TNF-beta) which can be a single chain trimeric version as described in WO 2015/007903, the entire contents of which are incorporated by reference.

In some embodiments, the modified agent is a TNF family member (*e.g.* TNF-alpha, TNF-beta) which has reduced affinity and/or activity, *i.e.* antagonistic activity (*e.g.* natural antagonistic activity or antagonistic activity that is the result of one or more mutations, *see, e.g.,* WO 2015/007520, the entire contents of which are hereby incorporated by reference) at TNFR1. In these embodiments, the modified agent is a TNF family member (*e.g.* TNF-alpha, TNF-beta) which also, optionally, has substantially reduced or ablated affinity and/or activity for TNFR2. In some embodiments, the modified agent is a TNF family member (*e.g.* TNF-alpha, TNF-beta) which has reduced affinity and/or activity, *i.e.* antagonistic activity (*e.g.* natural antagonistic activity or antagonistic activity that is the result of one or more mutations, *see, e.g.,* WO 2015/007520, the entire contents of which are hereby incorporated by reference) at TNFR2. In these embodiments, the modified agent is a TNF family member (*e.g.* TNF-alpha, TNF-beta) which also, optionally, has substantially reduced or ablated affinity and/or activity for TNFR1. The constructs of such embodiments find use in, for example, methods of dampening TNF response in a cell specific manner. In some embodiments, the antagonistic TNF family member (*e.g.* TNF-alpha, TNF-beta) is a single chain trimeric version as described in WO 2015/007903.

In an embodiment, the additional modified signaling agent is TRAIL. In some embodiments, the modified TRAIL agent has reduced affinity and/or activity for DR4 (TRAIL-RI) and/or DR5 (TRAIL-RII) and/or DcR1 and/or DcR2. In some embodiments, the modified TRAIL agent has substantially reduced or ablated affinity and/or activity for DR4 (TRAIL-RI) and/or DR5 (TRAIL-RII) and/or DcR1 and/or DcR2.

In an embodiment, the wild type TRAIL has the amino acid sequence of SEQ ID NO:239.

In such embodiments, the modified TRAIL agent may comprise a mutation at amino acid positions T127-R132, E144-R149, E155-H161, Y189-Y209, T214-1220, K224-A226, W231, E236-L239, E249-K251, T261-H264 and H270-E271 (Numbering based on the human sequence, Genbank accession number NP _003801, version 10 NP _003801.1, GI: 4507593; see above).

In an embodiment, the additional modified signaling agent is TGFα. In such embodiments, the modified TGFα agent has reduced affinity and/or activity for the epidermal growth factor receptor (EGFR). In some embodiments, the modified TGFα agent has substantially reduced or ablated affinity and/or activity for the epidermal growth factor receptor (EGFR).

In an embodiment, the additional modified signaling agent is TGFβ. In such embodiments, the modified signaling agent has reduced affinity and/or activity for TGFBR1 and/or TGFBR2. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for TGFBR1 and/or TGFBR2. In some embodiments, the modified signaling agent optionally has reduced or substantially reduced or ablated affinity and/or activity for TGFBR3 which, without wishing to be bound by theory, may act as a reservoir of ligand for TGF-beta receptors. In some embodiments, the TGFβ may favor TGFBR1 over TGFBR2 or TGFBR2 over TGFBR1. Similarly, LAP, without wishing to be bound by theory, may act as a reservoir of ligand for TGF-beta receptors. In some embodiments, the modified signaling agent has reduced affinity and/or activity for TGFBR1 and/or TGFBR2 and/or substantially reduced or ablated affinity and/or activity for Latency Associated Peptide (LAP). In some

embodiments, such chimeras find use in Camurati-Engelmann disease, or other diseases associated with inappropriate TGFβ signaling.

In some embodiments, the additional modified agent is a TGF family member (*e.g.* TGFα, TGFβ) which has reduced affinity and/or activity, *i.e.* antagonistic activity (*e.g.* natural antagonistic activity or antagonistic activity that is the result of one or more mutations, *see, e.g.,* WO 2015/007520, the entire contents of which are hereby incorporated by reference) at one or more of TGFBR1, TGFBR2, TGFBR3. In these embodiments, the modified agent is a TGF family member (*e.g.* TGFα, TGFβ) which also, optionally, has substantially reduced or ablated affinity and/or activity at one or more of TGFBR1, TGFBR2, TGFBR3.

In some embodiments, the additional modified agent is a TGF family member (*e.g.* TGFα, TGFβ) which has reduced affinity and/or activity, *i.e.* antagonistic activity (*e.g.* natural antagonistic activity or antagonistic activity that is the result of one or more mutations, *see, e.g.,* WO 2015/007520, the entire contents of which are hereby incorporated by reference) at TGFBR1 and/or TGFBR2. In these embodiments, the modified agent is a TGF family member (*e.g.* TGFα, TGFβ) which also, optionally, has substantially reduced or ablated affinity and/or activity at TGFBR3.

In an embodiment, the additional modified signaling agent is IL-1. In an embodiment, the modified signaling agent is IL-1α or IL-1β. In some embodiments, the modified signaling agent has reduced affinity and/or activity for IL-1R1 and/or IL-1RAcP. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for IL-1R1 and/or IL-1RAcP. In some embodiments, the modified signaling agent has reduced affinity and/or activity for IL-1R2. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for IL-1R2. For instance, in some embodiments, the present modified IL-1 agents avoid interaction at IL-1R2 and therefore substantially reduce its function as a decoy and/or sink for therapeutic agents.

In an embodiment, the wild type IL-1β has the amino acid sequence of SEQ ID NO:240.

IL-1β is a proinflammatory cytokine and an important immune system regulator. It is a potent activator of CD4 T cell responses, increases proportion of Th17 cells and expansion of IFNγ and IL-4 producing cells. IL-1β is also a potent regulator of $CD8^+$ T cells, enhancing antigen-specific $CD8^+$ T cell expansion, differentiation, migration to periphery and memory. IL-1β receptors comprise IL-1R1 and IL-1R2. Binding to and signaling through the IL-1R1 constitutes the mechanism whereby IL-1β mediates many of its biological (and pathological) activities. IL1-R2 can function as a decoy receptor, thereby reducing IL-1β availability for interaction and signaling through the IL-1R1.

In some embodiments, the modified IL-1β has reduced affinity and/or activity (*e.g.* agonistic activity) for IL-1R1. In some embodiments, the modified IL-1β has substantially reduced or ablated affinity and/or activity for IL-1R2. In such embodiments, there is inducible or restorable IL-1β / IL-1R1 signaling and prevention of loss of therapeutic chimeras at IL-R2 and therefore a reduction in dose of IL-1 that is required (*e.g.* relative to wild type or a chimera bearing only an attenuation mutation for IL-R1). Such constructs find use in, for example, methods of treating cancer, including, for example, stimulating the immune system to mount an anti-cancer response.

In some embodiments, the modified IL-1β has reduced affinity and/or activity (*e.g.* antagonistic activity, *e.g.* natural antagonistic activity or antagonistic activity that is the result of one or more mutations, *see, e.g.,* WO 2015/007520,

the entire contents of which are hereby incorporated by reference) for IL-1R1. In some embodiments, the modified IL-1β has substantially reduced or ablated affinity and/or activity for IL-1R2. In such embodiments, there is the IL-1β/IL-1R1 signaling is not inducible or restorable and prevention of loss of therapeutic chimeras at IL-R2 and therefore a reduction in dose of IL-1β that is required (*e.g.* relative to wild type or a chimera bearing only an attenuation mutation for IL-R1). Such constructs find use in, for example, methods of treating autoimmune diseases, including, for example, suppressing the immune system.

In such embodiments, the modified signaling agent has a deletion of amino acids 52-54 which produces a modified human IL-1β with reduced binding affinity for type I IL-1R and reduced biological activity. See, for example, WO 1994/000491, the entire contents of which are hereby incorporated by reference. In some embodiments, the modified human IL-1β has one or more substitution mutations selected from A117G/P118G, R120X, L122A, T125G/L126G, R127G, Q130X, Q131G, K132A, S137G/Q138Y, L145G, H146X, L145A/L147A, Q148X, Q148G/Q150G, Q150G/D151A, M152G, F162A, F162A/Q164E, F166A, Q164E/E167K, N169G/D170G, I172A, V174A, K208E, K209X, K209A/K210A, K219X, E221X, E221 S/N224A, N224S/K225S, E244K, N245Q (where X can be any change in amino acid, *e.g.*, a non-conservative change), which exhibit reduced binding to IL-1R, as described, for example, in WO2015/007542 and WO/2015/007536, the entire contents of which is hereby incorporated by reference (numbering base on the human IL-1 β sequence, Genbank accession number NP_000567, version NP-000567.1 , GI: 10835145). In some embodiments, the modified human IL-1β may have one or more mutations selected from R120A, R120G, Q130A, Q130W, H146A, H146G, H146E, H146N, H146R, Q148E, Q148G, Q148L, K209A, K209D, K219S, K219Q, E221S and E221K. In an embodiment, the modified human IL-1β comprises the mutations Q131G and Q148G. In an embodiment, the modified human IL-1β comprises the mutations Q148G and K208E. In an embodiment, the modified human IL-1β comprises the mutations R120G and Q131G. In an embodiment, the modified human IL-1β comprises the mutations R120G and H146G. In an embodiment, the modified human IL-1β comprises the mutations R120G and K208E. In an embodiment, the modified human IL-1β comprises the mutations R120G, F162A, and Q164E.

In an embodiment, the additional modified signaling agent is IL-2. In such an embodiment, the modified signaling agent has reduced affinity and/or activity for IL-2Rα and/or IL-2Rβ and/or IL-2Rγ. In some embodiments, the modified signaling agent has reduced affinity and/or activity for IL-2Rβ and/or IL-2Rγ. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for IL-2Rα. Such embodiments may be relevant for treatment of cancer, for instance when the modified IL-2 is agonistic at IL-2Rβ and/or IL-2Rγ. For instance, the present constructs may favor attenuated activation of $CD8^+$ T cells (which can provide an anti-tumor effect), which have IL2 receptors β and γ and disfavor T_{regs} (which can provide an immune suppressive, pro-tumor effect), which have IL2 receptors α, β, and γ. Further, in some embodiments, the preferences for IL-2Rβ and/or IL-2Rγ over IL-2Rα avoid IL-2 side effects such as pulmonary edema. Also, IL-2-based chimeras are useful for the treatment of autoimmune diseases, for instance when the modified IL-2 is antagonistic (*e.g.* natural antagonistic activity or antagonistic activity that is the result of one or more mutations, *see, e.g.,* WO 2015/007520, the entire contents of which are hereby incorporated by reference) at IL-2Rβ and/or IL-2Rγ. For instance, the

present constructs may favor attenuated suppression of $CD8^+$ T cells (and therefore dampen the immune response), which have IL2 receptors β and γ and disfavor T_{regs} which have IL2 receptors α, β, and γ. Alternatively, in some embodiments, the chimeras bearing IL-2 favor the activation of T_{regs}, and therefore immune suppression, and activation of disfavor of $CD8^+$ T cells. For instance, these constructs find use in the treatment of diseases or diseases that would benefit from immune suppression, *e.g.* autoimmune disorders.

In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex has targeting moieties as described herein directed to $CD8^+$ T cells as well as a modified IL-2 agent having reduced affinity and/or activity for IL-2Rβ and/or IL-2Rγ and/or substantially reduced or ablated affinity and/or activity for IL-2Rα. In some embodiments, these constructs provide targeted $CD8^+$ T cell activity and are generally inactive (or have substantially reduced activity) towards T_{reg} cells. In some embodiments, such constructs have enhanced immune stimulatory effect compared to wild type IL-2 (*e.g.*, without wishing to be bound by theory, by not stimulating Tregs), whilst eliminating or reducing the systemic toxicity associated with IL-2.

In an embodiment, the wild type IL-2 has the amino acid sequence of SEQ ID NO:241.

In such embodiments, the modified IL-2 agent has one or more mutations at amino acids L72 (L72G, L72A, L72S, L72T, L72Q, L72E, L72N, L72D, L72R, or L72K), F42 (F42A, F42G, F42S, F42T, F42Q, F42E, F42N, F42D, F42R, or F42K) and Y45 (Y45A, Y45G, Y45S, Y45T, Y45Q, Y45E, Y45N, Y45D, Y45R or Y45K). Without wishing to be bound by theory, it is believed that these modified IL-2 agents have reduced affinity for the high-affinity IL-2 receptor and preserves affinity to the intermediate-affinity IL-2 receptor, as compared to the wild-type IL-2. See, for example, US Patent Publication No. 2012/0244112, the entire contents of which are hereby incorporated by reference.

In an embodiment, the additional modified signaling agent is IL-3. In some embodiments, the modified signaling agent has reduced affinity and/or activity for the IL-3 receptor, which is a heterodimer with a unique alpha chain paired with the common beta (beta c or CD131) subunit. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for the IL-3 receptor, which is a heterodimer with a unique alpha chain paired with the common beta (beta c or CD131) subunit.

In an embodiment, the additional modified signaling agent is IL-4. In such an embodiment, the modified signaling agent has reduced affinity and/or activity for type 1 and/or type 2 IL-4 receptors. In such an embodiment, the modified signaling agent has substantially reduced or ablated affinity and/or activity for type 1 and/or type 2 IL-4 receptors. Type 1 IL-4 receptors are composed of the IL-4Rα subunit with a common γ chain and specifically bind IL-4. Type 2 IL-4 receptors include an IL-4Rα subunit bound to a different subunit known as IL-13Rα1. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity the type 2 IL-4 receptors.

In an embodiment, the wild type IL-4 has the amino acid sequence of SEQ ID NO:242.

In such embodiments, the modified IL-4 agent has one or more mutations at amino acids R121 (R121A, R121D, R121E, R121F, R121H, R121I, R121K, R121N, R121P, R121T, R121W), E122 (E122F), Y124 (Y124A, Y124Q, Y124R, Y124S, Y124T) and S125 (S125A). Without wishing to be bound by theory, it is believed that these modified IL-4 agents maintain the activity mediated by the type I receptor, but significantly reduces the biological activity

mediated by the other receptors. See, for example, US Patent No. 6,433,157, the entire contents of which are hereby incorporated by reference.

In an embodiment, the additional modified signaling agent is IL-6. IL-6 signals through a cell-surface type I cytokine receptor complex including the ligand-binding IL-6R chain (CD126), and the signal-transducing component gp130. IL-6 may also bind to a soluble form of IL-6R (sIL-6R), which is the extracellular portion of IL-6R. The sIL-6R/IL-6 complex may be involved in neurites outgrowth and survival of neurons and, hence, may be important in nerve regeneration through remyelination. Accordingly, in some embodiments, the modified signaling agent has reduced affinity and/or activity for IL-6R/gp130 and/or sIL-6R. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for IL-6R/gp130 and/or sIL-6R.

In an embodiment, the wild type IL-6 has the amino acid sequence of SEQ ID NO:243.

In such embodiments, the modified signaling agent has one or more mutations at amino acids 58, 160, 163, 171 or 177. Without wishing to be bound by theory, it is believed that these modified IL-6 agents exhibit reduced binding affinity to IL-6Ralpha and reduced biological activity. See, for example, WO 97/10338, the entire contents of which are hereby incorporated by reference.

In an embodiment, the additional modified signaling agent is IL-10. In such an embodiment, the modified signaling agent has reduced affinity and/or activity for IL-10 receptor-1 and IL-10 receptor-2. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for IL-10 receptor-1 and IL-10 receptor-2

In an embodiment, the additional modified signaling agent is IL-11. In such an embodiment, the modified signaling agent has reduced affinity and/or activity for IL-11Rα and/or IL-11Rβ and/or gp130. In such an embodiment, the modified signaling agent has substantially reduced or ablated affinity and/or activity for IL-11Rα and/or IL-11Rβ and/or gp130.

In an embodiment, the additional modified signaling agent is IL-12. In such an embodiment, the modified signaling agent has reduced affinity and/or activity for IL-12Rβ1 and/or IL-12Rβ2. In such an embodiment, the modified signaling agent has substantially reduced or ablated affinity and/or activity for IL-12Rβ1 and/or IL-12Rβ2.

In an embodiment, the additional modified signaling agent is IL-13. In such an embodiment, the modified signaling agent has reduced affinity and/or activity for the IL-4 receptor (IL-4Rα) and IL-13Rα1. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for IL-4 receptor (IL-4Rα) or IL-13Rα1.

In an embodiment, the wild type IL-13 has the amino acid sequence of SEQ ID NO:244.

In such embodiments, the modified IL-13 agent has one or more mutations at amino acids 13, 16, 17, 66, 69, 99, 102, 104, 105, 106, 107, 108, 109, 112, 113 and 114. Without wishing to be bound by theory, it is believed that these modified IL-13 agents exhibit reduced biological activity. See, for example, WO 2002/018422, the entire contents of which are hereby incorporated by reference.

In an embodiment, the signaling agent is a wild type or modified IL-15. In embodiments, the modified IL-15 has reduced affinity and/or activity for interleukin 15 receptor.

In an embodiment, the wild type IL-15 has the amino acid sequence of:

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISHESGDTDIHDTVENLIILANNILSSNGN ITESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 292).

In such embodiments, the modified IL-15 agent has one or more mutations at amino acids S7, D8, K10, K11, E46, L47, V49, I50, D61, N65, L66, I67, I68, L69, N72, Q108 with respect to SEQ ID NO: 292.

In an embodiment, the additional modified signaling agent is IL-18. In some embodiments, the modified signaling agent has reduced affinity and/or activity for IL-18Rα and/or IL-18Rβ. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for IL-18Rα and/or IL-18Rβ. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for IL-18Rα type II, which is an isoform of IL-18Rα that lacks the TIR domain required for signaling.

In an embodiment, the wild type IL-18 has the amino acid sequence of SEQ ID NO:245.

In such embodiments, the modified IL-18 agent may comprise one or more mutations in amino acids or amino acid regions selected from Y37-K44, R49-Q54, D59-R63, E67-C74, R80, M87-A97, N 127-K129, Q139-M149, K165-K171, R183 and Q190-N191, as described in WO/2015/007542, the entire contents of which are hereby incorporated by reference (numbering based on the human IL-18 sequence, Genbank accession number AAV38697, version AAV38697.1, GI: 54696650).

In an embodiment, the additional modified signaling agent is IL-33. In such an embodiment, the modified signaling agent has reduced affinity and/or activity for the ST-2 receptor and IL-1RAcP. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for the ST-2 receptor and IL-1RAcP.

In an embodiment, the wild type IL-33 has the amino acid sequence of SEQ ID NO:246.

In such embodiments, the modified IL-33 agent may comprise one or more mutations in amino acids or amino acid regions selected from I113-Y122, S127-E139, E144-D157, Y163-M183, E200, Q215, L220-C227 and T260-E269, as described in WO/2015/007542, the entire contents of which are hereby incorporated by reference (numbering based on the human sequence, Genbank accession number NP_254274, version NP_254274.1, GI:15559209).

In an embodiment, the modified signaling agent is epidermal growth factor (EGF). EGF is a member of a family of potent growth factors. Members include EGF, HB-EGF, and others such as TGFalpha, amphiregulin, neuregulins, epiregulin, betacellulin. EGF family receptors include EGFR (ErbB1), ErbB2, ErbB3 and ErbB4. These may function as homodimeric and /or heterodimeric receptor subtypes. The different EGF family members exhibit differential selectivity for the various receptor subtypes. For example, EGF associates with ErbB1/ErbB1, ErbB1/ErbB2, ErbB4/ErbB2 and some other heterodimeric subtypes. HB-EGF has a similar pattern, although it also associates with ErbB4/4. Modulation of EGF (EGF-like) growth factor signaling, positively or negatively, is of considerable therapeutic interest. For example, inhibition of EGFRs signaling is of interest in the treatment of various cancers where EGFR signaling constitutes a major growth promoting signal. Alternatively, stimulation of

EGFRs signaling is of therapeutic interest in, for example, promoting wound healing (acute and chronic), oral mucositis (a major side-effect of various cancer therapies, including, without limitation radiation therapy).

In some embodiments, the additional modified signaling agent has reduced affinity and/or activity for ErbB1, ErbB2, ErbB3, and/or ErbB4. Such embodiments find use, for example, in methods of treating wounds. In some embodiments, the modified signaling agent binds to one or more ErbB1, ErbB2, ErbB3, and ErbB4 and antagonizes the activity of the receptor. In such embodiments, the modified signaling agent has reduced affinity and/or activity for ErbB1, ErbB2, ErbB3, and/or ErbB4 which allows for the activity of the receptor to be antagonized in an attenuated fashion. Such embodiments find use in, for example, treatments of cancer. In an embodiment, the modified signaling agent has reduced affinity and/or activity for ErbB1. ErbB1 is the therapeutic target of kinase inhibitors -most have side effects because they are not very selective (*e.g.*, gefitinib, erlotinib, afatinib, brigatinib and icotinib). In some embodiments, attenuated antagonistic ErbB1 signaling is more on-target and has less side effects than other agents targeting receptors for EGF.

In some embodiments, the additional modified signaling agent has reduced affinity and/or activity (*e.g.* antagonistic *e.g.* natural antagonistic activity or antagonistic activity that is the result of one or more mutations, *see, e.g.,* WO 2015/007520, the entire contents of which are hereby incorporated by reference) for ErbB1 and/or substantially reduced or ablated affinity and/or activity for ErbB4 or other subtypes it may interact with. Through specific targeting via the targeting moiety, cell-selective suppression (antagonism *e.g.* natural antagonistic activity or antagonistic activity that is the result of one or more mutations, *see, e.g.,* WO 2015/007520, the entire contents of which are hereby incorporated by reference) of ErbB1/ErbB1 receptor activation would be achieved – while not engaging other receptor subtypes potentially associated with inhibition-associated side effects. Hence, in contrast to EGFR kinase inhibitors, which inhibit EGFR activity in all cell types in the body, such a construct would provide a cell-selective (*e.g.*, tumor cell with activated EGFR signaling due to amplification of receptor, overexpression *etc.*) anti-EGFR (ErbB1) drug effect with reduced side effects.

In some embodiments, the additional modified signaling agent has reduced affinity and/or activity (*e.g.* agonistic) for ErbB4 and/or other subtypes it may interact with. Through targeting to specific target cells through the targeting moiety, a selective activation of ErbB1 signaling is achieved (*e.g.* epithelial cells). Such a construct finds use, in some embodiments, in the treatment of wounds (promoting would healing) with reduced side effects, especially for treatment of chronic conditions and application other than topical application of a therapeutic (*e.g.* systemic wound healing).

In an embodiment, the modified signaling agent is insulin or insulin analogs. In some embodiments, the modified insulin or insulin analog has reduced affinity and/or activity for the insulin receptor and/or IGF1 or IGF2 receptor. In some embodiments, the modified insulin or insulin analog has substantially reduced or ablated affinity and/or activity for the insulin receptor and/or IGF1 or IGF2 receptor. Attenuated response at the insulin receptor allows for the control of diabetes, obesity, metabolic disorders and the like while directing away from IGF1 or IGF2 receptor avoids pro-cancer effects.

In an embodiment, the modified signaling agent is insulin-like growth factor-I or insulin-like growth factor-II (IGF-1 or IGF-2). In an embodiment, the modified signaling agent is IGF-1. In such an embodiment, the modified signaling agent has reduced affinity and/or activity for the insulin receptor and/or IGF1 receptor. In an embodiment, the modified signaling agent may bind to the IGF1 receptor and antagonize the activity of the receptor. In such an embodiment, the modified signaling agent has reduced affinity and/or activity for IGF1 receptor which allows for the activity of the receptor to be antagonized in an attenuated fashion. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for the insulin receptor and/or IGF1 receptor. In some embodiments, the modified signaling agent has reduced affinity and/or activity for IGF2 receptor which allows for the activity of the receptor to be antagonized in an attenuated fashion. In an embodiment, the modified signaling agent has substantially reduced or ablated affinity and/or activity for the insulin receptor and accordingly does not interfere with insulin signaling. In various embodiments, this applies to cancer treatment. In various embodiments, the present agents may prevent IR isoform A from causing resistance to cancer treatments.

In an embodiment, the modified signaling agent is EPO. In various embodiments, the modified EPO agent has reduced affinity and/or activity for the EPO receptor (EPOR) receptor and/or the ephrin receptor (EphR) relative to wild type EPO or other EPO based agents described herein. In some embodiments, the modified EPO agent has substantially reduced or ablated affinity and/or activity for the EPO receptor (EPOR) receptor and/or the Eph receptor (EphR). Illustrative EPO receptors include, but are not limited to, an EPOR homodimer or an EPOR/CD131 heterodimer. Also included as an EPO receptor is beta-common receptor (βcR). Illustrative Eph receptors include, but are not limited to, EPHA1, EPHA2, EPHA3, EPHA4, EPHA5, EPHA6, EPHA7, EPHA8, EPHA9, EPHA10, EPHB1, EPHB2, EPHB3, EPHB4, EPHB5, and EPHB6. In some embodiments, the modified EPO protein comprises one or more mutations that cause the EPO protein to have reduced affinity for receptors that comprise one or more different EPO receptors or Eph receptors (*e.g.* heterodimer, heterotrimers, *etc.*, including by way of non-limitation: EPOR-EPHB4, EPOR-βcR-EPOR). Also provided are the receptors of EP Patent Publication No. 2492355 the entire contents of which are hereby incorporated by reference, including by way of non-limitation, NEPORs.

In an embodiment, the human EPO has the amino acid sequence of SEQ ID NO:247 (first 27 amino acids are the signal peptide).

In an embodiment, the human EPO protein is the mature form of EPO (with the signal peptide being cleaved off) which is a glycoprotein of 166 amino acid residues having the sequence of SEQ ID NO:248.

The structure of the human EPO protein is predicted to comprise four-helix bundles including helices A, B, C, and D. In various embodiments, the modified EPO protein comprises one or more mutations located in four regions of the EPO protein which are important for bioactivity, *i.e.*, amino acid residues 10-20, 44-51, 96-108, and 142-156. In some embodiments, the one or more mutations are located at residues 11-15, 44-51, 100-108, and 147-151. These residues are localized to helix A (Val11, Arg14, and Tyr15), helix C (Ser100, Arg103, Ser104, and Leu108), helix D (Asn147, Arg150, Gly151, and Leu155), and the A/B connecting loop (residues 42-51). In some embodiments, the modified EPO protein comprises mutations in residues between amino acids 41-52 and amino

acids 147, 150, 151, and 155. Without wishing to be bound by theory, it is believed that mutations of these residues have substantial effects on both receptor binding and in vitro biological activity. In some embodiments, the modified EPO protein comprises mutations at residues 11, 14, 15, 100, 103, 104, and 108. Without wishing to be bound by theory, it is believed that mutations of these residues have modest effects on receptor binding activity and much greater effects on in vitro biological activity. Illustrative substitutions include, but are not limited to, one or more of Val11Ser, Arg14Ala, Arg14Gln, Tyr15Ile, Pro42Asn, Thr44Ile, Lys45Asp, Val46Ala, Tyr51Phe, Ser100Glu, Ser100Thr, Arg103Ala, Ser104Ile, Ser104Ala, Leu108Lys, Asn147Lys, Arg150Ala, Gly151Ala, and Leu155Ala.

In some embodiments, the modified EPO protein comprises mutations that effect bioactivity and not binding, *e.g.* those listed in Eliot, *et al.* Mapping of the Active Site of Recombinant Human Erythropoietin January 15, 1997; *Blood*: 89 (2), the entire contents of which are hereby incorporated by reference.

In some embodiments, the modified EPO protein comprises one or more mutations involving surface residues of the EPO protein which are involved in receptor contact. Without wishing to be bound by theory, it is believed that mutations of these surface residues are less likely to affect protein folding thereby retaining some biological activity. Illustrative surface residues that may be mutated include, but are not limited to, residues 147 and 150. In illustrative embodiments, the mutations are substitutions including, one or more of N147A, N147K, R150A and R150E.

In some embodiments, the modified EPO protein comprises one or more mutations at residues N59, E62, L67, and L70, and one or more mutations that affect disulfide bond formation. Without wishing to be bound by theory, it is believed that these mutations affect folding and/or are predicted be in buried positions and thus affects biological activity indirectly.

In an embodiment, the modified EPO protein comprises a K20E substitution which significantly reduces receptor binding. *See* Elliott, *et al.,* (1997) *Blood,* 89:493-502, the entire contents of which are hereby incorporated by reference.

Additional EPO mutations that may be incorporated into the chimeric EPO protein of the invention are disclosed in, for example, Elliott, *et al.,* (1997) *Blood,* 89:493-502, the entire contents of which are hereby incorporated by reference and Taylor *et al*., (2010) *PEDS*, 23(4): 251-260, the entire contents of which are hereby incorporated by reference.

In various embodiments, the signaling agent is a toxin or toxic enzyme. In some embodiments, the toxin or toxic enzyme is derived from plants and bacteria. Illustrative toxins or toxic enzymes include, but are not limited to, the diphtheria toxin, Pseudomonas toxin, anthrax toxin, ribosome-inactivating proteins (RIPs) such as ricin and saporin, modeccin, abrin, gelonin, and poke weed antiviral protein. Additional toxins include those disclosed in Mathew *et al.*, (2009) Cancer Sci 100(8): 1359-65, the entire disclosures are hereby incorporated by reference. In such embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention may be utilized to induce cell death in cell-type specific manner. In such embodiments, the toxin may be modified, *e.g.* mutated, to reduce affinity and/or activity of the toxin for an attenuated effect, as described with other signaling agents herein.

Linkers and Functional Groups

In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex optionally comprises one or more linkers. In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises a linker connecting the targeting moiety and the signaling agent (*e.g.*, IFNα1 or a variant thereof). In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises a linker within the signaling agent (*e.g.*, IFNα1 or a variant thereof). In some embodiments, the linker may be utilized to link various functional groups, residues, or moieties as described herein to the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex. In some embodiments, the linker is a single amino acid or a plurality of amino acids that does not affect or reduce the stability, orientation, binding, neutralization, and/or clearance characteristics of the binding regions and the binding protein. In various embodiments, the linker is selected from a peptide, a protein, a sugar, or a nucleic acid.

In some embodiments vectors encoding the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex linked as a single nucleotide sequence to any of the linkers described herein are provided and may be used to prepare such chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex. In embodiments, the substituents of the Fc-based chimeric protein complex are expressed as nucleotide sequences in a vector.

In some embodiments, the linker length allows for efficient binding of a targeting moiety and the signaling agent (*e.g.*, IFNα1 or a variant thereof) to their receptors. For instance, in some embodiments, the linker length allows for efficient binding of one of the targeting moieties and the signaling agent to receptors on the same cell.

In some embodiments the linker length is at least equal to the minimum distance between the binding sites of one of the targeting moieties and the signaling agent to receptors on the same cell. In some embodiments the linker length is at least twice, or three times, or four times, or five times, or ten times, or twenty times, or 25 times, or 50 times, or one hundred times, or more the minimum distance between the binding sites of one of the targeting moieties and the signaling agent to receptors on the same cell.

As described herein, the linker length allows for efficient binding of one of the targeting moieties and the signaling agent to receptors on the same cell, the binding being sequential, *e.g.* targeting moiety/receptor binding preceding signaling agent/receptor binding.

In some embodiments, there are two linkers in a single chimera, each connecting the signaling agent to a targeting moiety. In various embodiments, the linkers have lengths that allow for the formation of a site that has a disease cell and an effector cell without steric hindrance that would prevent modulation of the either cell.

The invention contemplates the use of a variety of linker sequences. In various embodiments, the linker may be derived from naturally-occurring multi-domain proteins or are empirical linkers as described, for example, in Chichili *et al.*, (2013), Protein Sci. 22(2):153-167, Chen *et al.*, (2013), Adv Drug Deliv Rev. 65(10):1357-1369, the entire contents of which are hereby incorporated by reference. In some embodiments, the linker may be designed using linker designing databases and computer programs such as those described in Chen *et al.*, (2013), Adv Drug Deliv Rev. 65(10):1357-1369 and Crasto *et al.*, (2000), Protein Eng. 13(5):309-312, the entire contents of which are

hereby incorporated by reference. In various embodiments, the linker may be functional. For example, without limitation, the linker may function to improve the folding and/or stability, improve the expression, improve the pharmacokinetics, and/or improve the bioactivity of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex.

In some embodiments, the linker is a polypeptide. In some embodiments, the linker is less than about 100 amino acids long. For example, the linker may be less than about 100, about 95, about 90, about 85, about 80, about 75, about 70, about 65, about 60, about 55, about 50, about 45, about 40, about 35, about 30, about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 12, about 11, about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, or about 2 amino acids long. In some embodiments, the linker is a polypeptide. In some embodiments, the linker is greater than about 100 amino acids long. For example, the linker may be greater than about 100, about 95, about 90, about 85, about 80, about 75, about 70, about 65, about 60, about 55, about 50, about 45, about 40, about 35, about 30, about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 12, about 11, about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, or about 2 amino acids long. In some embodiments, the linker is flexible. In another embodiment, the linker is rigid.

In some embodiments directed to chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes having two or more targeting moieties, a linker connects the two targeting moieties to each other and this linker has a short length and a linker connects a targeting moiety and a signaling agent this linker is longer than the linker connecting the two targeting moieties. For example, the difference in amino acid length between the linker connecting the two targeting moieties and the linker connecting a targeting moiety and a signaling agent may be about 100, about 95, about 90, about 85, about 80, about 75, about 70, about 65, about 60, about 55, about 50, about 45, about 40, about 35, about 30, about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 12, about 11, about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, or about 2 amino acids.

In various embodiments, the linker is substantially comprised of glycine and serine residues (*e.g.* about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 95%, or about 97% glycines and serines). For example, in some embodiments, the linker is $(Gly_4Ser)_n$, where n is from about 1 to about 8, *e.g.* 1, 2, 3, 4, 5, 6, 7, or 8 (SEQ ID NO:249 -SEQ ID NO:256, respectively). In an embodiment, the linker sequence is GGSGGSGGGGSGGGGS (SEQ ID NO:257). Additional illustrative linkers include, but are not limited to, linkers having the sequence LE, GGGGS (SEQ ID NO:249), $(GGGGS)_n$ (n=1-4) (SEQ ID NO:249 -SEQ ID NO:252), $(Gly)_8$ (SEQ ID NO:258), $(Gly)_6$ (SEQ ID NO:259), $(EAAAK)_n$ (n=1-3) (SEQ ID NO:260 -SEQ ID NO:262), $A(EAAAK)_nA$ (n = 2-5) (SEQ ID NO:263 – SEQ ID NO:266), AEAAAKEAAAKA (SEQ ID NO:263), $A(EAAAK)_4ALEA(EAAAK)_4A$ (SEQ ID NO:267), PAPAP (SEQ ID NO:268), KESGSVSSEQLAQFRSLD (SEQ ID NO:269), EGKSSGSGSESKST (SEQ ID NO:270), GSAGSAAGSGEF (SEQ ID NO:271), and $(XP)_n$, with X designating any amino acid, *e.g.*, Ala, Lys, or Glu. In various embodiments, the linker is GGS.

In some embodiments, the linker is one or more of GGGSE (SEQ ID NO: 272), GSESG (SEQ ID NO: 273), GSEGS (SEQ ID NO: 274), GEGGSGEGSSGEGSSSEGGGSEGGGSEGGGSEGGS (SEQ ID NO: 275), and a linker of randomly placed G, S, and E every 4 amino acid intervals.

In some embodiments, the linker is a hinge region of an antibody (*e.g.*, of IgG, IgA, IgD, and IgE, inclusive of subclasses (*e.g.* IgG1, IgG2, IgG3, and IgG4, and IgA1 and IgA2)). In various embodiments, the linker is a hinge region of an antibody (*e.g.*, of IgG, IgA, IgD, and IgE, inclusive of subclasses (*e.g.* IgG1, IgG2, IgG3, and IgG4, and IgA1 and IgA2)). The hinge region, found in IgG, IgA, IgD, and IgE class antibodies, acts as a flexible spacer, allowing the Fab portion to move freely in space. In contrast to the constant regions, the hinge domains are structurally diverse, varying in both sequence and length among immunoglobulin classes and subclasses. For example, the length and flexibility of the hinge region varies among the IgG subclasses. The hinge region of IgG1 encompasses amino acids 216-231 and, because it is freely flexible, the Fab fragments can rotate about their axes of symmetry and move within a sphere centered at the first of two inter-heavy chain disulfide bridges. IgG2 has a shorter hinge than IgG1, with 12 amino acid residues and four disulfide bridges. The hinge region of IgG2 lacks a glycine residue, is relatively short, and contains a rigid poly-proline double helix, stabilized by extra inter-heavy chain disulfide bridges. These properties restrict the flexibility of the IgG2 molecule. IgG3 differs from the other subclasses by its unique extended hinge region (about four times as long as the IgG1 hinge), containing 62 amino acids (including 21 prolines and 11 cysteines), forming an inflexible poly-proline double helix. In IgG3, the Fab fragments are relatively far away from the Fc fragment, giving the molecule a greater flexibility. The elongated hinge in IgG3 is also responsible for its higher molecular weight compared to the other subclasses. The hinge region of IgG4 is shorter than that of IgG1 and its flexibility is intermediate between that of IgG1 and IgG2. The flexibility of the hinge regions reportedly decreases in the order IgG3>IgG1>IgG4>IgG2.

According to crystallographic studies, the immunoglobulin hinge region can be further subdivided functionally into three regions: the upper hinge region, the core region, and the lower hinge region. *See* Shin *et al.*, 1992 *Immunological Reviews* 130:87. The upper hinge region includes amino acids from the carboxyl end of C_{H1} to the first residue in the hinge that restricts motion, generally the first cysteine residue that forms an interchain disulfide bond between the two heavy chains. The length of the upper hinge region correlates with the segmental flexibility of the antibody. The core hinge region contains the inter-heavy chain disulfide bridges, and the lower hinge region joins the amino terminal end of the C_{H2} domain and includes residues in C_{H2}. *Id.* The core hinge region of wild-type human IgG1 contains the sequence Cys-Pro-Pro-Cys (SEQ ID NO: 276), which when dimerized by disulfide bond formation, results in a cyclic octapeptide believed to act as a pivot, thus conferring flexibility. In various embodiments, the present linker comprises, one, or two, or three of the upper hinge region, the core region, and the lower hinge region of any antibody (*e.g.*, of IgG, IgA, IgD, and IgE, inclusive of subclasses (*e.g.* IgG1, IgG2, IgG3, and IgG4, and IgA1 and IgA2)). The hinge region may also contain one or more glycosylation sites, which include a number of structurally distinct types of sites for carbohydrate attachment. For example, IgA1 contains five glycosylation sites within a 17-amino-acid segment of the hinge region, conferring resistance of the hinge region polypeptide to intestinal proteases, considered an advantageous property for a secretory immunoglobulin.

In various embodiments, the linker of the present invention comprises one or more glycosylation sites. In various embodiments, the linker is a hinge-CH2-CH3 domain of a human IgG4 antibody.

In some embodiments, the linker is a synthetic linker such as PEG.

In various embodiments, the linker may be functional. For example, without limitation, the linker may function to improve the folding and/or stability, improve the expression, improve the pharmacokinetics, and/or improve the bioactivity of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex. In another example, the linker may function to target the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex to a particular cell type or location.

In various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may include one or more functional groups, residues, or moieties. In various embodiments, the one or more functional groups, residues, or moieties are attached or genetically fused to any of the signaling agents or targeting moieties described herein. In some embodiments, such functional groups, residues or moieties confer one or more desired properties or functionalities to the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention. Examples of such functional groups and of techniques for introducing them into the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex are known in the art, for example, see *Remington's Pharmaceutical Sciences,* 16th ed., Mack Publishing Co., Easton, Pa. (1980).

In various embodiments, each of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may by conjugated and/or fused with another agent to extend half-life or otherwise improve pharmacodynamic and pharmacokinetic properties. In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be fused or conjugated with one or more of PEG, XTEN (*e.g.,* as rPEG), polysialic acid (POLYXEN), albumin (*e.g.,* human serum albumin or HAS), elastin-like protein (ELP), PAS, HAP, GLK, CTP, transferrin, and the like. In various embodiments, each of the individual chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is fused to one or more of the agents described in BioDrugs (2015) 29:215–239, the entire contents of which are hereby incorporated by reference.

In some embodiments, the functional groups, residues, or moieties comprise a suitable pharmacologically acceptable polymer, such as poly(ethyleneglycol) (PEG) or derivatives thereof (such as methoxypoly(ethyleneglycol) or mPEG). In some embodiments, attachment of the PEG moiety increases the half-life and/or reduces the immunogenecity of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex. Generally, any suitable form of pegylation can be used, such as the pegylation used in the art for antibodies and antibody fragments (including but not limited to single domain antibodies such as VHHs); see, for example, Chapman, *Nat. Biotechnol.,* 54, 531-545 (2002); by Veronese and Harris, *Adv. Drug Deliv. Rev.* 54, 453-456 (2003), by Harris and Chess, *Nat. Rev. Drug. Discov.,* 2, (2003) and in WO04060965, the entire contents of which are hereby incorporated by reference. Various reagents for pegylation of proteins are also commercially available, for example, from Nektar Therapeutics, USA. In some embodiments, site-directed

pegylation is used, in particular via a cysteine-residue (see, for example, Yang *et al.*, Protein Engineering, 16, 10, 761-770 (2003), the entire contents of which is hereby incorporated by reference). In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention is modified so as to suitably introduce one or more cysteine residues for attachment of PEG, or an amino acid sequence comprising one or more cysteine residues for attachment of PEG may be fused to the amino-and/or carboxy-terminus of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex, using techniques known in the art.

In some embodiments, the functional groups, residues, or moieties comprise N-linked or O-linked glycosylation. In some embodiments, the N-linked or O-linked glycosylation is introduced as part of a co-translational and/or post-translational modification.

In some embodiments, the functional groups, residues, or moieties comprise one or more detectable labels or other signal-generating groups or moieties. Suitable labels and techniques for attaching, using and detecting them are known in the art and, include, but are not limited to, fluorescent labels (such as fluorescein, isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, and fluorescamine and fluorescent metals such as Eu or others metals from the lanthanide series), phosphorescent labels, chemiluminescent labels or bioluminescent labels (such as luminal, isoluminol, theromatic acridinium ester, imidazole, acridinium salts, oxalate ester, dioxetane or GFP and its analogs), radio-isotopes, metals, metals chelates or metallic cations or other metals or metallic cations that are particularly suited for use in *in vivo*, *in vitro* or *in situ* diagnosis and imaging, as well as chromophores and enzymes (such as malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, biotinavidin peroxidase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-VI-phosphate dehydrogenase, glucoamylase and acetylcholine esterase). Other suitable labels include moieties that can be detected using NMR or ESR spectroscopy. Such labeled VHHs and polypeptides of the invention may, for example, be used for in vitro, in vivo or in situ assays (including immunoassays known per se such as ELISA, RIA, EIA and other "sandwich assays," *etc.*) as well as in vivo diagnostic and imaging purposes, depending on the choice of the specific label.

In some embodiments, the functional groups, residues, or moieties comprise a tag that is attached or genetically fused to the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex. In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may include a single tag or multiple tags. The tag for example is a peptide, sugar, or DNA molecule that does not inhibit or prevent binding of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex to its target or any other antigen of interest such as tumor antigens. In various embodiments, the tag is at least about: three to five amino acids long, five to eight amino acids long, eight to twelve amino acids long, twelve to fifteen amino acids long, or fifteen to twenty amino acids long. Illustrative tags are described for example, in U.S. Patent Publication No. US2013/0058962. In some embodiment, the tag is an affinity tag such as glutathione-S-

transferase (GST) and histidine (His) tag. In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises a His tag.

In some embodiments, the functional groups, residues, or moieties comprise a chelating group, for example, to chelate one of the metals or metallic cations. Suitable chelating groups, for example, include, without limitation, diethyl-enetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

In some embodiments, the functional groups, residues, or moieties comprise a functional group that is one part of a specific binding pair, such as the biotin-(strept)avidin binding pair. Such a functional group may be used to link the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention to another protein, polypeptide or chemical compound that is bound to the other half of the binding pair, *i.e.*, through formation of the binding pair. For example, a chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention may be conjugated to biotin, and linked to another protein, polypeptide, compound or carrier conjugated to avidin or streptavidin. For example, such a conjugated chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be used as a reporter, for example, in a diagnostic system where a detectable signal-producing agent is conjugated to avidin or streptavidin. Such binding pairs may, for example, also be used to bind the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex to a carrier, including carriers suitable for pharmaceutical purposes. One non-limiting example are the liposomal formulations described by Cao and Suresh, *Journal of Drug Targeting,* 8, 4, 257 (2000). Such binding pairs may also be used to link a therapeutically active agent to the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention.

Production of Chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein Complex

Methods for producing the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention are described herein. For example, DNA sequences encoding the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention (*e.g.*, DNA sequences encoding the signaling agent (*e.g.*, IFNα1 or a variant thereof) and the targeting moiety and the linker) can be chemically synthesized using methods known in the art. Synthetic DNA sequences can be ligated to other appropriate nucleotide sequences, including, *e.g.*, expression control sequences, to produce gene expression constructs encoding the desired chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex. Accordingly, in various embodiments, the present invention provides for isolated nucleic acids comprising a nucleotide sequence encoding the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention.

Nucleic acids encoding the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention can be incorporated (ligated) into expression vectors, which can be introduced into host cells through transfection, transformation, or transduction techniques. For example, nucleic acids encoding the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention can be introduced into host cells by retroviral transduction. Illustrative host cells are *E. coli* cells, Chinese hamster ovary (CHO) cells, human embryonic kidney 293 (HEK 293) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey

kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), and myeloma cells. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention. Accordingly, in various embodiments, the present invention provides expression vectors comprising nucleic acids that encode the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention. In various embodiments, the present invention additional provides host cells comprising such expression vectors.

Specific expression and purification conditions will vary depending upon the expression system employed. For example, if a gene is to be expressed in *E. coli*, it is first cloned into an expression vector by positioning the engineered gene downstream from a suitable bacterial promoter, *e.g.*, Trp or Tac, and a prokaryotic signal sequence. In another example, if the engineered gene is to be expressed in eukaryotic host cells, *e.g.*, CHO cells, it is first inserted into an expression vector containing for example, a suitable eukaryotic promoter, a secretion signal, enhancers, and various introns. The gene construct can be introduced into the host cells using transfection, transformation, or transduction techniques.

The chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention can be produced by growing a host cell transfected with an expression vector encoding the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex under conditions that permit expression of the protein. Following expression, the protein can be harvested and purified using techniques well known in the art, *e.g.*, affinity tags such as glutathione-S-transferase (GST) and histidine tags or by chromatography.

Accordingly, in various embodiments, the present invention provides for a nucleic acid encoding a chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the present invention. In various embodiments, the present invention provides for a host cell comprising a nucleic acid encoding a chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the present invention.

In various embodiments, IFNα1, its variant, or a chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprising the IFNα1 or its variant may be expressed *in vivo*, for instance, in a patient. For example, in various embodiments, the IFNα1, its variant, or a chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprising the IFNα1 or its variant may administered in the form of nucleic acid which encodes for the IFNα1 or its variant or chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprising IFNα1 or its variant. In various embodiments, the nucleic acid is DNA or RNA. In some embodiments, the IFNα1, its variant, or a chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprising the IFNα1 or its variant is encoded by a modified mRNA, *i.e.* an mRNA comprising one or more modified nucleotides. In some embodiments, the modified mRNA comprises one or modifications found in U.S. Patent No. 8,278,036, the entire contents of which are hereby incorporated by reference. In some embodiments, the modified mRNA comprises one or more of m5C, m5U, m6A, s2U, Ψ, and 2'-O-methyl-U. In some embodiments, the present invention relates to administering a modified mRNA encoding one or more of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex. In some embodiments, the present invention relates to gene therapy vectors comprising the same. In some

embodiments, the present invention relates to gene therapy methods comprising the same. In various embodiments, the nucleic acid is in the form of an oncolytic virus, *e.g.* an adenovirus, reovirus, measles, herpes simplex, Newcastle disease virus or vaccinia.

In various embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises a targeting moiety that is a VHH. In various embodiments, the VHH is not limited to a specific biological source or to a specific method of preparation. For example, the VHH can generally be obtained: (1) by isolating the V_HH domain of a naturally occurring heavy chain antibody; (2) by expression of a nucleotide sequence encoding a naturally occurring V_HH domain; (3) by "humanization" of a naturally occurring V_HH domain or by expression of a nucleic acid encoding a such humanized V_HH domain; (4) by "camelization" of a naturally occurring VH domain from any animal species, such as from a mammalian species, such as from a human being, or by expression of a nucleic acid encoding such a camelized VH domain; (5) by "camelization" of a "domain antibody" or "Dab" as described in the art, or by expression of a nucleic acid encoding such a camelized VH domain; (6) by using synthetic or semi-synthetic techniques for preparing proteins, polypeptides or other amino acid sequences known in the art; (7) by preparing a nucleic acid encoding a VHH using techniques for nucleic acid synthesis known in the art, followed by expression of the nucleic acid thus obtained; and/or (8) by any combination of one or more of the foregoing.

In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises a VHH that corresponds to the V_HH domains of naturally occurring heavy chain antibodies directed against a target of interest. In some embodiments, such V_HH sequences can generally be generated or obtained by suitably immunizing a species of Camelid with a molecule of based on the target of interest (*e.g.*, XCR1, Clec9a, CD8, SIRP1α, FAP, *etc.*) (*i.e.*, so as to raise an immune response and/or heavy chain antibodies directed against the target of interest), by obtaining a suitable biological sample from the Camelid (such as a blood sample, or any sample of B-cells), and by generating V_HH sequences directed against the target of interest, starting from the sample, using any suitable known techniques. In some embodiments, naturally occurring V_HH domains against the target of interest can be obtained from naive libraries of Camelid V_HH sequences, for example, by screening such a library using the target of interest or at least one part, fragment, antigenic determinant or epitope thereof using one or more screening techniques known in the art. Such libraries and techniques are, for example, described in WO 9937681, WO 0190190, WO 03025020 and WO 03035694, the entire contents of which are hereby incorporated by reference. In some embodiments, improved synthetic or semi-synthetic libraries derived from naive V_HH libraries may be used, such as V_HH libraries obtained from naive V_HH libraries by techniques such as random mutagenesis and/or CDR shuffling, as for example, described in WO 0043507, the entire contents of which are hereby incorporated by reference. In some embodiments, another technique for obtaining V_HH sequences directed against a target of interest involves suitably immunizing a transgenic mammal that is capable of expressing heavy chain antibodies (*i.e.*, so as to raise an immune response and/or heavy chain antibodies directed against the target of interest), obtaining a suitable biological sample from the transgenic mammal (such as a blood sample, or any sample of B-cells), and then generating V_HH sequences directed against XCR1 starting from the sample, using

any suitable known techniques. For example, for this purpose, the heavy chain antibody-expressing mice and the further methods and techniques described in WO 02085945 and in WO 04049794 (the entire contents of which are hereby incorporated by reference) can be used.

In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises a VHH that has been "humanized" *i.e.*, by replacing one or more amino acid residues in the amino acid sequence of the naturally occurring V_HH sequence (and in particular in the framework sequences) by one or more of the amino acid residues that occur at the corresponding position(s) in a VH domain from a conventional 4-chain antibody from a human being. This can be performed using humanization techniques known in the art. In some embodiments, possible humanizing substitutions or combinations of humanizing substitutions may be determined by methods known in the art, for example, by a comparison between the sequence of a VHH and the sequence of a naturally occurring human VH domain. In some embodiments, the humanizing substitutions are chosen such that the resulting humanized VHHs still retain advantageous functional properties. Generally, as a result of humanization, the VHHs of the invention may become more "human-like," while still retaining favorable properties such as a reduced immunogenicity, compared to the corresponding naturally occurring V_HH domains. In various embodiments, the humanized VHHs of the invention can be obtained in any suitable manner known in the art and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring V_HH domain as a starting material.

In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex comprises a VHH that has been "camelized," *i.e.*, by replacing one or more amino acid residues in the amino acid sequence of a naturally occurring VH domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a V_HH domain of a heavy chain antibody of a camelid. In some embodiments, such "camelizing" substitutions are inserted at amino acid positions that form and/or are present at the VH-VL interface, and/or at the so-called Camelidae hallmark residues (see, for example, WO9404678, the entire contents of which are hereby incorporated by reference). In some embodiments, the VH sequence that is used as a starting material or starting point for generating or designing the camelized VHH is a VH sequence from a mammal, for example, the VH sequence of a human being, such as a VH3 sequence. In various embodiments, the camelized VHHs can be obtained in any suitable manner known in the art (*i.e.*, as indicated under points (1)-(8) above) and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring VH domain as a starting material.

In various embodiments, both "humanization" and "camelization" can be performed by providing a nucleotide sequence that encodes a naturally occurring V_HH domain or VH domain, respectively, and then changing, in a manner known in the art, one or more codons in the nucleotide sequence in such a way that the new nucleotide sequence encodes a "humanized" or "camelized" VHH, respectively. This nucleic acid can then be expressed in a manner known in the art, so as to provide the desired VHH of the invention. Alternatively, based on the amino acid sequence of a naturally occurring V_HH domain or VH domain, respectively, the amino acid sequence of the desired humanized or camelized VHH of the invention, respectively, can be designed and then synthesized de novo using

techniques for peptide synthesis known in the art. Also, based on the amino acid sequence or nucleotide sequence of a naturally occurring V_HH domain or VH domain, respectively, a nucleotide sequence encoding the desired humanized or camelized VHH, respectively, can be designed and then synthesized de novo using techniques for nucleic acid synthesis known in the art, after which the nucleic acid thus obtained can be expressed in a manner known in the art, so as to provide the desired VHH of the invention. Other suitable methods and techniques for obtaining the VHHs of the invention and/or nucleic acids encoding the same, starting from naturally occurring VH sequences or V_HH sequences, are known in the art, and may, for example, comprise combining one or more parts of one or more naturally occurring VH sequences (such as one or more FR sequences and/or CDR sequences), one or more parts of one or more naturally occurring V_HH sequences (such as one or more FR sequences or CDR sequences), and/or one or more synthetic or semi-synthetic sequences, in a suitable manner, so as to provide a VHH of the invention or a nucleotide sequence or nucleic acid encoding the same.

Pharmaceutically Acceptable Salts and Excipients

The chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex described herein can possess a sufficiently basic functional group, which can react with an inorganic or organic acid, or a carboxyl group, which can react with an inorganic or organic base, to form a pharmaceutically acceptable salt. A pharmaceutically acceptable acid addition salt is formed from a pharmaceutically acceptable acid, as is well known in the art. Such salts include the pharmaceutically acceptable salts listed in, for example, *Journal of Pharmaceutical Science*, 66, 2-19 (1977) and *The Handbook of Pharmaceutical Salts; Properties, Selection, and Use*. P. H. Stahl and C. G. Wermuth (eds.), Verlag, Zurich (Switzerland) 2002, which are hereby incorporated by reference in their entirety.

Pharmaceutically acceptable salts include, by way of non-limiting example, sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, camphorsulfonate, pamoate, phenylacetate, trifluoroacetate, acrylate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, isobutyrate, phenylbutyrate, α-hydroxybutyrate, butyne-1,4-dicarboxylate, hexyne-1,4-dicarboxylate, caprate, caprylate, cinnamate, glycollate, heptanoate, hippurate, malate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, phthalate, teraphthalate, propiolate, propionate, phenylpropionate, sebacate, suberate, p-bromobenzenesulfonate, chlorobenzenesulfonate, ethylsulfonate, 2-hydroxyethylsulfonate, methylsulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, naphthalene-1,5-sulfonate, xylenesulfonate, and tartarate salts.

The term “pharmaceutically acceptable salt” also refers to a salt of the compositions of the present invention having an acidic functional group, such as a carboxylic acid functional group, and a base. Suitable bases include, but are not limited to, hydroxides of alkali metals such as sodium, potassium, and lithium; hydroxides of alkaline earth metal such as calcium and magnesium; hydroxides of other metals, such as aluminum and zinc; ammonia, and

organic amines, such as unsubstituted or hydroxy-substituted mono-, di-, or tri-alkylamines, dicyclohexylamine; tributyl amine; pyridine; N-methyl, N-ethylamine; diethylamine; triethylamine; mono-, bis-, or tris-(2-OH-lower alkylamines), such as mono-; bis-, or tris-(2-hydroxyethyl)amine, 2-hydroxy-tert-butylamine, or tris-(hydroxymethyl)methylamine, N,N-di-lower alkyl-N-(hydroxyl-lower alkyl)-amines, such as N,N-dimethyl-N-(2-hydroxyethyl)amine or tri-(2-hydroxyethyl)amine; N-methyl-D-glucamine; and amino acids such as arginine, lysine, and the like.

In some embodiments, the compositions described herein are in the form of a pharmaceutically acceptable salt.

Pharmaceutical Compositions and Formulations

In various embodiments, the present invention pertains to pharmaceutical compositions comprising the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex described herein and a pharmaceutically acceptable carrier or excipient. Any pharmaceutical compositions described herein can be administered to a subject as a component of a composition that comprises a pharmaceutically acceptable carrier or vehicle. Such compositions can optionally comprise a suitable amount of a pharmaceutically acceptable excipient so as to provide the form for proper administration.

In various embodiments, pharmaceutical excipients can be liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical excipients can be, for example, saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea and the like. In addition, auxiliary, stabilizing, thickening, lubricating, and coloring agents can be used. In one embodiment, the pharmaceutically acceptable excipients are sterile when administered to a subject. Water is a useful excipient when any agent described herein is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid excipients, specifically for injectable solutions. Suitable pharmaceutical excipients also include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Any agent described herein, if desired, can also comprise minor amounts of wetting or emulsifying agents, or pH buffering agents. Other examples of suitable pharmaceutical excipients are described in *Remington's Pharmaceutical Sciences* 1447-1676 (Alfonso R. Gennaro eds., 19th ed. 1995), incorporated herein by reference.

The present invention includes the described pharmaceutical compositions (and/or additional therapeutic agents) in various formulations. Any inventive pharmaceutical composition (and/or additional therapeutic agents) described herein can take the form of solutions, suspensions, emulsion, drops, tablets, pills, pellets, capsules, capsules containing liquids, gelatin capsules, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, lyophilized powder, frozen suspension, dessicated powder, or any other form suitable for use. In one embodiment, the composition is in the form of a capsule. In another embodiment, the composition is in the form of a tablet. In yet another embodiment, the pharmaceutical composition is formulated in the form of a soft-gel capsule. In a further embodiment, the pharmaceutical composition is formulated in the form of a gelatin capsule. In yet another embodiment, the pharmaceutical composition is formulated as a liquid.

Where necessary, the inventive pharmaceutical compositions (and/or additional agents) can also include a solubilizing agent. Also, the agents can be delivered with a suitable vehicle or delivery device as known in the art. Combination therapies outlined herein can be co-delivered in a single delivery vehicle or delivery device.

The formulations comprising the inventive pharmaceutical compositions (and/or additional agents) of the present invention may conveniently be presented in unit dosage forms and may be prepared by any of the methods well known in the art of pharmacy. Such methods generally include the step of bringing the therapeutic agents into association with a carrier, which constitutes one or more accessory ingredients. Typically, the formulations are prepared by uniformly and intimately bringing the therapeutic agent into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into dosage forms of the desired formulation (*e.g.*, wet or dry granulation, powder blends, *etc.*, followed by tableting using conventional methods known in the art).

In various embodiments, any pharmaceutical compositions (and/or additional agents) described herein is formulated in accordance with routine procedures as a composition adapted for a mode of administration described herein.

Routes of administration include, for example: oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by inhalation, or topically. Administration can be local or systemic. In some embodiments, the administering is effected orally. In another embodiment, the administration is by parenteral injection. The mode of administration can be left to the discretion of the practitioner, and depends in-part upon the site of the medical condition. In most instances, administration results in the release of any agent described herein into the bloodstream.

In one embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex described herein is formulated in accordance with routine procedures as a composition adapted for oral administration. Compositions for oral delivery can be in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs, for example. Orally administered compositions can comprise one or more agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions can be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving any chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex described herein are also suitable for orally administered compositions. In these latter platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time-delay material such as glycerol monostearate or glycerol stearate can also be useful. Oral compositions can include standard excipients such as mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, and magnesium carbonate. In one embodiment, the excipients

are of pharmaceutical grade. Suspensions, in addition to the active compounds, may contain suspending agents such as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, tragacanth, *etc.*, and mixtures thereof.

Dosage forms suitable for parenteral administration (*e.g.* intravenous, intramuscular, intraperitoneal, subcutaneous and intra-articular injection and infusion) include, for example, solutions, suspensions, dispersions, emulsions, and the like. They may also be manufactured in the form of sterile solid compositions (*e.g.* lyophilized composition), which can be dissolved or suspended in sterile injectable medium immediately before use. They may contain, for example, suspending or dispersing agents known in the art. Formulation components suitable for parenteral administration include a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose.

For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). The carrier should be stable under the conditions of manufacture and storage, and should be preserved against microorganisms. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol), and suitable mixtures thereof.

The compositions provided herein, alone or in combination with other suitable components, can be made into aerosol formulations (*i.e.*, "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Any inventive pharmaceutical compositions (and/or additional agents) described herein can be administered by controlled-release or sustained-release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Patent Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; and 5,733,556, each of which is incorporated herein by reference in its entirety. Such dosage forms can be useful for providing controlled-or sustained-release of one or more active ingredients using, for example, hydropropyl cellulose, hydropropylmethyl cellulose, polyvinylpyrrolidone, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled- or sustained-release formulations known to those skilled in the art, including those described herein, can be readily selected for use with the active ingredients of the agents described herein. The invention thus provides single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled- or sustained-release.

Controlled- or sustained-release of an active ingredient can be stimulated by various conditions, including but not limited to, changes in pH, changes in temperature, stimulation by an appropriate wavelength of light, concentration or availability of enzymes, concentration or availability of water, or other physiological conditions or compounds.

In another embodiment, a controlled-release system can be placed in proximity of the target area to be treated, thus requiring only a fraction of the systemic dose (see, *e.g.,* Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)). Other controlled-release systems discussed in the review by Langer, 1990, *Science* 249:1527-1533) may be used.

Pharmaceutical formulations preferably are sterile. Sterilization can be accomplished, for example, by filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.

Administration and Dosage

It will be appreciated that the actual dose of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex to be administered according to the present invention will vary according to the particular dosage form, and the mode of administration. Many factors that may modify the action of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex (*e.g.*, body weight, gender, diet, time of administration, route of administration, rate of excretion, condition of the subject, drug combinations, genetic disposition and reaction sensitivities) can be taken into account by those skilled in the art. Administration can be carried out continuously or in one or more discrete doses within the maximum tolerated dose. Optimal administration rates for a given set of conditions can be ascertained by those skilled in the art using conventional dosage administration tests.

In some embodiments, a suitable dosage of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is in a range of about 0.01 μg/kg to about 100 mg/kg of body weight of the subject, about 0.01 μg/kg to about 10 mg/kg of body weight of the subject, or about 0.01 μg/kg to about 1 mg/kg of body weight of the subject for example, about 0.01 μg/kg, about 0.02 μg/kg, about 0.03 μg/kg, about 0.04 μg/kg, about 0.05 μg/kg, about 0.06 μg/kg, about 0.07 μg/kg, about 0.08 μg/kg, about 0.09 μg/kg, about 0.1 mg/kg, about 0.2 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, about 1.5 mg/kg, about 1.6 mg/kg, about 1.7 mg/kg, about 1.8 mg/kg, 1.9 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg body weight, or about 100 mg/kg body weight, inclusive of all values and ranges therebetween.

Individual doses of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex can be administered in unit dosage forms (*e.g.*, tablets, capsules, or liquid formulations) containing, for example, from about 1 μg to about 100 mg, from about 1 μg to about 90 mg, from about 1 μg to about 80 mg, from about 1 μg to about 70 mg, from about 1 μg to about 60 mg, from about 1 μg to about 50 mg, from about 1 μg to about 40 mg, from about 1 μg to about 30 mg, from about 1 μg to about 20 mg, from about 1 μg to about 10 mg, from about 1 μg to about 5 mg, from about 1 μg to about 3 mg, from about 1 μg to about 1 mg per unit dosage form, or from about 1 μg to about 50 μg per unit dosage form. For example, a unit dosage form can be about 1 μg, about 2 μg, about 3 μg, about 4 μg, about 5 μg, about 6 μg, about 7 μg, about 8 μg, about 9 μg, about 10 μg, about 11 μg, about 12 μg, about 13 μg, about 14 μg, about 15 μg, about 16 μg, about 17 μg, about 18 μg, about 19

μg, about 20 μg, about 21 μg, about 22 μg, about 23 μg, about 24 μg, about 25 μg, about 26 μg, about 27 μg, about 28 μg, about 29, about 30 μg, about 35 μg, about 40 μg, about 45 μg, about 50 μg, about 60 μg, about 70 μg, about 80 μg, about 90 μg, about 0.1 mg, about 0.2 mg, about 0.3 mg, about 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1 mg, about 2 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, or about 100 mg, inclusive of all values and ranges therebetween.

In one embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is administered at an amount of from about 1 μg to about 100 mg daily, from about 1 μg to about 90 mg daily, from about 1 μg to about 80 mg daily, from about 1 μg to about 70 mg daily, from about 1 μg to about 60 mg daily, from about 1 μg to about 50 mg daily, from about 1 μg to about 40 mg daily, from about 1 μg to about 30 mg daily, from about 1 μg to about 20 mg daily, from about 01 μg to about 10 mg daily, from about 1 μg to about 5 mg daily, from about 1 μg to about 3 mg daily, or from about 1 μg to about 1 mg daily. In various embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is administered at a daily dose of about 1 μg, about 2 μg, about 3 μg, about 4 μg, about 5 μg, about 6 μg, about 7 μg, about 8 μg, about 9 μg, about 10 μg, about 11 μg, about 12 μg, about 13 μg, about 14 μg, about 15 μg, about 16 μg, about 17 μg, about 18 μg, about 19 μg, about 20 μg,, about 21 μg, about 22 μg, about 23 μg, about 24 μg, about 25 μg, about 26 μg, about 27 μg, about 28 μg, about 29, about 30 μg, about 35 μg, about 40 μg, about 45 μg, about 50 μg, about 60 μg, about 70 μg, about 80 μg, about 90 μg, about 0.1 mg, about 0.2 mg, about 0.3 mg, about 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1 mg, about 2 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, or about 100 mg, inclusive of all values and ranges therebetween.

In accordance with certain embodiments of the invention, the pharmaceutical composition comprising the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be administered, for example, more than once daily (*e.g.*, about two times, about three times, about four times, about five times, about six times, about seven times, about eight times, about nine times, or about ten times daily), about once per day, about every other day, about every third day, about once a week, about once every two weeks, about once every month, about once every two months, about once every three months, about once every six months, or about once every year. In an embodiment, the pharmaceutical composition comprising the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is administered about three times a week.

In various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be administered for a prolonged period. For example, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be administered as described herein for at least about

1 week, at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, at least about 5 weeks, at least about 6 weeks, at least about 7 weeks, at least about 8 weeks, at least about 9 weeks, at least about 10 weeks, at least about 11 weeks, or at least about 12 weeks. For example, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be administered for 12 weeks, 24 weeks, 36 weeks or 48 weeks. In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is administered for at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, or at least about 12 months. n some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be administered for at least about 1 year, at least about 2 years, at least about 3 years, at least about 4 years, or at least about 5 years.

Combination Therapy and Additional Therapeutic Agents

In various embodiments, the pharmaceutical composition of the present invention is co-administered in conjunction with additional therapeutic agent(s). Co-administration can be simultaneous or sequential.

In one embodiment, the additional therapeutic agent and the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the present invention are administered to a subject simultaneously. The term “simultaneously” as used herein, means that the additional therapeutic agent and the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex are administered with a time separation of no more than about 60 minutes, such as no more than about 30 minutes, no more than about 20 minutes, no more than about 10 minutes, no more than about 5 minutes, or no more than about 1 minute. Administration of the additional therapeutic agent and the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex can be by simultaneous administration of a single formulation (*e.g.*, a formulation comprising the additional therapeutic agent and the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex) or of separate formulations (*e.g.*, a first formulation including the additional therapeutic agent and a second formulation including the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex).

Co-administration does not require the therapeutic agents to be administered simultaneously, if the timing of their administration is such that the pharmacological activities of the additional therapeutic agent and the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex overlap in time, thereby exerting a combined therapeutic effect. For example, the additional therapeutic agent and the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex can be administered sequentially. The term “sequentially” as used herein means that the additional therapeutic agent and the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex are administered with a time separation of more than about 60 minutes. For example, the time between the sequential administration of the additional therapeutic agent and the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex can be more than about 60 minutes, more than about 2 hours, more than about 5 hours, more than about

10 hours, more than about 1 day, more than about 2 days, more than about 3 days, more than about 1 week apart, more than about 2 weeks apart, or more than about one month apart. The optimal administration times will depend on the rates of metabolism, excretion, and/or the pharmacodynamic activity of the additional therapeutic agent and the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex being administered. Either the additional therapeutic agent or the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex cell may be administered first.

Co-administration also does not require the therapeutic agents to be administered to the subject by the same route of administration. Rather, each therapeutic agent can be administered by any appropriate route, for example, parenterally or non-parenterally.

In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex described herein acts synergistically when co-administered with another therapeutic agent. In such embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex and the additional therapeutic agent may be administered at doses that are lower than the doses employed when the agents are used in the context of monotherapy.

In some embodiments, the present invention pertains to chemotherapeutic agents as additional therapeutic agents. For example, without limitation, such combination of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex and chemotherapeutic agent find use in the treatment of cancers, as described elsewhere herein. Examples of chemotherapeutic agents include, but are not limited to, alkylating agents such as thiotepa and CYTOXAN cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (*e.g.*, bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; cally statin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (*e.g.*, cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB 1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammall and calicheamicin omegall (*see, e.g.*, Agnew, Chem. Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN doxorubicin (including morpholino- doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxy doxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such

as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as minoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (*e.g.*, T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, TAXOL paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, 111.), and TAXOTERE doxetaxel (Rhone-Poulenc Rorer, Antony, France); chloranbucil; GEMZAR gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE. vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11) (including the treatment regimen of irinotecan with 5-FU and leucovorin); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; combretastatin; leucovorin (LV); oxaliplatin, including the oxaliplatin treatment regimen (FOLFOX); lapatinib (Tykerb); inhibitors of PKC-α, Raf, H-Ras, EGFR (*e.g.*, erlotinib (Tarceva)) and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above. In addition, the methods of treatment can further include the use of radiation. In addition, the methods of treatment can further include the use of photodynamic therapy.

In some embodiments, inclusive of, without limitation, infectious disease applications, the present invention pertains to anti-infectives as additional therapeutic agents. In some embodiments, the anti-infective is an anti-viral agent including, but not limited to, Abacavir, Acyclovir, Adefovir, Amprenavir, Atazanavir, Cidofovir, Darunavir, Delavirdine, Didanosine, Docosanol, Efavirenz, Elvitegravir, Emtricitabine, Enfuvirtide, Etravirine, Famciclovir, and Foscarnet. In some embodiments, the anti-infective is an anti-bacterial agent including, but not limited to, cephalosporin antibiotics (cephalexin, cefuroxime, cefadroxil, cefazolin, cephalothin, cefaclor, cefamandole, cefoxitin, cefprozil, and ceftobiprole); fluoroquinolone antibiotics (cipro, Levaquin, floxin, tequin, avelox, and norflox); tetracycline antibiotics (tetracycline, minocycline, oxytetracycline, and doxycycline); penicillin antibiotics (amoxicillin, ampicillin, penicillin V, dicloxacillin, carbenicillin, vancomycin, and methicillin); monobactam antibiotics (aztreonam); and carbapenem antibiotics (ertapenem, doripenem, imipenem/cilastatin, and meropenem). In some

embodiments, the anti-infectives include anti-malarial agents (*e.g.*, chloroquine, quinine, mefloquine, primaquine, doxycycline, artemether/lumefantrine, atovaquone/proguanil and sulfadoxine/pyrimethamine), metronidazole, tinidazole, ivermectin, pyrantel pamoate, and albendazole.

In illustrative embodiments, the present invention pertains to the use of hepatitis therapeutics as additional therapeutic agents. In various embodiments, the hepatitis therapeutics include, but are not limited to, IFN-α such as INTRON A or pegylated IFN-α such as Pegasys or PEG-INTRON, ribavirin, boceprevir, simeprevir, sofosbuvir, simeprevir, daclatasvir, ledipasvir/sofosbuvir (Harvoni), ombitasvir/paritaprevir/ritonavir (Technivie), ombitasvir/paritaprevir/ritonavir/dasabuvir (Viekira Pak), lamivudine, adefovir, entecavir, telbivudine, entecavir, tenofovir, velpatasvir, elbasvir, grazoprevir, dasabuvir, and any combinations thereof. In an embodiment, the additional therapeutic agent is IFN-α (*e.g.*, INTRON A) or pegylated IFN-α (*e.g.*, Pegasys or PEG-INTRON). In another embodiment, the additional therapeutic agent is ribavirin.

In some embodiments, the present invention relates to combination therapies using the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex and an immunosuppressive agent. In some embodiments, the present invention relates to administration of the Clec9A binding agent to a patient undergoing treatment with an immunosuppressive agent.

In an embodiment, the immunosuppressive agent is TNF. In illustrative embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex act synergistically when co-administered with TNF. In an illustrative embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex acts synergistically when co-administered with TNF for use in treating tumor or cancer. For example, co-administration of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the present invention and TNF may act synergistically to reduce or eliminate the tumor or cancer, or slow the growth and/or progression and/or metastasis of the tumor or cancer. In some embodiments, the combination of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex and TNF may exhibit improved safety profiles when compared to the agents used alone in the context of monotherapy. In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex and TNF may be administered at doses that are lower than the doses employed when the agents are used in the context of monotherapy.

In some embodiments, inclusive, without limitation, of autoimmune applications, the additional therapeutic agent is an immunosuppressive agent that is an anti-inflammatory agent such as a steroidal anti-inflammatory agent or a non-steroidal anti-inflammatory agent (NSAID). Steroids, particularly the adrenal corticosteroids and their synthetic analogues, are well known in the art. Examples of corticosteroids useful in the present invention include, without limitation, hydroxyltriamcinolone, alpha-methyl dexamethasone, beta-methyl betamethasone, beclomethasone dipropionate, betamethasone benzoate, betamethasone dipropionate, betamethasone valerate, clobetasol valerate, desonide, desoxymethasone, dexamethasone, diflorasone diacetate, diflucortolone valerate, fluadrenolone, fluclorolone acetonide, flumethasone pivalate, fluosinolone acetonide, fluocinonide, flucortine butylester, fluocortolone, fluprednidene (fluprednylidene) acetate, flurandrenolone, halcinonide, hydrocortisone

acetate, hydrocortisone butyrate, methylprednisolone, triamcinolone acetonide, cortisone, cortodoxone, flucetonide, fludrocortisone, difluorosone diacetate, fluradrenolone acetonide, medrysone, amcinafel, amcinafide, betamethasone and the balance of its esters, chloroprednisone, clocortelone, clescinolone, dichlorisone, difluprednate, flucloronide, flunisolide, fluoromethalone, fluperolone, fluprednisolone, hydrocortisone, meprednisone, paramethasone, prednisolone, prednisone, beclomethasone dipropionate. (NSAIDS) that may be used in the present invention, include but are not limited to, salicylic acid, acetyl salicylic acid, methyl salicylate, glycol salicylate, salicylmides, benzyl-2,5-diacetoxybenzoic acid, ibuprofen, fulindac, naproxen, ketoprofen, etofenamate, phenylbutazone, and indomethacin. In some embodiments, the immunosupressive agent may be cytostatics such as alkylating agents, antimetabolites (*e.g.*, azathioprine, methotrexate), cytotoxic antibiotics, antibodies (*e.g.*, basiliximab, daclizumab, and muromonab), anti-immunophilins (*e.g.*, cyclosporine, tacrolimus, sirolimus), inteferons, opioids, TNF binding proteins, mycophenolates, and small biological agents (*e.g.*, fingolimod, myriocin). Additional anti-inflammatory agents are described, for example, in U.S. Patent No. 4,537,776, the entire contents of which is incorporated by reference herein.

In some embodiments, the present invention pertains to various agents used for treating obesity as additional therapeutic agents. Illustrative agents used for treating obesity include, but are not limited to, orlistat (*e.g.* ALL1, XENICAL), loracaserin (*e.g.* BELVIQ), phentermine-topiramate (*e.g.* QSYMIA), sibutramme (*e.g.* REDUCTIL or MERJDIA), rimonabant (ACOMPLLA), exenatide (*e.g.* BYETTA), pramlintide (*e.g.* SYMLIN) phentermine, benzphetamine, diethylpropion, phendimetrazme, bupropion, and metformin. Agents that interfere with the body's ability to absorb specific nutrients in food are among the additional agents, *e.g.* orlistat (*e.g.* ALU, XENICAL), glucomannan, and guar gum. Agents that suppress apetite are also among the additional agents, *e.g.* catecholamines and their derivatives (such as phenteimine and other amphetamine-based drugs), various antidepressants and mood stabilizers (*e.g.* bupropion and topiramate), anorectics (*e.g.* dexedrine, digoxin). Agents that increase the body's metabolism are also among the additional agents.

In some embodiments, additional therapeutic agents may be selected from among appetite suppressants, neurotransmitter reuptake inhibitors, dopaminergic agonists, serotonergic agonists, modulators of GABAergic signaling, anticonvulsants, antidepressants, monoamine oxidase inhibitors, substance P (NK1) receptor antagonists, melanocortin receptor agonists and antagonists, lipase inhibitors, inhibitors of fat absorption, regulators of energy intake or metabolism, cannabinoid receptor modulators, agents for treating addiction, agents for treating metabolic syndrome, peroxisome proliferator-activated receptor (PPAR) modulators; dipeptidyl peptidase 4 (DPP- 4) antagonists, agents for treating cardiovascular disease, agents for treating elevated triglyceride levels, agents for treating low HDL, agents for treating hypercholesterolemia, and agents for treating hypertension. Some agents for cardiovascular disease include statins (*e.g.* lovastatin, atorvastatin, fluvastatin, rosuvastatin, simvastatin and pravastatin) and omega-3 agents (*e.g.* LOVAZA, EPANQVA, VASCEPA, esterified omega-3's in general, fish oils, krill oils, algal oils). In some embodiments, additional agents may be selected from among amphetamines, benzodiazepines, suifonyl ureas, meglitinides, thiazolidinediones, biguanides, beta-blockers, XCE inhibitors, diuretics, nitrates, calcium channel blockers, phenlermine, sibutramine, iorcaserin,

cetilistat, rimonabant, taranabant, topiramate, gabapentin, valproate, vigabatrin, bupropion, tiagabine, sertraline, fluoxetine, trazodone, zonisamide, methylphenidate, varenicline, naltrexone, diethylpropion, phendimetrazine, rcpaglini.de, nateglinide, glimepiride, metformin, pioglitazone, rosiglilazone, and sitagliptin.

In some embodiments, the present invention pertains to an agent used for treating diabetes as additional therapeutic agents. Illustrative anti-diabetic agents include, but are not limited to, sulfonylurea (*e.g.*, DYMELOR (acetohexamide), DIABINESE (chlorpropamide), ORINASE (tolbutamide), and TOLINASE (tolazamide), GLUCOTROL (glipizide), GLUCOTROL XL (extended release), DIABETA (glyburide), MICRONASE (glyburide), GLYNASE PRESTAB (glyburide), and AMARYL (glimepiride)); a Biguanide (*e.g.* metformin (GLUCOPHAGE, GLUCOPHAGE XR, RIOMET, FORTAMET, and GLUMETZA)); a thiazolidinedione (*e.g.* ACTOS (pioglitazone) and AVANDIA (rosiglitazone); an alpha-glucosidase inhibitor (*e.g.*, PRECOSE (acarbose) and GLYSET (miglitol); a Meglitinide (*e.g.*, PRANDIN (repaglinide) and STARLIX (nateglinide)); a Dipeptidyl peptidase IV (DPP-IV) inhibitor (*e.g.*, JANUVIA (sitagliptin), NESINA (alogliptin), ONGLYZA (saxagliptin), and TRADJENTA (linagliptin)); Sodium-glucose co-transporter 2 (SGLT2) inhibitor (*e.g.* INVOKANA (canaglifozin)); and a combination pill (*e.g.* GLUCOVANCE, which combines glyburide (a sulfonylurea) and metformin, METAGLIP, which combines glipizide (a sulfonylurea) and metformin, and AVANDAMET, which uses both metformin and rosiglitazone (AVANDIA) in one pill, KAZANO (alogliptin and metformin), OSENI (alogliptin plus pioglitazone), METFORMIN oral, ACTOS oral, BYETTA subcutaneous, JANUVIA oral, WELCHOL oral, JANUMET oral, glipizide oral, glimepiride oral, GLUCOPHAGE oral, LANTUS subcutaneous, glyburide oral, ONGLYZA oral, AMARYl oral, LANTUS SOLOSTAR subcutaneous, BYDUREON subcutaneous, LEVEMIR FLEXPEN subcutaneous, ACTOPLUS MET oral, GLUMETZA oral, TRADJENTA oral, bromocriptine oral, KOMBIGLYZE XR oral, INVOKANA oral, PRANDIN oral, LEVEMIR subcutaneous, PARLODEL oral, pioglitazone oral, NOVOLOG subcutaneous, NOVOLOG FLEXPEN subcutaneous, VICTOZA 2-PAK subcutaneous, HUMALOG subcutaneous, STARLIX oral, FORTAMET oral, GLUCOVANCE oral, GLUCOPHAGE XR oral, NOVOLOG Mix 70-30 FLEXPEN subcutaneous, GLYBURIDE-METFORMIN oral, acarbose oral, SYMLINPEN 60 subcutaneous, GLUCOTROl XL oral, NOVOLIN R inj, GLUCOTROL oral, DUETACT oral, sitagliptin oral, SYMLINPEN 120 subcutaneous, HUMALOG KWIKPEN subcutaneous, JANUMET XR oral, GLIPIZIDE-METFORMIN oral, CYCLOSET oral, HUMALOG MIX 75-25 subcutaneous, nateglinide oral, HUMALOG Mix 75-25 KWIKPEN subcutaneous, HUMULIN 70/30 subcutaneous, PRECOSE oral, APIDRA subcutaneous, Humulin R inj, Jentadueto oral, Victoza 3-Pak subcutaneous, Novolin 70/30 subcutaneous, NOVOLIN N subcutaneous, insulin detemir subcutaneous, glyburide micronized oral, GLYNASE oral, HUMULIN N subcutaneous, insulin glargine subcutaneous, RIOMET oral, pioglitazone-metformin oral, APIDRA SOLOSTAR subcutaneous, insulin lispro subcutaneous, GLYSET oral, HUMULIN 70/30 Pen subcutaneous, colesevelam oral, sitagliptin-metformin oral, DIABETA oral, insulin regular human inj, HUMULIN N Pen subcutaneous, exenatide subcutaneous, HUMALOG Mix 50-50 KWIKPEN subcutaneous, liraglutide subcutaneous, KAZANO oral, repaglinide oral, chlorpropamide oral, insulin aspart subcutaneous, NOVOLOG Mix 70-30 subcutaneous, HUMALOG Mix 50-50 subcutaneous, saxagliptin oral, ACTOPLUS Met XR oral, miglitol oral, NPH insulin human recomb subcutaneous, insulin NPH and regular human subcutaneous, tolazamide oral, mifepristone oral, insulin aspart protam-insulin aspart subcutaneous, repaglinide-metformin oral, saxagliptin-

metformin oral, linagliptin-metformin oral, NESINA oral, OSENI oral, tolbutamide oral, insulin lispro protamine and lispro subcutaneous, pramlintide subcutaneous, insulin glulisine subcutaneous, pioglitazone-glimepiride oral, PRANDIMET oral, NOVOLOG PenFill subcutaneous, linagliptin oral, exenatide microspheres subcutaneous, KORLYM oral, alogliptin oral, alogliptin-pioglitazone oral, alogliptin-metformin oral, canagliflozin oral, Lispro (HUMALOG); Aspart (NOVOLOG); Glulisine (APIDRA); Regular (NOVOLIN R or HUMULIN R); NPH (NOVOLIN N or HUMULIN N); Glargine (LANTUS); Detemir (LEVEMIR); HUMULIN or NOVOLIN 70/30; and NOVOLOG Mix 70/30 HUMALOG Mix 75/25 or 50/50.

In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the present invention act synergistically when used in combination with Chimeric Antigen Receptor (CAR) T-cell therapy. In an illustrative embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex acts synergistically when used in combination with CAR T-cell therapy in treating tumor or cancer. In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex agent acts synergistically when used in combination with CAR T-cell therapy in treating blood-based tumors. In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex acts synergistically when used in combination with CAR T-cell therapy in treating solid tumors. For example, use of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex and CAR T-cells may act synergistically to reduce or eliminate the tumor or cancer, or slow the growth and/or progression and/or metastasis of the tumor or cancer. In various embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention induces CAR T-cell division. In various embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention induces CAR T-cell proliferation. In various embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention prevents anergy of the CAR T cells.

In various embodiments, the CAR T-cell therapy comprises CAR T cells that target antigens (*e.g.*, tumor antigens) such as, but not limited to, carbonic anhydrase IX (CAIX), 5T4, CD19, CD20, CD22, CD30, CD33, CD38, CD47, CS1, CD138, Lewis-Y, L1-CAM, MUC16, ROR-1, IL13Rα2, gp100, prostate stem cell antigen (PSCA), prostate-specific membrane antigen (PSMA), B-cell maturation antigen (BCMA), human papillomavirus type 16 E6 (HPV-16 E6), CD171, folate receptor alpha (FR-α), GD2, human epidermal growth factor receptor 2 (HER2), mesothelin, EGFRvIII, fibroblast activation protein (FAP), carcinoembryonic antigen (CEA), and vascular endothelial growth factor receptor 2 (VEGF-R2), as well as other tumor antigens well known in the art. Additional illustrative tumor antigens include, but are not limited to MART-1/Melan-A, gp100, Dipeptidyl peptidase IV (DPPIV), adenosine deaminase-binding protein (ADAbp), cyclophilin b, Colorectal associated antigen (CRC)-0017-1A/GA733, Carcinoembryonic Antigen (CEA) and its immunogenic epitopes CAP-1 and CAP-2, etv6, aml1, Prostate Specific Antigen (PSA) and its immunogenic epitopes PSA-1, PSA-2, and PSA-3, T-cell receptor/CD3-zeta chain, MAGE-family of tumor antigens (*e.g.*, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3),

MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, MAGE-C3, MAGE-C4, MAGE-C5), GAGE-family of tumor antigens (*e.g.*, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9), BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1, CDK4, tyrosinase, p53, MUC family, HER2/neu, p21ras, RCAS1, α-fetoprotein, E-cadherin, α-catenin, β-catenin and γ-catenin, p120ctn, gp100 Pmel117, PRAME, NY-ESO-1, cdc27, adenomatous polyposis coli protein (APC), fodrin, Connexin 37, Ig-idiotype, p15, gp75, GM2 and GD2 gangliosides, viral products such as human papilloma virus proteins, Smad family of tumor antigens, Imp-1, NA, EBV-encoded nuclear antigen (EBNA)-1, brain glycogen phosphorylase, SSX-1, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-1 CT-7, c-erbB-2, CD19, CD37, CD56, CD70, CD74, CD138, AGS16, MUC1 , GPNMB, Ep-CAM, PD-L1, and PD-L2.

Illustrative CAR T-cell therapy include, but are not limited to, JCAR014 (Juno Therapeutics), JCAR015 (Juno Therapeutics), JCAR017 (Juno Therapeutics), JCAR018 (Juno Therapeutics), JCAR020 (Juno Therapeutics), JCAR023 (Juno Therapeutics), JCAR024 (Juno Therapeutics), CTL019 (Novartis), KTE-C19 (Kite Pharma), BPX-401 (Bellicum Pharmaceuticals), BPX-501 (Bellicum Pharmaceuticals), BPX-601 (Bellicum Pharmaceuticals), bb2121 (Bluebird Bio), CD-19 Sleeping Beauty cells (Ziopharm Oncology), UCART19 (Cellectis), UCART123 (Cellectis), UCART38 (Cellectis), UCARTCS1 (Cellectis), OXB-302 (Oxford BioMedica, MB-101 (Mustang Bio) and CAR T-cells developed by Innovative Cellular Therapeutics.

In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the present invention is used in a method of treating multiple sclerosis (MS) in combination with one or more MS therapeutics including, but not limited to, 3-interferons, glatiramer acetate, T-interferon, IFN-ß-2 (U. S. Patent Publication No. 2002/0025304), spirogermaniums (*e.g.*, N-(3-dimethylaminopropyl)-2-aza-8,8-dimethyl-8-germanspiro [4:5] decane, N-(3-dimethylaminopropyl)-2-aza-8,8-diethyl-8- germaspiro [4:5] decane, N-(3-dimethylaminopropyl)-2-aza-8,8-dipropyl-8-germaspiro [4:5] decane, and N-(3-dimethylaminopropyl)-2-aza-8, 8-dibutyl-8-germaspiro [4:5] decane), vitamin D analogs (*e.g.*, 1,25 (OH) 2D3, (see, *e.g.*, U.S. Patent No. 5,716,946)), prostaglandins (*e.g.*, latanoprost, brimonidine, PGE1, PGE2 and PGE3, see, *e.g.*, U. S. Patent Publication No. 2002/0004525), tetracycline and derivatives (*e.g.*, minocycline and doxycycline, see, *e.g.*, U.S. Patent Publication No. 20020022608), a VLA-4 binding antibody (see, *e.g.*, U.S. Patent Publication No. 2009/0202527), adrenocorticotrophic hormone, corticosteroid, prednisone, methylprednisone, 2-chlorodeoxyadenosine, mitoxantrone, sulphasalazine, methotrexate, azathioprine, cyclophosphamide, cyclosporin, fumarate, anti-CD20 antibody (*e.g.*, rituximab), and tizanidine hydrochloride.

In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is used in combination with one or more therapeutic agents that treat one or more symptoms or side effects of MS. Such agents include, but are not limited to, amantadine, baclofen, papaverine, meclizine, hydroxyzine, sulfamethoxazole, ciprofloxacin, docusate, pemoline, dantrolene, desmopressin, dexamethasone, tolterodine, phenyloin, oxybutynin, bisacodyl, venlafaxine, amitriptyline, methenamine, clonazepam, isoniazid, vardenafil, nitrofurantoin, psyllium hydrophilic mucilloid, alprostadil, gabapentin, nortriptyline, paroxetine,

propantheline bromide, modafinil, fluoxetine, phenazopyridine, methylprednisolone, carbamazepine, imipramine, diazepam, sildenafil, bupropion, and sertraline.

In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is used in a method of treating multiple sclerosis in combination with one or more of the disease modifying therapies (DMTs) described herein (*e.g.* the agents of **Table 6**). In some embodiments, the present invention provides an improved therapeutic effect as compared to use of one or more of the DMTs described herein (*e.g.* the agents listed in **Table 6** below) without the one or more disclosed binding agent. In an embodiment, the combination of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex and the one or more DMTs produces synergistic therapeutic effects.

Illustrative disease modifying therapies include, but are not limited to:

Table 6

Generic Name	Branded Name/Company	Frequency/Route of Delivery/Usual Dose
teriflunomide	AUBAGIO (GENZYME)	Every day; pill taken orally; 7 mg or 14 mg.
interferon beta-1a	AVONEX (BIOGEN IDEC)	Once a week; intramuscular (into the muscle) injection; 30 mcg
interferon beta-1b	BETASERON (BAYER HEALTHCARE PHARMACEUTICALS, INC.)	Every other day; subcutaneous (under the skin) injection; 250 mcg.
glatiramer acetate	COPAXONE (TEVA NEUROSCIENCE)	Every day; subcutaneous (under the skin) injection; 20 mg (20,000 mcg) OR Three times a week; subcutaneous (under the skin) injection; 40 mg (40,000 mcg)
interferon beta-1b	EXTAVIA (NOVARTIS PHARMACEUTICALS CORP.)	Every other day; subcutaneous (under the skin) injection; 250 mcg.
fingolimod	GILENYA (NOVARTIS PHARMACEUTICALS CORP.)	Every day; capsule taken orally; 0.5 mg.
Alemtuzumab (anti-CD52 monoclonal antibody)	LEMTRADA (GENZYME)	Intravenous infusion on five consecutive days, followed by intravenous infusion on three consecutive days one year later (12 mg)
mitoxantrone	NOVANTRONE (EMD SERONO)	Four times a year by IV infusion in a medical facility. Lifetime cumulative dose limit of approximately 8–12 doses over 2–3 years (140 mg/m2).
pegylated interferon beta-1a	PLEGRIDY (BIOGEN IDEC)	Every 14 days; subcutaneous (under the skin) injection; 125 mcg
interferon beta-1a	REBIF (EMD SERONO, INC.)	Three times a week; subcutaneous (under the skin) injection; 44 mcg
dimethyl fumarate (BG-12)	TECFIDERA (BIOGEN IDEC)	Twice a day; capsule taken orally; 120 mg for one week and 240 mg therafter
Natalizumab (humanized monoclonal antibody VLA-4 antagonist)	TYSABRI (BIOGEN IDEC)	Every four weeks by IV infusion in a registered infusion facility; 300 mg
DMTs in Development		
Amiloride (targets Acid-sensing ion channel-1 Epithelial sodium channel Na+/H+ exchanger)	PAR PHARMACEUTICAL, PERRIGO COMPANY , SIGMAPHARM LABORATORIES	Oral

Table 6		
Generic Name	**Branded Name/Company**	**Frequency/Route of Delivery/Usual Dose**
ATX-MS-1467 (targets Major histocompatibility complex class II T cell responses to myelin basic protein)	APITOPE / MERCK SERONO	Intradermal Subcutaneous
BAF312 (targets Sphingosine 1-phosphate (S1P) receptor subtypes S1P1 and S1P5 B cell distrubution T cell distribution)	NOVARTIS PHARMA	Oral
BGC20-0134 (targets Proinflammatory and anti-inflammatory cytokines)	BTG PLC	Oral
BIIB033 (targets LINGO-1 ("leucine-rich repeat and immunoglobulin-like domain-containing, Nogo receptor-interacting protein"))	BIOGEN	Intravenous infusion used in Phase I and Phase II trials Subcutaneous injection used in Phase I trial
Cladribine (targets CD4+ T cells DNA synthesis and repair E-selectin Intracellular adhesion molecule-1 Pro-inflammatory cytokines interleukin 2 and interleukin 2R Pro-inflammatory cytokines interleukin 8 and RANTES Cytokine secretion Monocyte and lymphocyte migration)	MERCK SERONO	Oral
Cyclophosphamide (targets T cells, particularly CD4+ helper T cells B cells)	BAXTER HEALTHCARE CORPORATION	Oral, monthly intravenous pulses
Daclizumab (humanized monoclonal antibody targeting CD25 Immune modulator of T cells)	BIOGEN IDEC/ABBVIE BIOTHERAPEUTICS	Projected to be IM injection once monthly
Dalfampridine (targets Voltage-gated potassium channels Degenerin/epithelial sodium channels L-type calcium channels that contain subunit Cavbeta3)	ACORDA THERAPEUTICS / BIOGEN IDEC	One tablet every 12 hours (extended release), 10 mg twice a day
Dronabinol (targets Cannabinoid receptor CB1 Cannabinoid receptor CB2)	ABBVIE INC.	Oral
Firategrast (targets Alpha4beta1 integrin)	GLAXOSMITHKLINE	Oral
GNbAC1MSRV-Env (targets envelope protein of the MS-associated retrovirus)	GENEURO SA / SERVIER	Intravenous infusion

Table 6		
Generic Name	**Branded Name/Company**	**Frequency/Route of Delivery/Usual Dose**
Idebenone (targets Reactive oxygen species)	SANTHERA PHARMACEUTICALS	Oral Dose in clinical trial for PPMS is 2250 mg per day (750 mg dose, 3 times per day)
Imilecleucel-T (targets Myelin-specific, autoreactive T cells)	OPEXA THERAPEUTICS / MERCK SERONO	Subcutaneous Given 5 times per year, according to information from the manufacturer
Laquinimod	TEVA	Projected to be 0.6 mg or 1.2 mg oral tablet taken daily
Masitinib (targets KIT (a stem cell factor, also called c-KIT) receptor as well as select other tyrosine kinases Mast cells)	AB SCIENCE	Oral
MEDI-551 (targets CD19, a B cell-specific antigen that is part of the B cell receptor complex and that functions in determining the threshold for B cell activation B cells Plasmablasts, B cells that express CD19 (but not CD20) and that secrete large quantities of antibodies; depletion of plasmablasts may be useful in autoimmune diseases involving pathogenic autoantibodies)	MEDIMMUNE	Intravenous Subcutaneous
Minocycline (targets T cells Microglia Leukocyte migration Matrix metalloproteinases)	VARIOUS	Oral Available as pellet-filled capsules and an oral suspension
MIS416 (targets Innate immune system Pathogen-associated molecular pattern recognition receptors of the innate immune system Myeloid cells of the innate immune system, which might be able to remodel the deregulated immune system activity that occurs in SPMS)	INNATE IMMUNOTHERAPEUTICS	Intravenous
Mycophenolate mofetil (targets Purine synthesis)	MANUFACTURED BY GENENTECH	Oral
Naltrexone (targets Opioid receptors Toll-like receptor 4)	VARIOUS	Given at low doses (3 to 4.5 mg per day) in oral form as"Low-dose naltrexone" (or "LDN")
Ocrelizumab and Ofatumumab (humanized monoclonal antibodies targeting CD20 B cell suppression	ROCHE / GSK	Projected to be IV infusion

Table 6		
Generic Name	**Branded Name/Company**	**Frequency/Route of Delivery/Usual Dose**
ONO-4641 (targets Sphingosine 1-phosphate receptor)	ONO PHARMACEUTICAL CO.	Oral
Phenytoin (targets Sodium channels)	PFIZER	Intravenous Intramuscular (less favored option) Oral
Ponesimod	ACTELION	To be determined
Raltegravir (targets Retroviral integrase Herpesvirus DNA packaging terminase)	MERCK	Oral 400 mg tablet twice daily, according to information from the manufacturer
RHB-104	REDHILL BIOPHARMA LIMITED	95 mg clarithromycin, 45 mg rifabutin, and 10 mg clofazimine
Riluzole (targets Glutamatergic neurotransmission Glutamate uptake and release Voltage-gated sodium channels Protein kinase C)	COVIS PHARMA / SANOFI	Oral

In some embodiments, the present invention relates to combination therapy with a blood transfusion. For instance, the present compositions may supplement a blood transfusion. In some embodiments, the present invention relates to combination therapy with iron supplements.

In some embodiments, the present invention relates to combination therapy with one or more EPO-based agents. For example, the present compositions may be used as an adjuvant to other EPO-based agents. In some embodiments, the present compositions are used as a maintenance therapy to other EPO-based agents. Other EPO-based agents include the following: epoetin alfa, including without limitation, DARBEPOETIN (ARANESP), EPOCEPT (LUPIN PHARMA), NANOKINE (NANOGEN PHARMACEUTICAL), EPOFIT (INTAS PHARMA), EPOGEN (AMGEN), EPOGIN, EPREX, (JANSSEN-CILAG), BINOCRIT7 (SANDOZ), PROCRIT; epoetin beta, including without limitation, NEORECORMON (HOFFMANN–LA ROCHE), RECORMON, Methoxy polyethylene glycol-epoetin beta (MIRCERA, ROCHE); epoetin delta, including without limitation, DYNEPO (erythropoiesis stimulating protein, SHIRE PLC); epoetin omega, including without limitation, EPOMAX; epoetin zeta, including without limitation, SILAPO (STADA) and RETACRIT (HOSPIRA) and other EPOs, including without limitation, EPOCEPT (LUPIN PHARMACEUTICALS), EPOTRUST (PANACEA BIOTEC LTD), ERYPRO SAFE (BIOCON LTD.), REPOITIN (SERUM INSTITUTE OF INDIA LIMITED), VINTOR (EMCURE PHARMACEUTICALS), EPOFIT (INTAS PHARMA), ERYKINE (INTAS BIOPHARMACEUTICA), WEPOX (WOCKHARDT BIOTECH), ESPOGEN (LG LIFE SCIENCES), RELIPOIETIN (RELIANCE LIFE SCIENCES), SHANPOIETIN (SHANTHA BIOTECHNICS LTD), ZYROP (CADILA HEALTHCARE LTD.), EPIAO (RHUEPO) (SHENYANG SUNSHINE PHARMACEUTICAL CO. LTD), CINNAPOIETIN (CINNAGEN).

In some embodiments, the present invention relates to combination therapy with one or more immune-modulating agents, for example, without limitation, agents that modulate immune checkpoint. In various embodiments, the

immune-modulating agent targets one or more of PD-1, PD-L1, and PD-L2. In various embodiments, the immune-modulating agent is PD-1 inhibitor. In various embodiments, the immune-modulating agent is an antibody specific for one or more of PD-1, PD-L1, and PD-L2. For instance, in some embodiments, the immune-modulating agent is an antibody such as, by way of non-limitation, nivolumab, (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), pidilizumab (CT-011, CURE TECH), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), MPDL328OA (ROCHE). In some embodiments, the immune-modulating agent targets one or more of CD137 or CD137L. In various embodiments, the immune-modulating agent is an antibody specific for one or more of CD137 or CD137L. For instance, in some embodiments, the immune-modulating agent is an antibody such as, by way of non-limitation, urelumab (also known as BMS-663513 and anti-4-1BB antibody). In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is combined with urelumab (optionally with one or more of nivolumab, lirilumab, and urelumab) for the treatment of solid tumors and/or B-cell non-Hodgkins lymphoma and/or head and neck cancer and/or multiple myeloma. In some embodiments, the immune-modulating agent is an agent that targets one or more of CTLA-4, AP2M1, CD80, CD86, SHP-2, and PPP2R5A. In various embodiments, the immune-modulating agent is an antibody specific for one or more of CTLA-4, AP2M1, CD80, CD86, SHP-2, and PPP2R5A. For instance, in some embodiments, the immune-modulating agent is an antibody such as, by way of non-limitation, ipilimumab (MDX-010, MDX-101, Yervoy, BMS) and/or tremelimumab (Pfizer). In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is combined with ipilimumab (optionally with bavituximab) for the treatment of one or more of melanoma, prostate cancer, and lung cancer. In various embodiments, the immune-modulating agent targets CD20. In various embodiments, the immune-modulating agent is an antibody specific CD20. For instance, in some embodiments, the immune-modulating agent is an antibody such as, by way of non-limitation, Ofatumumab (GENMAB), obinutuzumab (GAZYVA), AME-133v (APPLIED MOLECULAR EVOLUTION), Ocrelizumab (GENENTECH), TRU-015 (TRUBION/EMERGENT), veltuzumab (IMMU-106).

In some embodiments, the present invention relates to combination therapy with one or more chimeric agents described in WO 2013/10779, WO 2015/007536, WO 2015/007520, WO 2015/007542, and WO 2015/007903, the entire contents of which are hereby incorporated by reference in their entireties.

In some embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex described herein, include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the composition such that covalent attachment does not prevent the activity of the composition. For example, but not by way of limitation, derivatives include composition that have been modified by, *inter alia*, glycosylation, lipidation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, *etc*. Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, *etc.*

In still other embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex described herein further comprise a cytotoxic agent, comprising, in illustrative embodiments, a toxin, a chemotherapeutic agent, a radioisotope, and an agent that causes apoptosis or cell death. Such agents may be conjugated to a composition described herein.

The chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex described herein may thus be modified post-translationally to add effector moieties such as chemical linkers, detectable moieties such as for example fluorescent dyes, enzymes, substrates, bioluminescent materials, radioactive materials, and chemiluminescent moieties, or functional moieties such as for example streptavidin, avidin, biotin, a cytotoxin, a cytotoxic agent, and radioactive materials.

Illustrative cytotoxic agents include, but are not limited to, methotrexate, aminopterin, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine; alkylating agents such as mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU), mitomycin C, lomustine (CCNU), 1-methylnitrosourea, cyclothosphamide, mechlorethamine, busulfan, dibromomannitol, streptozotocin, mitomycin C, cis-dichlorodiamine platinum (II) (DDP) cisplatin and carboplatin (paraplatin); anthracyclines include daunorubicin (formerly daunomycin), doxorubicin (adriamycin), detorubicin, carminomycin, idarubicin, epirubicin, mitoxantrone and bisantrene; antibiotics include dactinomycin (actinomycin D), bleomycin, calicheamicin, mithramycin, and anthramycin (AMC); and antimytotic agents such as the vinca alkaloids, vincristine and vinblastine. Other cytotoxic agents include paclitaxel (taxol), ricin, pseudomonas exotoxin, gemcitabine, cytochalasin B, gramicidin D, ethidium bromide, emetine, etoposide, tenoposide, colchicin, dihydroxy anthracin dione, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), interferons, and mixtures of these cytotoxic agents.

Further cytotoxic agents include, but are not limited to, chemotherapeutic agents such as carboplatin, cisplatin, paclitaxel, gemcitabine, calicheamicin, doxorubicin, 5-fluorouracil, mitomycin C, actinomycin D, cyclophosphamide, vincristine, bleomycin, VEGF antagonists, EGFR antagonists, platins, taxols, irinotecan, 5-fluorouracil, gemcytabine, leucovorine, steroids, cyclophosphamide, melphalan, vinca alkaloids (*e.g.*, vinblastine, vincristine, vindesine and vinorelbine), mustines, tyrosine kinase inhibitors, radiotherapy, sex hormone antagonists, selective androgen receptor modulators, selective estrogen receptor modulators, PDGF antagonists, TNF antagonists, IL-1 antagonists, interleukins (*e.g.* IL-12 or IL-2), IL-12R antagonists, Toxin conjugated monoclonal antibodies, tumor antigen specific monoclonal antibodies, Erbitux, Avastin, Pertuzumab, anti-CD20 antibodies, Rituxan, ocrelizumab, ofatumumab, DXL625, HERCEPTIN®, or any combination thereof. Toxic enzymes from plants and bacteria such as ricin, diphtheria toxin and Pseudomonas toxin may be conjugated to the therapeutic agents (*e.g.* antibodies) to generate cell-type-specific-killing reagents (Youle, *et al.*, Proc. Nat'l Acad. Sci. USA 77:5483 (1980); Gilliland, *et al.*, Proc. Nat'l Acad. Sci. USA 77:4539 (1980); Krolick, *et al.*, Proc. Nat'l Acad. Sci. USA 77:5419 (1980)).

Other cytotoxic agents include cytotoxic ribonucleases as described by Goldenberg in U.S. Pat. No. 6,653,104. Embodiments of the invention also relate to radioimmunoconjugates where a radionuclide that emits alpha or beta

particles is stably coupled to the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex, with or without the use of a complex-forming agent. Such radionuclides include beta-emitters such as Phosphorus-32, Scandium-47, Copper-67, Gallium-67, Yttrium-88, Yttrium-90, Iodine-125, Iodine-131, Samarium-153, Lutetium-177, Rhenium-186 or Rhenium-188, and alpha-emitters such as Astatine-211, Lead-212, Bismuth-212, Bismuth-213 or Actinium-225.

Illustrative detectable moieties further include, but are not limited to, horseradish peroxidase, acetylcholinesterase, alkaline phosphatase, beta-galactosidase and luciferase. Further illustrative fluorescent materials include, but are not limited to, rhodamine, fluorescein, fluorescein isothiocyanate, umbelliferone, dichlorotriazinylamine, phycoerythrin and dansyl chloride. Further illustrative chemiluminescent moieties include, but are not limited to, luminol. Further illustrative bioluminescent materials include, but are not limited to, luciferin and aequorin. Further illustrative radioactive materials include, but are not limited to, Iodine-125, Carbon-14, Sulfur-35, Tritium and Phosphorus-32.

Methods of Treatment

Methods and compositions described herein have application to treating various diseases and disorders, including, but not limited to cancer, infections, immune disorders, anemia, autoimmune diseases, cardiovascular diseases, wound healing, ischemia-related diseases, neurodegenerative diseases, metabolic diseases and many other diseases and disorders.

Further, any of the present agents may be for use in the treating, or the manufacture of a medicament for treating, various diseases and disorders, including, but not limited to cancer, infections, immune disorders, inflammatory diseases or conditions, and autoimmune diseases.

In some embodiments, the present invention relates to the treatment of, or a patient having one or more of chronic granulomatous disease, osteopetrosis, idiopathic pulmonary fibrosis, Friedreich's ataxia, atopic dermatitis, Chagas disease, cancer, heart failure, autoimmune disease, sickle cell disease, thalassemia, blood loss, transfusion reaction, diabetes, vitamin B12 deficiency, collagen vascular disease, Shwachman syndrome, thrombocytopenic purpura, Celiac disease, endocrine deficiency state such as hypothyroidism or Addison's disease, autoimmune disease such as Crohn's Disease, systemic lupus erythematosis, rheumatoid arthritis or juvenile rheumatoid arthritis, ulcerative colitis immune disorders such as eosinophilic fasciitis, hypoimmunoglobulinemia, or thymoma/thymic carcinoma, graft versus host disease, preleukemia, Nonhematologic syndrome (*e.g.* Down's, Dubowwitz, Seckel), Felty syndrome, hemolytic uremic syndrome, myelodysplasic syndrome, nocturnal paroxysmal hemoglobinuria, osteomyelofibrosis, pancytopenia, pure red-cell aplasia, Schoenlein-Henoch purpura, malaria, protein starvation, menorrhagia, systemic sclerosis, liver cirrhosis, hypometabolic states, and congestive heart failure.

In some embodiments, the present invention is related to a method for treating cancer, comprising administering an effective amount of i) the chimeric protein, the chimeric protein complex and/or the Fc-based chimeric protein complex to a patient in need thereof; ii) a recombinant nucleic acid encoding the chimeric protein, the chimeric protein complex and/or the Fc-based chimeric protein complex to a patient in need thereof; or iii) a host cell

comprising the recombinant nucleic acid encoding the chimeric protein, the chimeric protein complex and/or the Fc-based chimeric protein complex to a patient in need thereof.

In some embodiments, the present invention relates to the treatment of, or a patient having one or more of chronic granulomatous disease, osteopetrosis, idiopathic pulmonary fibrosis, Friedreich's ataxia, atopic dermatitis, Chagas disease, mycobacterial infections, cancer, scleroderma, hepatitis, hepatitis C, septic shock, and rheumatoid arthritis.

In some embodiments, the present invention relates to the treatment of, or a patient having cancer. As used herein, cancer refers to any uncontrolled growth of cells that may interfere with the normal functioning of the bodily organs and systems, and includes both primary and metastatic tumors. Primary tumors or cancers that migrate from their original location and seed vital organs can eventually lead to the death of the subject through the functional deterioration of the affected organs. A metastasis is a cancer cell or group of cancer cells, distinct from the primary tumor location, resulting from the dissemination of cancer cells from the primary tumor to other parts of the body. Metastases may eventually result in death of a subject. For example, cancers can include benign and malignant cancers, polyps, hyperplasia, as well as dormant tumors or micrometastases.

Illustrative cancers that may be treated include, but are not limited to, carcinomas, *e.g.* various subtypes, including, for example, adenocarcinoma, basal cell carcinoma, squamous cell carcinoma, and transitional cell carcinoma), sarcomas (including, for example, bone and soft tissue), leukemias (including, for example, acute myeloid, acute lymphoblastic, chronic myeloid, chronic lymphocytic, and hairy cell), lymphomas and myelomas (including, for example, Hodgkin and non-Hodgkin lymphomas, light chain, non-secretory, MGUS, and plasmacytomas), and central nervous system cancers (including, for example, brain (*e.g.* gliomas (*e.g.* astrocytoma, oligodendroglioma, and ependymoma), meningioma, pituitary adenoma, and neuromas, and spinal cord tumors (*e.g.* meningiomas and neurofibroma).

Illustrative cancers that may be treated include, but are not limited to, basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.*, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma (*e.g.*, Kaposi's sarcoma); skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL;

mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (*e.g.* that associated with brain tumors), and Meigs' syndrome. In an embodiment, the present invention relates to the treatment of leukemia including hairy cell leukemia. In another embodiment, the present invention relates to the treatment of melanoma including malignant melanoma. In a further embodiment, the present invention relates to the treatment of Kaposi's sarcoma including AIDS-related Kaposi's sarcoma.

In some embodiments, the present invention relates to the treatment of, or a patient having a microbial infection and/or chronic infection. Illustrative infections include, but are not limited to, Chagas disease, HIV/AIDS, tuberculosis, osteomyelitis, hepatitis B, hepatitis C, Epstein-Barr virus or parvovirus, T cell leukemia virus, bacterial overgrowth syndrome, fungal or parasitic infections.

In some embodiments, the present invention relates to the treatment of hepatitis. Illustrative hepatitis that may be treated include, but is not limited to, hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E, autoimmune hepatitis, alcoholic hepatitis, acute hepatitis, and chronic hepatitis.

In an illustrative embodiment, the present invention relates to the treatment of chronic hepatitis C. In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention may be utilized to treat a patient infected with any one of the hepatitis C genotypes, including genotype 1 (*e.g.*, 1a, 1b), genotype 2 (*e.g.* 2a, 2b, 2c and 2d), genotype 3 (*e.g.*, 3a, 3b, 3c, 3d, 3e, and 3f), genotype 4 (*e.g.*, 4a, 4b, 4c, 4d, 4e, 4f, 4g, 4h, 4i and 4j), genotype 5a, and genotype 6a.

In various embodiments, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention may be utilized to treat patients who are poorly or non-responsive to standard of care antiviral therapy or who are otherwise difficult to treat with standard of care hepatitis C treatment. In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be utilized to treat a patient who shows low or no response to IFN-α therapy (*e.g.*, IFN-α2a or IFN-α2b or pegylated IFN-α) with or without ribavirin. In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be utilized to treat a patient who shows low or no response to combination therapy of pegylated interferon and ribavirin. In an embodiment, the present invention is directed to the treatment of patients infected with hepatitis C genotype 1 or any other genotype who did not respond to previous IFN-α therapy. In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention may be used to treat a patient with high baseline viral load (*e.g.*, greater than 800,000 IU/mL). In an embodiment, the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention may be utilized to treat patients with severe liver damage including those patients with advanced liver fibrosis and/or liver cirrhosis.

In some embodiments, the present invention relates to the treatment of patients who are naive to antiviral therapy. In other embodiments, the present invention relates to the treatment of patients who did not respond to previous

antiviral therapy. In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be used to treat relapsed patients.

In some embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be effective in treating hepatitis infection in all ethnic groups including white, African-American, Hispanic, and Asian. In an embodiment, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be particularly effective in treating African-Americans who are otherwise poorly responsive to IFN-α therapy with or without ribavirin.

In various embodiments, the targeted chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention provides improved safety compared to, *e.g.*, untargeted IFNα1 or an unmodified, wildtype IFNα1 or a modified IFNα1 (*e.g.*, pegylated IFNα1). In illustrative embodiments, administration of the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex is associated with minimal side effects such as those side effects associated with the use of the untargeted IFNα1 or an unmodified, wildtype IFN-α or a modified IFN-α (*e.g.*, influenza-like symptoms, myalgia, leucopenia, thrombocytopenia, neutropenia, depression, and weight loss).

In some embodiments, the targeted chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention shows improved therapeutic activity compared to untargeted IFNα1 or an unmodified, wildtype IFNα1, or a modified IFNα1 (*e.g.*, pegylated IFNα1). In some embodiments, the targeted chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex of the invention shows improved pharmacokinetic profile (*e.g.*, longer serum half-life and stability) compared to untargeted IFNα1 or an unmodified, wildtype IFNα1 or a modified IFNα1 (*e.g.*, pegylated IFNα1).

Without wishing to be bound by theory, it is believed that due to such advantageous safety and pharmacokinetic and therapeutic profiles, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be used to treat patients at high dosages and/or for prolonged periods of time. For example, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be used at high dosages for initial induction therapy against chronic hepatitis C infection. In another example, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex may be used for long-term maintenance therapy to prevent disease relapse.

In various embodiments, the present compositions are used to treat or prevent one or more inflammatory diseases or conditions, such as inflammation, acute inflammation, chronic inflammation, respiratory disease, atherosclerosis, restenosis, asthma, allergic rhinitis, atopic dermatitis, septic shock, rheumatoid arthritis, inflammatory bowel disease, inflammatory pelvic disease, pain, ocular inflammatory disease, celiac disease, Leigh Syndrome, Glycerol Kinase Deficiency, Familial eosinophilia (FE), autosomal recessive spastic ataxia, laryngeal inflammatory disease; Tuberculosis, Chronic cholecystitis, Bronchiectasis, Silicosis and other pneumoconioses.

In various embodiments, the present compositions are used to treat or prevent one or more autoimmune diseases or conditions, such as multiple sclerosis, diabetes mellitus, lupus, celiac disease, Crohn's disease, ulcerative colitis, Guillain-Barre syndrome, scleroderms, Goodpasture's syndrome, Wegener's granulomatosis, autoimmune

epilepsy, Rasmussen's encephalitis, Primary biliary sclerosis, Sclerosing cholangitis, Autoimmune hepatitis, Addison's disease, Hashimoto's thyroiditis, Fibromyalgia, Menier's syndrome; transplantation rejection (*e.g.*, prevention of allograft rejection) pernicious anemia, rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, Sjogren's syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, Reiter's syndrome, Grave's disease, and other autoimmune diseases.

In various embodiments, the present compositions are used to treat, control or prevent cardiovascular disease, such as a disease or condition affecting the heart and vasculature, including but not limited to, coronary heart disease (CHD), cerebrovascular disease (CVD), aortic stenosis, peripheral vascular disease, atherosclerosis, arteriosclerosis, myocardial infarction (heart attack), cerebrovascular diseases (stroke), transient ischemic attacks (TIA), angina (stable and unstable), atrial fibrillation, arrhythmia, vavular disease, and/or congestive heart failure.

In various embodiments, the present compositions are used to treat or prevent one or more metabolic-related disorders. In various embodiments, the present invention is useful for the treatment, controlling or prevention of diabetes, including Type 1 and Type 2 diabetes and diabetes associated with obesity. The compositions and methods of the present invention are useful for the treatment or prevention of diabetes-related disorders, including without limitation diabetic nephropathy, hyperglycemia, impaired glucose tolerance, insulin resistance, obesity, lipid disorders, dyslipidemia, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, low HDL levels, high LDL levels, atherosclerosis and its sequelae, vascular restenosis, irritable bowel syndrome, inflamatory bowel disease, including Crohn's disease and ulcerative colitis, other inflammatory conditions, pancreatitis, abdominal obesity, neurodegenerative disease, retinopathy, neoplastic conditions, adipose cell tumors, adipose cell carcinomas, such as liposarcoma, prostate cancer and other cancers, including gastric, breast, bladder and colon cancers, angiogenesis, Alzheimer's disease, psoriasis, high blood pressure, Metabolic Syndrome (*e.g.* a person has three or more of the following disorders: abdominal obesity, hypertriglyceridemia, low HDL cholesterol, high blood pressure, and high fasting plasma glucose), ovarian hyperandrogenism (polycystic ovary syndrome), and other disorders where insulin resistance is a component, such as sleep apnea. The compositions and methods of the present invention are useful for the treatment, control, or prevention of obesity, including genetic or environmental, and obesity-related disorders. The obesity-related disorders herein are associated with, caused by, or result from obesity. Examples of obesity-related disorders include obesity, diabetes, overeating, binge eating, and bulimia, hypertension, elevated plasma insulin concentrations and insulin resistance, dyslipidemia, hyperlipidemia, endometrial, breast, prostate, kidney and colon cancer, osteoarthritis, obstructive sleep apnea, gallstones, heart disease, abnormal heart rhythms and arrythmias, myocardial infarction, congestive heart failure, coronary heart disease, sudden death, stroke, polycystic ovary disease, craniopharyngioma, Prader-Willi Syndrome, Frohlich's syndrome, GH-deficient subjects, normal variant short stature, Turner's syndrome, and other pathological conditions showing reduced metabolic activity or a decrease in resting energy expenditure as a percentage of total fat-free mass, e.g, children with acute lymphoblastic leukemia. Further examples of obesity-related disorders are Metabolic Syndrome, insulin resistance syndrome, reproductive hormone abnormalities, sexual and reproductive dysfunction, such as impaired fertility, infertility, hypogonadism in males and hirsutism in females, fetal defects

associated with maternal obesity, gastrointestinal motility disorders, such as obesity-related gastro-esophageal reflux, respiratory disorders, such as obesity-hypoventilation syndrome (Pickwickian syndrome), breathlessness, cardiovascular disorders, inflammation, such as systemic inflammation of the vasculature, arteriosclerosis, hypercholesterolemia, lower back pain, gallbladder disease, hyperuricemia, gout, and kidney cancer, and increased anesthetic risk. The compositions and methods of the present invention are also useful to treat Alzheimer's disease.

In various embodiments, the present compositions are used to treat or prevent one or more respiratory diseases, such as idiopathic pulmonary fibrosis (IPF), asthma, chronic obstructive pulmonary disease (COPD), bronchiectasis, allergic rhinitis, sinusitis, pulmonary vasoconstriction, inflammation, allergies, impeded respiration, respiratory distress syndrome, cystic fibrosis, pulmonary hypertension, pulmonary vasoconstriction, emphysema, Hantavirus pulmonary syndrome (HPS), Loeffler's syndrome, Goodpasture's syndrome, Pleurisy, pneumonitis, pulmonary edema, pulmonary fibrosis, Sarcoidosis, complications associated with respiratory syncitial virus infection, and other respiratory diseases.

In some embodiments, the present invention is used to treat or prevent one or more neurodegenerative disease. Illustrative neurodegenerative diseases include, but are not limited to, Friedreich's Ataxia, multiple sclerosis (including without limitation, benign multiple sclerosis; relapsing-remitting multiple sclerosis (RRMS); secondary progressive multiple sclerosis (SPMS); progressive relapsing multiple sclerosis (PRMS); and primary progressive multiple sclerosis (PPMS)), Alzheimer's. disease (including, without limitation, Early-onset Alzheimer's, Late-onset Alzheimer's, and Familial Alzheimer's disease (FAD), Parkinson's disease and parkinsonism (including, without limitation, Idiopathic Parkinson's disease, Vascular parkinsonism, Drug-induced parkinsonism, Dementia with Lewy bodies, Inherited Parkinson's, Juvenile Parkinson's), Huntington's disease, Amyotrophic lateral sclerosis (ALS, including, without limitation, Sporadic ALS, Familial ALS, Western Pacific ALS, Juvenile ALS, Hiramaya Disease).

In various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex finds use in treating wounds, *e.g.*, a non-healing wound, an ulcer, a burn, or frostbite, a chronic or acute wound, open or closed wound, internal or external wound (illustrative external wounds are penetrating and non-penetrating wound.

In various embodiments, the present chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complexes find use in treating ischemia, by way of non-limiting example, ischemia associated with acute coronary syndrome, acute lung injury (ALI), acute myocardial infarction (AMI), acute respiratory distress syndrome (ARDS), arterial occlusive disease, arteriosclerosis, articular cartilage defect, aseptic systemic inflammation, atherosclerotic cardiovascular disease, autoimmune disease, bone fracture, bone fracture, brain edema, brain hypoperfusion, Buerger's disease, burns, cancer, cardiovascular disease, cartilage damage, cerebral infarct, cerebral ischemia, cerebral stroke, cerebrovascular disease, chemotherapy-induced neuropathy, chronic infection, chronic mesenteric ischemia, claudication, congestive heart failure, connective tissue damage, contusion, coronary artery disease (CAD), critical limb ischemia (CLI), Crohn's disease, deep vein thrombosis, deep wound, delayed

ulcer healing, delayed wound-healing, diabetes (type I and type II), diabetic neuropathy, diabetes induced ischemia, disseminated intravascular coagulation (DIC), embolic brain ischemia, frostbite, graft-versus-host disease, hereditary hemorrhagic telengiectasiaischemic vascular disease, hyperoxic injury, hypoxia, inflammation, inflammatory bowel disease, inflammatory disease, injured tendons, intermittent claudication, intestinal ischemia, ischemia, ischemic brain disease, ischemic heart disease, ischemic peripheral vascular disease, ischemic placenta, ischemic renal disease, ischemic vascular disease, ischemic-reperfusion injury, laceration, left main coronary artery disease, limb ischemia, lower extremity ischemia, myocardial infarction, myocardial ischemia, organ ischemia, osteoarthritis, osteoporosis, osteosarcoma, Parkinson's disease, peripheral arterial disease (PAD), peripheral artery disease, peripheral ischemia, peripheral neuropathy, peripheral vascular disease, pre-cancer, pulmonary edema, pulmonary embolism, remodeling disorder, renal ischemia, retinal ischemia, retinopathy, sepsis, skin ulcers, solid organ transplantation, spinal cord injury, stroke, subchondral-bone cyst, thrombosis, thrombotic brain ischemia, tissue ischemia, transient ischemic attack (TIA), traumatic brain injury, ulcerative colitis, vascular disease of the kidney, vascular inflammatory conditions, von Hippel-Lindau syndrome, or wounds to tissues or organs

In various embodiments, the present invention relates to the treatment of one or more of anemia, including anemia resulting from chronic kidney disease (*e.g.* from dialysis) and/or an anti-cancer agent (*e.g.* chemotherapy and/or HIV treatment (*e.g.* Zidovudine (INN) or azidothymidine (AZT)), inflammatory bowel disease (*e.g.* Crohn's disease and ulcer colitis), anemia linked to inflammatory conditions (*e.g.* arthritis, lupus, IBD), anemia linked to diabetes, schizophrenia, cerebral malaria, as aplastic anemia, and myelodysplasia from the treatment of cancer (*e.g.* chemotherapy and/or radiation), and various myelodysplastic syndrome diseases (*e.g.* sickle cell anemia, hemoglobin SC disease, hemoglobin C disease, alpha- and beta-thalassemias, neonatal anemia after premature birth, and comparable conditions).

In some embodiments, the present invention relates to the treatment of, or a patient having anemia, *i.e.* a condition in which the number of red blood cells and/or the amount of hemoglobin found in the red blood cells is below normal. In various embodiments, the anemia may be acute or chronic. For example, the present anemias include but are not limited to iron deficiency anemia, renal anemia, anemia of chronic diseases/inflammation, pernicious anemia such as macrocytic achylic anemia, juvenile pernicious anemia and congenital pernicious anemia, cancer-related anemia, anti-cancer-related anemia (*e.g.* chemotherapy-related anemia, radiotherapy-related anemia), pure red cell aplasia, refractory anemia with excess of blasts, aplastic anemia, X-lined siderobalstic anemia, hemolytic anemia, sickle cell anemia, anemia caused by impaired production of ESA, myelodysplasia syndromes, hypochromic anemia, microcytic anemia, sideroblastic anemia, autoimmune hemolytic anemia, Cooley's anemia, Mediterranean anemia, Diamond Blackfan anemia, Fanconi's anemia and drug-induced immune hemolytic anemia. Anemia may cause serious symptoms, including hypoxia, chronic fatigue, lack of concentration, pale skin, low blood pressure, dizziness and heart failure.

In some embodiments, the present invention relates to the treatment of anemia resulting from chronic renal failure. In some embodiments, the present invention relates to the treatment of anemia resulting from the use of one or

more renal replacement therapies, inclusive of dialysis, hemodialysis, peritoneal dialysis, hemofiltration, hemodiafiltration, and renal transplantation.

In some embodiments, the present invention relates to the treatment of anemia in patients with chronic kidney disease who are not on dialysis. For instance, the present invention relates to patients in stage 1 CKD, or stage 2 CKD, or stage 3 CKD, or stage 4 CKD, or stage 5 CKD. In some embodiments, the present patient is stage 4 CKD or stage 5 CKD. In some embodiments, the present patient has undergone a kidney transplant. In some embodiments, the present invention relates to the treatment of anemia is a patient having an acute kidney injury (AKI).

In some embodiments, the anemia is induced by chemotherapy. For instance, the chemotherapy may be any myelosuppressive chemotherapy. In some embodiment, the chemotherapy is one or more of Revlimid, Thalomid, dexamethasone, Adriamycin and Doxil. In some embodiments, the chemotherapy is one or more platinum-based drugs including cisplatin (*e.g.* PLATINOL) and carboplatin (*e.g.* PARAPLATIN). In some embodiments, the chemotherapy is any one of the chemotherapeutic agents described herein. In some embodiments, the chemotherapy is any agent described in Groopman *et al.* J Natl Cancer Inst (1999) 91 (19): 1616-1634, the contents of which are hereby incorporated by reference in their entireties. In some embodiments, the present compositions and methods are used in the treatment of chemotherapy-related anemia in later stage cancer patients (*e.g.* a stage IV, or stage III, or stage II cancer). In some embodiments, the present compositions and methods are used in the treatment of chemotherapy-related anemia in cancer patients receiving dose-dense chemotherapy or other aggressive chemotherapy regimens.

In some embodiments, the present invention relates to the treatment of anemia in a patient having one or more blood-based cancers, such as leukemia, lymphoma, and multiple myeloma. Such cancers may affect the bone marrow directly. Further, the present invention relates to metastatic cancer that has spread to the bone or bone marrow. In some embodiments, the present invention relates to the treatment of anemia in a patient undergoing radiation therapy. Such radiation therapy may damage the bone marrow, lowering its ability to make red blood cells. In further embodiments, the present invention relates to the treatment of anemia in a patient having a reduction or deficiency of one or more of iron, vitamin B12, and folic acid. In further embodiments, the present invention relates to the treatment of anemia in a patient having excessive bleeding including without limitation, after surgery or from a tumor that is causing internal bleeding. In further embodiments, the present invention relates to the treatment of anemia in a patient having anemia of chronic disease.

In some embodiments, the present methods and compositions stimulate red blood cell production. In some embodiments, the present methods and compositions stimulate division and differentiation of committed erythroid progenitors in the bone marrow.

Certain embodiments of the present invention are particularly useful for treating chemotherapy-induced anemia in cancer patients. In some embodiments, the present methods and compositions allows for continued administration of the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex after a cancer patient's chemotherapy is finished. In some embodiments, the present methods and compositions allows for

treatment of a cancer patient without dose reduction relative to a non-cancer patient. In some embodiments, the present methods and compositions allows for treatment of a cancer patient receiving chemotherapy and considered curable. In various embodiments, the cancer patient has one or more of a history of blood clots, recent surgery, prolonged periods of bed rest or limited activity, and treatment with a chemotherapeutic agent.

Kits

The invention also provides kits for the administration of any agent described herein (*e.g.* the chimeric proteins or chimeric protein complexes such as Fc-based chimeric protein complex with or without various additional therapeutic agents). The kit is an assemblage of materials or components, including at least one of the inventive pharmaceutical compositions described herein. Thus, in some embodiments, the kit contains at least one of the pharmaceutical compositions described herein.

The exact nature of the components configured in the kit depends on its intended purpose. In one embodiment, the kit is configured for the purpose of treating human subjects.

Instructions for use may be included in the kit. Instructions for use typically include a tangible expression describing the technique to be employed in using the components of the kit to effect a desired outcome, such as to treat cancer. Optionally, the kit also contains other useful components, such as, diluents, buffers, pharmaceutically acceptable carriers, syringes, catheters, applicators, pipetting or measuring tools, bandaging materials or other useful paraphernalia as will be readily recognized by those of skill in the art.

The materials and components assembled in the kit can be provided to the practitioner stored in any convenience and suitable ways that preserve their operability and utility. For example, the components can be provided at room, refrigerated or frozen temperatures. The components are typically contained in suitable packaging materials. In various embodiments, the packaging material is constructed by well-known methods, preferably to provide a sterile, contaminant-free environment. The packaging material may have an external label which indicates the contents and/or purpose of the kit and/or its components.

Definitions

As used herein, “a,” “an,” or “the” can mean one or more than one.

Unless specifically stated or obvious from context, as used herein, the term “or” is understood to be inclusive and covers both “or” and “and”.

Further, the term “about” when used in connection with a referenced numeric indication means the referenced numeric indication plus or minus up to 10% of that referenced numeric indication, *e.g.*, within (plus or minus) 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. For example, the language “about 50” covers the range of 45 to 55.

An “effective amount,” when used in connection with medical uses is an amount that is effective for providing a measurable treatment, prevention, or reduction in the rate of pathogenesis of a disease of interest.

As used herein, something is “decreased” if a read-out of activity and/or effect is reduced by a significant amount, such as by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at

least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, or more, up to and including at least about 100%, in the presence of an agent or stimulus relative to the absence of such modulation. As will be understood by one of ordinary skill in the art, in some embodiments, activity is decreased and some downstream read-outs will decrease but others can increase.

Conversely, activity is "increased" if a read-out of activity and/or effect is increased by a significant amount, for example by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, or more, up to and including at least about 100% or more, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 50-fold, at least about 100-fold, in the presence of an agent or stimulus, relative to the absence of such agent or stimulus.

As referred to herein, all compositional percentages are by weight of the total composition, unless otherwise specified. As used herein, the word "include," and its variants, is intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that may also be useful in the compositions and methods of this technology. Similarly, the terms "can" and "may" and their variants are intended to be non-limiting, such that recitation that an embodiment can or may comprise certain elements or features does not exclude other embodiments of the present technology that do not contain those elements or features.

Although the open-ended term "comprising," as a synonym of terms such as including, containing, or having, is used herein to describe and claim the invention, the present invention, or embodiments thereof, may alternatively be described using alternative terms such as "consisting of" or "consisting essentially of."

As used herein, the words "preferred" and "preferably" refer to embodiments of the technology that afford certain benefits, under certain circumstances. However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful, and is not intended to exclude other embodiments from the scope of the technology.

The amount of compositions described herein needed for achieving a therapeutic effect may be determined empirically in accordance with conventional procedures for the particular purpose. Generally, for administering therapeutic agents for therapeutic purposes, the therapeutic agents are given at a pharmacologically effective dose. A "pharmacologically effective amount," "pharmacologically effective dose," "therapeutically effective amount," or "effective amount" refers to an amount sufficient to produce the desired physiological effect or amount capable of achieving the desired result, particularly for treating the disorder or disease. An effective amount as used herein would include an amount sufficient to, for example, delay the development of a symptom of the disorder or disease, alter the course of a symptom of the disorder or disease (*e.g.*, slow the progression of a symptom of the disease), reduce or eliminate one or more symptoms or manifestations of the disorder or disease, and reverse a symptom of a disorder or disease. Therapeutic benefit also includes halting or slowing the progression of the underlying disease or disorder, regardless of whether improvement is realized.

Effective amounts, toxicity, and therapeutic efficacy can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to about 50% of the population) and the ED50 (the dose therapeutically effective in about 50% of the population). The dosage can vary depending upon the dosage form employed and the route of administration utilized. The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio LD50/ED50. In some embodiments, compositions and methods that exhibit large therapeutic indices are preferred. A therapeutically effective dose can be estimated initially from in vitro assays, including, for example, cell culture assays. Also, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 as determined in cell culture, or in an appropriate animal model. Levels of the described compositions in plasma can be measured, for example, by high performance liquid chromatography. The effects of any particular dosage can be monitored by a suitable bioassay. The dosage can be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment.

In certain embodiments, the effect will result in a quantifiable change of at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 70%, or at least about 90%. In some embodiments, the effect will result in a quantifiable change of about 10%, about 20%, about 30%, about 50%, about 70%, or even about 90% or more. Therapeutic benefit also includes halting or slowing the progression of the underlying disease or disorder, regardless of whether improvement is realized.

As used herein, “methods of treatment” are equally applicable to use of a composition for treating the diseases or disorders described herein and/or compositions for use and/or uses in the manufacture of a medicaments for treating the diseases or disorders described herein. This invention is further illustrated by the following non-limiting examples.

EXAMPLES

The term "AcTaferon" or “AFN” is occasionally used herein to reference an INFα1-based chimeric protein or chimeric protein complex described herein (details are provided in the Examples regarding the format of the chimeric protein, occasionally an INFα2-based chimeric protein or chimeric protein complex is described, as noted).

“IFNα1” and “IFNa1” may be used interchangeably to refer to interferon alpha 1.

“IFNα2” and “IFNa2” may be used interchangeably to refer to interferon alpha 2.

Example 1: Generation, Production and Purification of IFNα1 AcTaferons (AFNs)

To generate AFNs based on IFNα1 fusion proteins, a nucleic acid sequence encoding for IFNα1 was linked, *via* a nucleic acid sequence encoding a flexible 20*GGS flexible linker, to a nucleic acid sequence encoding a VHH targeting human CD20 in pHEN6C vector (under control of the PelB signal peptide) for bacterial expression. A His_6 tag was added at the end for purification.

AFN expression was induced overnight with 1 mM IPTG, cells were pelleted, and periplasmic extracts prepared using TES (0.2 M Tris pH 8.0, 0.5 mM EDTA, 0.5 M sucrose) and TES/4 buffers. Proteins were purified from

extracts using the TALON Metal affinity resin according to the manufacturer's guidelines and imidazole was removed from the samples using PD10 columns (GE HEALTHCARE).

A similar process was used to generate AFNs based on IFN-α2.

Structure and Sequence of IFNα1 AFN

The structure of the IFNα1 AFN is shown below:

CD20 VHH - *(GGS)$_{20}$* - hIFNα1 - His$_6$

The amino acid sequence of the IFNα1 AFN is shown below (the sequence of CD20 VHH is shown in bold letters, the sequence of (GGS)$_{20}$ is shown in italicized letters, and the sequence of hIFNα1 is shown in underlined letters):

QVQLQESGGGLAQAGGSLRLSCAASGRTFSMGWFRQAPGKEREFVAAITYSGGSPYYASSVRGRFTISRDNA KNTVYLQMNSLKPEDTAVYYCAANPTYGSDWNAENWGQGTQVTVSS*VDGGSGGSGGSGGSGGSGGSRSG GSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGS*AAACDLPETHSLDNRRTLMLLAQMSRISPSSCL MDRHDFGFPQEEFDGNQFQKAPAISVLHELIQQIFNLFTTKDSSAAWDEDLLDKFCTELYQQLNDLEACVMQEE RVGETPLMNADSILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRRKELEHHHHHH (SEQ ID NO: 286).

Structure and Sequence of IFN-α2 AFN

The structure of the IFN-α2 AFN is shown below:

CD20 VHH - *(GGS)$_{20}$* – hIFNα2 - His$_6$

The amino acid sequence of the IFN-α2 AFN is shown below (the sequence of CD20 VHH is shown in bold letters, the sequence of (GGS)$_{20}$ is shown in italicized letters, and the sequence of hIFNα2 is shown in underlined letters):

QVQLQESGGGLAQAGGSLRLSCAASGRTFSMGWFRQAPGKEREFVAAITYSGGSPYYASSVRGRFTISRDNA KNTVYLQMNSLKPEDTAVYYCAANPTYGSDWNAENWGQGTQVTVSS*VDGGSGGSGGSGGSGGSGGSRSG GSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGS*AAAMCDLPQTHSLGSRRTLMLLAQMRRISLFS CLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQGV GVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKELEHHHHHH (SEQ ID NO: 287).

Example 2: Methods for STAT1 Phosphorylation in Peripheral Blood Mononuclear Cells (PBMCs) and IFN-responsive reporter activity in HL116 cells

PBMCs from buffy coats of healthy donors were isolated using density gradient centrifugation using Lymphoprep (STEMCELL TECHNOLOGIES). Cells were washed twice with FACS buffer (2% FBS, 1 mM EDTA in PBS) and stained with anti-human CD20 FITC (SINOBIOLOGICALS) for 20 minutes at 4°C. After two washes, cells were stimulated with a serial dilution wild type IFNα2, CD20 VHH-IFNα2, IFNα1 and CD20 VHH-IFNα1 for 15 minutes at 37°C. After fixation (10 minutes, 37°C, Fix Buffer I; BD BIOSCIENCES), permeabilization (30 minutes, on ice, Perm III Buffer I; BD BIOSCIENCES) and washing, cells were stained with anti-STAT1 pY701 Ab (BD Biosciences). Samples were acquired with a Macsquant X instrument (MILTENYI BIOTEC) and analyzed using the FlowLogic software (MILTENYI BIOTEC). Induction of pSTAT1 reflects activation of IFNAR by interferons. The HL116 clone

is derived from the human HT1080 cell line (ATCC CCL-121). It contains the firefly luciferase gene controlled by the IFN-inducible 6-16 promoter. Thus, induction of luciferase reporter expression reflects activation of IFNAR by interferons. Parental HL116 cells were transfected with an expression vector encoding the human CD20 sequence. Stable transfected clones were selected in G418-containing medium. Parental HL116 and HL116-huCD20 cells were seeded overnight at 20.000 cells per 96-well, before stimulation with a serial dilution of IFNα2 and IFNα1 variants for 6 hours. Luciferase activity in cell lysates was measured on an EnSight Multimode Plate Reader (Perkin Elmer).

Example 3: STAT1 Phosphorylation in Peripheral Blood Mononuclear Cells (PBMCs) and IFN-responsive reporter activity in HL116 cells

Data in **FIGs. 21A-D, Figures 22A-D** and **Table 7** clearly illustrate that incorporation of wild type IFNα1 into a chimeric fusion protein, exemplified here by linking IFNα1 to an anti-CD20 VHH, results in reduced IFNAR-stimulatory activity of IFNα1 compared to wild type IFNα1 (as shown here for CD20 negative cells, both for PBMCs and HL116). However, IFNα1 activity was induced/restored specifically on target cells (CD20 positive, for both PBMC and HL116-huCD20). Importantly, the activation of IFNAR signaling was highly selective for targeted (CD20-positive) versus non-targeted (CD20-negative) cells, with about a 200-600 fold targeting selectivity. Instead, in the case of IFNα2, a targeting selectivity of only 20-60-fold was observed in CD20-positive versus CD20 negative PBMCs and HL116 cells, thus approximately 10-fold less favorable for the intended target (CD20-positive cells) than observed for the comparable IFNα1 constructs. Furthermore, it is notable that targeted IFNα1 chimeric fusion protein was substantially more potent than wild type IFNα1 itself in activating IFNAR signaling in target cells (CD20-positive). Indeed, and remarkably, IFNα1 chimeric protein was 30-150 fold more potent than wild type IFNα1 on target cells (depending on cell type). Surprisingly also, both CD20 VHH-IFNα1 and CD20 VHH-IFNα2 chimeric proteins have a similar potency on CD20-positive target cells, while IFNα1 without fusion is significantly less potent compared to IFNα2. Stated another way, wild type IFNα1 is far less potent than IFNα2; however, unexpectedly, when coupled to a targeting moiety, such as a VHH, its on-target activity is similar to the on-target activity of targeted IFNα2, while the off-target activity is higher for targeted IFNα2. Thus, IFNAR activators that combine high potency and high selectivity may be created *de novo* by incorporating wild type IFNα1 in targeted chimeric fusion proteins—in a way and with a potency/selectivity index that is not achieved through use of wild type IFNα2.

Table 7: IFNα1, IFNα2 and variants signalling in PBMC and HL116 cells that express (positive) or do not express (negative) CD20. Note that in the first experiment on PBMC the curves for IFNα2 did not reach a bottom and hence the EC50 needed to be extrapolated.

	Biological activity IFNα1 AFN's					
Exp 1	**EC50 pSTAT1 in PBMC's (ng/ml)**		ratio	**EC50 6-16 reporter activity (ng/ml)**		ratio
	CD20 negative	CD20 positive	CD20 neg/pos	HL116	HL116-huCD20	CD20 neg/pos
IFNα2	0.103	±0.019	±5	0.1756	0.4598	0.4
CD20-IFNα2	3.019	0.149	20	0.9641	0.01567	62
IFNα1	7.515	3.966	2	2.375	3.249	0.7
CD20-IFNα1	24.57	0.1144	215	14.12	0.02408	586
Exp 2	**EC50 pSTAT1 in PBMC's (ng/ml)**		ratio			
	CD20 negative	CD20 positive	CD20 neg/pos			
IFNα2	0.106	0.039	3			
CD20-IFNα2	2.786	0.109	26			
IFNα1	6.774	2.723	2.5			
CD20-IFNα1	27.67	0.085	325			

Data in **Figures 22A-D** and **Table 7** for induction of luciferase activity in HL116 cells are qualitatively comparable with data for pSTAT1 induction in PBMCs data in **Figures 21A-D.** That is CD20 targeting of IFNα1 results in a more pronounced relative increase in IFNAR signalling compared to CD20 targeting wild type IFNα2 (586 versus 62-fold respectively in HL116 cells, and 215 versus 20-fold, respectively, in PBMCs). Remarkably, both CD20-IFNα1 and CD20-IFNα2 have a similar potency on respective CD20-positive PBMC and HL116 target cells, while IFNα1 without fusion is significantly less potent compared to IFNα2 in CD20-positive cells (as well as CD20-negative cells).

Surprisingly, without limitation, the data show an IFNα1-based fusion/chimeric protein to be as potent as a comparable fusion/chimeric protein based on WT IFNα2, despite the reduced activity of IFNα1 as compared to IFNα2 in an unfused, non-chimeric protein setting. Moreover, without limitation, the data show that the IFNα1 fusion is superior over the IFNα2 fusion regarding degree of selectivity for intended target cells versus non-target cells.

Example 4: Generation, Production, Purification and Characterization of Mutant IFNα1 AcTaFerons (AFNs)

Chimeric proteins based on mutant IFNα1 are generated using the following protocol. A nucleic acid sequence encoding for mutant IFNα1 is fused/linked, *via* a nucleic acid sequence encoding a flexible 20*GGS flexible linker,

to a nucleic acid sequence encoding a VHH antibody targeting human CD20 in pHEN6C vector (under control of the PelB signal peptide) for bacterial expression. A sequence encoding a His tag is added at the end for purification. AFN expression is induced overnight with 1 mM IPTG, cells were pelleted, and periplasmic extracts prepared using TES (0.2 M Tris pH 8.0, 0.5 mM EDTA, 0.5 M sucrose) and TES/4 buffers. Proteins were purified from extracts using the TALON Metal affinity resin according to the manufacturer's guidelines and imidazole is removed from the samples using PD10 columns (GE HEALTHCARE).

The mutants for IFNα1 that are prepared are shown in **Table 8** below:

Reduced Cysteine Based Aggregation Mutants	C86S, C88Y, or C86A (knocking out unpaired cysteine)
Attenuated Mutants (or variants) for IFNα1 with single mutations	L15A, A19W, R23A, S25A, L30A, L30V, D32A, R33K, R33A, R33Q, H34A, Q40A, D115R, L118A, K121A, K121E, R126A, R126E, E133A, K134A, K135A, R145A, R145D, R145E, R145G, R145H, R145I, R145K, R145L, R145N, R145Q, R145S, R145T, R145V, R145Y, A146D, A146E, A146G, A146H, A146I, A146K, A146L, A146M, A146N, A146Q, A146R, A146S, A146T, A146V, A146Y, M149A, R150A, S153A, L154A, N157A
Attenuated Mutants (or variants) for IFNα1 with multiple mutations	L30A/H58Y/E59N/Q62S, R33A/H58Y/E59N/Q62S, M149A/H58Y/E59N/Q62S, L154A/H58Y/E59N/Q62S, R145A/H58Y/E59N/Q62S, D115A/R121A, L118A/R121A, L118A/R121A/K122A, R121A/K122A, R121E/K122E

The STAT1 phosphorylation in PBMCs for each of these mutants, in the context of chimeras, identified in **Table 8**, is assessed using the protocol specified in Example 2. Similarly, reporter activity in HL116 wells is characterized for each of the mutants identified in **Table 8** using the protocol identified in Example 2.

Example 5: Generation, Production, Purification and Characterization of Further Mutant IFNα1 AFNs

In this example, we evaluated the activity of IFNα1 fused to a non-antibody type of targeting moiety – specifically FLT3L, which is a known ligand for FLT3, the receptor for FLT3L. The resulting chimeric molecule is therefore also an example of a bifunctional IFNα1 chimeric fusion protein, in the sense that it comprises two effector domains (IFNα1 and FLT3L), of which one of them (FLT3L) also serves as a targeting moiety. FLT3 is expressed on human dendritic cells, such a cDC1 dendritic cells. cDC1 cells are potent activators of T cells and important in promoting immune system-mediated antitumor responses. To evaluate the in vivo efficacy of Flt3-targeted IFNα1 in a humanized mouse (mouse with a human immune system), a Flt3L_linker_humanIFNα1_GGS_his9 fusion protein was expressed in HEKT cells and purified by metal affinity chromatography.

The sequence of the fusion protein contains the sequence of the extracellular domain of Flt3L (bold below; the final 21 residues of the bold residues are part of an extracellular region and not part of receptor binding domain):

Mature Sequence Flt3L (bold)_linker_(underline) humanIFNα1_(bold and underline)LEGGS_his9

TQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQDEELCGGLWRLVLAQRWMERLKTVAGSKMQGLL ERVNTEIHFVTKCAFQPPPSCLRFVQTNISRLLQETSEQLVALKPWITRQNFSRCLELQCQPDSSTLPPPWSPR PLEATAPTAVDGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGS AAA**CDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHDFGFPQEEFDGNQFQKAPAISVLHELIQQIFNLFTTK DSSAAWDEDLLDKFCTELYQQLNDLEACVMQEERVGETPLMNADSILAVKKYFRRITLYLTEKKYSPCAWEVV RAEIMRSLSLSTNLQERLRRKE**LEGGSHHHHHHHHH (SEQ ID NO: 289).

The purified protein was subsequently evaluated in a tumor model in a humanized mouse. In brief, newborn NSG mice (1-2 days of age) were sublethal irradiated with 100 cGy prior to intrahepatic delivery of $1x10^5$ CD34+ human stem cells (from HLA-A2 positive cord bloods). At week 13 after stem cell transfer mice were subcutaneously inoculated with $25x10^5$ human RL follicular lymphoma cells (ATCC CRL-2261; not sensitive to the direct anti-proliferative effect of IFN). Mice were treated daily intraperitoneally with 30 µg of human Flt3L protein, from day 8 to day 18 after tumor inoculation. Daily perilesional injection with buffer or Flt3L-IFNα1 (30 µg) was initiated at day 10 after tumor inoculation, when a palpable tumor was visible (n=5 or 6 mice per group). Tumor size (caliper measurements), body weight and temperature were assessed daily.

Data in **FIG. 23** show the tumor growth until 2 days after the last treatment and demonstrates that targeted-IFNα1 strongly suppresses tumor growth. Data on body weight and temperature did not show any major difference between buffer treatment and IFNα1 treatment supporting that the IFNα1 treatment was well tolerated.

Example 6: Fc-Based Chimeric Protein Complexes with Interferon Alpha 1

This Example evaluates and generates chimer protein complexes based on wild type interferon alpha 1 (IFNα1) and targeted via a human Clec9A specific VHH (clone R1CHCL50). For this purpose, a heterodimeric Fc protein based on knob-into-hole technology was designed.

Constructs:

- R1CHCL50-**20*GGS**-human IgG1 Fc (with hole mutations and effector knock-out mutations) (**P-1451**)

QVQLVESGGGLVHPGGSLRLSCAASGSFSSINVMGWYRQAPGKERELVARITNLGLPNYADSVTGRFTIS RDNAKNTVYLQMNSLKPEDTAVYYCYLVALKAEYWGQGTQVTVSS**GGSGGSGGSGGSGGSGGSGGSG GSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGS**DKTHTCPPCPAPEAAGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCQVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 290)

- human IgG1 Fc (with knob mutations and effector knock-out mutations) -**20*GGS**-IFNα1 (**P-1852**)

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCQVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQ
VSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
HNHYTQKSLSLSPGK**GGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGS**
GGSGGSGGSCDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHDFGFPQEEFDGNQFQKAPAISVLHELIQ
QIFNLFTTKDSSAAWDEDLLDKFCTELYQQLNDLEACVMQEERVGETPLMNADSILAVKKYFRRITLYLTEKK
YSPCAWEVVRAEIMRSLSLSTNLQERLRRKE (SEQ ID NO: 291)

Production and purification of IFNα1 AFN

Constructs R1CHCL50-20*GGS-Fc and Fc--20*GGS-IFNα1 were combined, resulting in an AFN with a structure outlined in **FIG. 7B**, and transiently expressed in the ExpiCHO expression system (Thermo Fisher) according to the manufacturer's guidelines. One week after transfection, supernatant was collected, and cells removed by centrifugation. Recombinant proteins were purified from the supernatant using the Pierce Protein A spin plates (Thermo Fisher).

Biological activity on HL116 reporter cell-lines

The HL116 clone is derived from the human HT1080 cell line (ATCC CCL-121). It contains the firefly luciferase gene controlled by the IFN-inducible 6-16 promoter. Parental HL116 cells were transfected with an expression vector encoding the human Clec9A sequence. Stable transfected clones were selected in G418-containing medium. Parental HL116 and HL116-hClec9A (target) cells were seeded overnight at 20,000 cells per 96-well and subsequently stimulated with a serial dilution of wild type human IFNα1 or the Fc-based CLEC9A targeted IFNα1 construct for 6 hours. Luciferase activity was measured in cell lysates. Data in the **FIG. 20** illustrate that both cell-lines are comparably sensitive to wild type IFNα1. The EC50 of IFNα1-based Fc AFN signaling in targeted cells is 0.3 ng/ml, while no luciferase induction could be measured in parental HL116 cells.

Without wishing to be bound by theory, multiple surprising observations can be made from data shown in **FIG. 20**. While wild type IFNα1 is comparably active on both cell lines (HL116 and HL116-hClec9A cells), the IFNα1-based Fc AFN is in comparison substantially inactive in HLL16 parental cells (no detectable signal at 100 ng/ml, while the EC50 for wild type IFNα1 on these cells is about 2 ng/ml). This shows, inter alia, that fusion of or linking of wild type IFNα1 to an Fc polypeptide and incorporating IFNα1 in a Fc-based chimeric protein complex, leads to a substantial loss of IFNα1 potency in IFNAR-activation. This is similar to observations made for other forms of IFNα1 fusions/chimeric proteins, as shown in Example 2 and Example 3. However, as also observed for other forms of IFNα1 fusions and chimeric proteins, IFNα1 activity is induced and/or restored on target cells (HL116-hClec9A cells). Furthermore, and surprisingly, the Fc-based IFNα1, whose intrinsic IFNα1 activity is severely reduced (to undetectable levels in dose range tested on HL116 cells), is even more potent than wild type IFNa on target cells (while wild type IFNα1 has an EC50 of about 1.6 ng/ml on HL116-CLEC9A cells this improves to 0.3 ng/ml for the fusion protein or 84 and 3.3 pM respectively). In summary, IFNAR activators that combine high potency and high selectivity may be created de novo by incorporating wild type IFNα1 in a targeted (Fc-based chimeric) fusion protein complex – in a way and with a potency/selectivity index that is not achieved through use of wild type IFNα2.

Example 7: Fc-Based Chimeric Protein Complexes Having Interferon Alpha 1 Variants

This Example evaluates and generates chimeric proteins and chimeric protein complexes based on interferon alpha 1 (IFNα1), including modified and activity-attenuated variants thereof, and targeted via a human Clec9A antigen-specific VHH antibody (clone R1CHCL50) to Clec9A-positive cells. For this purpose, illustrative heterodimeric Fc -based proteins based on knob-into-hole Fc-technology were designed, made and evaluated for bioactivity characteristics. This includes IFNα1 variants that comprise one of different mutations that cause change of a cysteine residue (C86) to reduce undesired disulfide pairing, to improve product homogeneity and pharmaceutical properties of the chimeric protein and chimeric protein complexes. The objective was to also evaluate whether such modifications would not substantially interfere with induction of, and/or allow for maintaining or avoiding substantial loss of induction of IFNAR-activation at the target cell (for both IFNα1 attenuated by fusion alone, and IFNα1 that is activity-attenuated by additional mutation). The results show that a unique combination of highly potent and highly cell target-selective IFNAR-signaling activation can be achieved with IFNα1 compositions, and variants thereof, described herein.

Constructs:

- R1CHCL50-**5*GGS**-human IgG1 Fc (with hole mutations and effector knock-out mutations); **P-1479**

qvqlvesggglvhpggslrlscaasgsfssinvmgwyrqapgkerelvaritnlglpnyadsvtgrftisrdnakntvylqmnslkpedtavyycylvalkaeyw gqgtqvtvss**GGSGGSGGSGGSGGS**DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCQVSNKALPAPIEKTISKAKGQPRE PQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 293)

- human IgG1 Fc (with knob mutations and effector knock-out mutations) -**10*GGS**-IFNα1(C86S); **P-2213**

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCQVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK**GGSGGSGGSGGSGGSGGSGGSGGSGGSGGS**CDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHD FGFPQEEFDGNQFQKAPAISVLHELIQQIFNLFTTKDSSAAWDEDLLDKFSTELYQQLNDLEACVMQEERVGETP LMNADSILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRRKE (SEQ ID NO: 294)

- human IgG1 Fc (with knob mutations and effector knock-out mutations) -**10*GGS**-IFNα1(C86S, A146G); **P-2214**

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCQVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK**GGSGGSGGSGGSGGSGGSGGSGGSGGSGGS**CDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHD

FGFPQEEFDGNQFQKAPAISVLHELIQQIFNLFTTKDSSAAWDEDLLDKFSTELYQQLNDLEACVMQEERVGETP
LMNADSILAVKKYFRRITLYLTEKKYSPCAWEVVRGEIMRSLSLSTNLQERLRRKE (SEQ ID NO: 295)

- human IgG1 Fc (with knob mutations and effector knock-out mutations) -**10*GGS**-IFNα1(C86S, M149V); **P-2215**

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCQVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK**GGSGGSGGSGGSGGSGGSGGSGGSGGSGGS**CDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHD
FGFPQEEFDGNQFQKAPAISVLHELIQQIFNLFTTKDSSAAWDEDLLDKFSTELYQQLNDLEACVMQEERVGETP
LMNADSILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIVRSLSLSTNLQERLRRKE (SEQ ID NO: 296)

- human IgG1 Fc (with knob mutations and effector knock-out mutations) -**10*GGS**-IFNα1(C86Y); **P-2216**

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCQVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK**GGSGGSGGSGGSGGSGGSGGSGGSGGSGGS**CDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHD
FGFPQEEFDGNQFQKAPAISVLHELIQQIFNLFTTKDSSAAWDEDLLDKFYTELYQQLNDLEACVMQEERVGETP
LMNADSILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRRKE (SEQ ID NO: 297)

- human IgG1 Fc (with knob mutations and effector knock-out mutations) -**10*GGS**-IFNα1(C86Y, A146G); **P-2217**

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCQVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK**GGSGGSGGSGGSGGSGGSGGSGGSGGSGGS**CDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHD
FGFPQEEFDGNQFQKAPAISVLHELIQQIFNLFTTKDSSAAWDEDLLDKFYTELYQQLNDLEACVMQEERVGETP
LMNADSILAVKKYFRRITLYLTEKKYSPCAWEVVRGEIMRSLSLSTNLQERLRRKE (SEQ ID NO: 298)

- human IgG1 Fc (with knob mutations and effector knock-out mutations) -**10*GGS**-IFNα1(C86Y, M149V); **P-2218**

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCQVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK**GGSGGSGGSGGSGGSGGSGGSGGSGGSGGS**CDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHD
FGFPQEEFDGNQFQKAPAISVLHELIQQIFNLFTTKDSSAAWDEDLLDKFYTELYQQLNDLEACVMQEERVGETP
LMNADSILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIVRSLSLSTNLQERLRRKE (SEQ ID NO: 299)

The construct R1CHCL50-5*GGS-Fc is combined with each of the Fc-10*GGS-IFNα1 variants, resulting in six different AFNs with a structure outlined in **FIG. 7B**, and transiently expressed in the ExpiCHO expression system (Thermo Fisher) according to the manufacturer's guidelines. One week after transfection, supernatant is collected, and cells removed by centrifugation. Recombinant proteins are purified from the supernatant using the Pierce Protein A spin plates (Thermo Fisher) and potency tested as described in Example 6.

Data in Figure 24A-G and Table 8 clearly illustrate, inter alia, that:

(i) Parental HL116 and the derived HL116-Clec9A cells (expressing human Clec9A) are comparably responsive/sensitive to wild type IFNα2a with an EC50 of 8 pM and 6 pM for reporter induction observed in HL116 and HL116-hCLEC9A, respectively. The same was previously observed for IFNα1 (as shown in Example 6, Fig. 20), with IFNα1 being approximately 10-15-fold less potent compared to IFNα2 (IFNα1 EC50 of 105 pM and 85 pM for reporter induction in HL116 and HL116-hCLEC9A, respectively).

(ii) All tested IFNα1 construct variants are substantially less active compared to wild type IFNα2 (and IFNα1 when comparing to Example 6, Fig. 20) on non-target cells (HL116).

(iii) Activity of all tested IFNα1 protein variants can be induced and/or restored at the target cells (Clec9A-positive), being markedly more active on targeted (i.e., Clec9A expressing) cells compared to non-targeted cells (i.e., exhibit high selectivity for target cells).

(iv) Remarkably, induction of IFNAR-activation in target cells by IFNα1 proteins harboring mutation of the free C86 (C to S, or C to Y), and otherwise wild type for IFNα1, is far superior to that obtained with wild type IFNα1 (non-fused, unmodified IFNα1). Even more surprising, activity on target cells is similar to that observed for IFNα2, which is among the most potent type I interferons. Similar observations were made for an Fc-based IFNα1 construct incorporating wild type IFNα1 that does not incorporate a mutation in C86 (see Fig. 20), as also observed for non-Fc chimeric proteins incorporating IFNα1 (see Table 7 for example).

(v) All the Fc-IFNα1 constructs exhibit remarkable selectivity for target cells (which is not observed for non-fused, unmodified wild type IFNα1 or IFNα2).

(vi) IFNα1 constructs with mutation of the free C86, to either S or Y (one amino acid change being conservative, the other non-conservative), have similar bioactivity, and similar to that of equivalent construct without mutation of C86 (see Fig. 20).

(vii) The activity of IFNα1 constructs with mutations A146G or M149V in IFNα1, while further attenuated for IFNAR-activation compared to a fusion without these mutations, is induced and/or restorable at a target cell (Clec9-positive cells). Remarkably, potency of IFNAR-activation is similar to or greater than potency of wild type IFNα1 at target cells.

(viii) IFNα1 chimeric constructs incorporating mutation of the free C86 to S or Y are highly inducible and active at target cells, including constructs harboring additional mutation A146G or M149V.

Table 8: EC50 of IFNAR signaling in HL116 cells expressed in pM. If the reporter activity at the highest concentration was detectable but did not reach EC50 levels this is indicated with ">". In case at the highest concentration no reporter activity was detected this is indicated by ">>".

	EC50 6-16 reporter activity (pM)		
	HL116	**HL116-CLEC9A**	**ratio**
IFNα1 (Example 6)	105	84	1.2
R1CHCL50-Fc3 + Fc-hIFNα1 (Example 6)	>1000	3	>330
IFNα2	8	6	1.3
R1CHCL50-Fc3 + Fc-hIFNα1(C86S)	>1000	curve does not allow EC50 calculation	
R1CHCL50-Fc3 + Fc-hIFNα1(C86Y)	>1000	1	>1000
R1CHCL50-Fc3 + Fc-hIFNα1(C86S-A146G)	>>1000	19	>>50
R1CHCL50-Fc3 + Fc-hIFNα1(C86Y-A146G)	>>1000	7	>>140
R1CHCL50-Fc3 + Fc-hIFNα1(C86S-M149V)	>>1000	60	>>15
R1CHCL50-Fc3 + Fc-hIFNα1(C86Y-M149V)	>>1000	15	>>65

In a second series of experiments, IFNα1 AFNs were tested for ability to promote STAT1 phosphorylation in primary human cDC1 cells (naturally express Clec9A, and the target of the IFNα1 chimeric constructs) compared to other PBMC populations (Clec9A negative cells). In brief, PBMCs from buffy coats of healthy donors were isolated using density gradient centrifugation using Lymphoprep (StemCell technologies). Cells were washed twice with FACS buffer (2% FBS, 1 mM EDTA in PBS) and stained with anti-Clec9A and anti-CD141 Ab's (both Miltenyi) to identify the cDC1 population for 20 minutes at 4°C. After two washes, cells were stimulated with a serial dilution of wild type IFNα2 or IFNα1 AFNs for 15 minutes at 37°C. After fixation (10 minutes, 37°C, Fix Buffer I; BD Biosciences), permeabilization (30 minutes, on ice, Perm III Buffer I; BD Biosciences) and washing, cells were stained with anti-STAT1 pY701 Ab (BD Biosciences). Samples were acquired with a MACSQuant X instrument (Miltenyi Biotec) and analyzed using the FlowLogic software (Miltenyi Biotec). Data in **Figs. 25A-E** are qualitatively comparable to results obtained with the HL-116/HL-116-hClec9A reporter cell lines (see **Figs. 24A-G**), and similar overall conclusion can be drawn from these data. For example, without limitation, the data show that (i) Clec9A-/CD141- and Clec9A+/CD141+ cells are comparably responsive/sensitive to wild type IFN, (ii) all tested targeted IFNα1 variants instead are markedly more active on targeted Clec9A+/CD141+ cells compared to non-targeted (Clec9A-/CD141-) cells, i.e., high targeting and selectivity index is observed, (iii) induction of IFNAR-activation in target cells by IFNα1 proteins harboring mutation of the free C86 (C to S, or C to Y), and otherwise wild type for IFNα1, is far superior to that obtained with wild type, non-fused, unmodified IFNα2. Even more surprising, activity on target cells is similar to that observed for IFNα2, which is among the most potent type I interferons, (iv) IFNα1 activity for IFN1a chimeric constructs harboring mutation A146G, while intrinsically attenuated, is induced and/or

restored in target cells. v) IFNα1 chimeric constructs incorporating mutation of the free C86 to S or Y are highly inducible and active at target cells, including constructs harboring an additional mutation such as A146G.

Together, both datasets illustrate, inter alia, that it is possible to selectively target IFNα1 activity to Clec9A expressing target cells including primary human cells, and achieve high potency of IFNAR-activation, without detectable IFNAR-activation and signaling in non-target cells at equivalent dose/concentration. Both C86S and C86Y mutations allow for removal of the free cysteine residue in the IFNα1 sequence, and hence undesired disulfide pairing, and still achieve a maximum signaling restoration. Surprisingly, on primary cDC1 which are the key target cells for a CLEC9A-targeted construct, the potency of the C86Y IFNα1 variant construct matched the potency of wild type IFNα2 (EC50 of 350 pM for P-1479/P-2216 versus 240 pM for IFNα2) while the selectivity window of the C86Y IFNα1 construct versus non-cDC1 cells was >100 fold.

Even more surprisingly is the finding that on primary cDC1 the potency of the C86S IFNα1 variant construct exceeded the potency of wild type IFNα2 (EC50 of 20 pM for P-1479/P-2213 versus 240 pM for IFNα2) while the selectivity window of the C86S IFNα1 construct versus non-cDC1 cells was about >300 fold.

In summary, the results show that a unique combination of high potency and high cell target-selectivity for IFNAR-signaling activation can be achieved with IFNα1 chimeric protein and protein complex compositions, and variants thereof.

Example 8: PD-L1 Targeted IFNα1 and Variants Thereof

In this example, constructs for Fc-based IFNα1, and variants thereof, and their targeting to PD-L1 expressing cells was evaluated. This includes constructs that incorporate wild type IFNα1 with a C86S mutation (as in some constructs in previous Example 7) and PD-L1 direct VHH antibodies. Two PD-L1-specific VHHs, termed 2LIG99 and 2LIG189, were used in different constructs. Comparable constructs based on use of an activity-attenuated and target-specific restorable mutant IFNα2 were also included, for comparison. Illustrative chimeric proteins and protein complexes were generated that are based on a heterodimeric, 'knob-in-hole' Fc-based AFN format. In the pcDNA3.4 expression vector, the sequence encoding one or two PD-L1 VHHs was, via a flexible 5*GGS-linker, fused/linked to the human IgG1 Fc sequence containing the L234A_L235A_K322Q effector mutations and the 'hole' modifications Y349C_T366S_L368A_Y407V (see sequence below). The second polypeptide that is part of and incorporated into the chimeric Fc-based protein complex, was also cloned in the pcDNA3.4 vector, and consists of the fusion/linkage between the human IgG1 Fc sequence containing the L234A_L235A_K322Q effector mutations and the 'knob' modifications S354C_T366W and the C86S variant of IFNα1.

To produce heterodimeric 'knob-in-hole' Fc-based chimeric protein and protein complexes, the following combinations of both 'hole' and 'knob' plasmids was transfected in ExpiCHO cells (ThermoFisher) according to the manufacturer's instructions:

- **P-1542**: Fc3 + **P-2213**: Fc4-**10*GGS**-hIFNα1_C86S;
- **P-2204**: 2LIG99-**5*GGS**-Fc3 + **P-2213**: Fc4-**10*GGS**-hIFNα1_C86S;

- **P-2206**: 2LIG189-**5*GGS**-Fc3 + **P-2213**: Fc4-**10*GGS**-hIFNα1_C86S;
- **P-2399**: 2LIG99-**20*GGS**-2LIG99-**5*GGS**-Fc3 + **P-2213**: Fc4-**10*GGS**-hIFNα1_C86S; (note: in this construct the PD-L1 VHH 2LIGG99 is repeated in tandem)

In addition, the equivalent constructs for the IFNα2 mutant were generated:

- **P-1542**: Fc3 + Fc4-**10*GGS**-hIFNα2_mut;
- **P-2204**: 2LIG99-**5*GGS**-Fc3 + Fc4-**10*GGS**-hIFNα2_mut;
- **P-2206**: 2LIG189-**5*GGS**-Fc3 + Fc4-**10*GGS**-hIFNα2_mut;
- **P-2399**: 2LIG99-**20*GGS**-2LIG99-**5*GGS**-Fc3 + + Fc4-**10*GGS**-hIFNα2_mut; (note: in this construct the PD-L1 VHH 2LIGG99 is repeated in tandem)

Seven days post transfection, recombinant protein complexes were sequentially purified on a Protein A column and a Superdex 200 Increase 10/300 column (both GE Healthcare) on an ÄKTA pure instrument (GE Healthcare). Proteins were quantified and purity checked on SDS-PAGE. Biological activity of the Fc-based constructs was tested on HL116 cells (an IFN responsive cell-line stably transfected with an IFN-inducible p6-16 luciferase reporter gene) that express PD-L1. Cells were seeded overnight and stimulated for 6 hours with a serial dilution of the different protein construct preparations. Luciferase activity was measured on an EnSight Multimode Plate Reader (Perkin Elmer).

Data in **Fig. 26A** illustrates clearly that Fc-based, PD-L1 targeted IFNα1 constructs are far more active than the untargeted variant, which is substantially inactive, also compared to wild type IFNα1 (unfused, unmodified as shown in Tables 7 and 8 and **Figs. 20** and **22C**). Thus, PD-L1 targeting induces and/or restores IFNα1-mediated IFNAR activation. This behavior is similar to that observed with targeting of IFNα1 that is incorporated into chimeric proteins or protein complexes, and variants thereof, to other target antigens (e.g., Clec9A and CD20: see Examples 2, 3, 7 and Tables 7 and 8), indicating that targeting is a generic approach to induce quenched or attenuated IFNα1 activity at target cells in a selective manner. Most surprisingly, induction of activity can reach or substantially overshoot that of wild type IFNα1. Interestingly, activity may be further enhanced by creating constructs that incorporate more than one copy of a targeting moiety, as shown for the (2LIG99)2-Fc3 + Fc4-IFNα1 construct (which comprises two copies of the PD-L1 VHH 2LIG99), which was able to reach potency of wild type human IFNα2 for IFNAR activation (while an untargeted Fc-IFNα1 construct has no significantly detectable activity over a large concentration range.

Fig. 26B shows similar analysis for constructs that incorporate a mutant form of IFNα2 instead of IFNα1. The mutant IFNα2 is a variant of IFNα2 that is substantially attenuated for IFNAR binding and activity, as indicated by the loss in IFNAR-activation and signaling activity of the untargeted Fc-based IFNα2 mutant (e.g., compared to wild type IFNα2 or other constructs). When targeted to PD-L1, IFNα2 activity is induced and/or restored. This is enhanced by integrating two copies of the PD-L1 VHH in the construct. Surprisingly, all comparable IFNα1 Fc-based constructs were more potent than the respective constructs incorporating mutant IFNα2: with EC50s on average 6-fold lower than those for respective IFNα2_mut constructs.

These findings, in conjunction with similar findings for various IFNα1 chimeric protein and chimeric protein complex constructs, and variants thereof, further demonstrate the extraordinary, unexpected and unique combination of potency and selectivity for IFNAR-activation that can be achieved on target cells when incorporating IFNα1, and variants thereof, in chimeric proteins and protein complexes.

Sequences:

1. **P-1542**: Fc3
DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCQVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 300)

2. **P-2204**: 2LIG99-**5*GGS**-Fc3
DVQLVESGGGLVQPGGSLRLSCTASGTIFSINRMDWFRQAPGKQRELVALITSGGTPAYADSAKGRFTISRDNSKNTVYLQMNSLRPEDTAVYYCHVSSGVYNYWGQGTLVTVSS**GGSGGSGGSGGSGGS**DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCQVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 301)

3. **P-2206**: 2LIG189-**5*GGS**-Fc3
DVQLVESGGGLVQPGGSLRLSCAASGKIFSGNHMGWYRQAPGKQRELVGIITSGGITDYADSVKGRFTISRDNSKNTVYLQMNSLRPEDTAVYYCNVRDRTIWWGQGTLVTVSS**GGSGGSGGSGGSGGS**DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCQVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 302)

4. **P-2399**: 2LIG99-**20*GGS**-2LIG99-**5*GGS**-Fc3
DVQLQESGGGLVQPGGSLRLSCTASGTIFSINRMDWFRQAPGKQRELVALITSGGTPAYADSAKGRFTISRDNSKNTVYLQMNSLRPEDTAVYYCHVSSGVYNYWGQGTLVTVSS**GGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGS**DVQLVESGGGLVQPGGSLRLSCTASGTIFSINRMDWFRQAPGKQRELVALITSGGTPAYADSAKGRFTISRDNSKNTVYLQMNSLRPEDTAVYYCHVSSGVYNYWGQGTLVTVSS**GGSGGSGGSGGSGGS**DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCQVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 303)

5. **P-2213**: Fc4-**10*GGS**-hIFNα1_C86S

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCQVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK**GGSGGSGGSGGSGGSGGSGGSGGSGGSGGS**CDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHD
FGFPQEEFDGNQFQKAPAISVLHELIQQIFNLFTTKDSSAAWDEDLLDKF**S**TELYQQLNDLEACVMQEERVGETP
LMNADSILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRRKE (SEQ ID NO: 294)

EQUIVALENTS

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific embodiments described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

INCORPORATION BY REFERENCE

All patents and publications referenced herein are hereby incorporated by reference in their entireties.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

As used herein, all headings are simply for organization and are not intended to limit the disclosure in any manner. The content of any individual section may be equally applicable to all sections.

CLAIMS

What is claimed is:

1. A chimeric protein comprising:

(a) an interferon alpha 1 (IFNα1) or a variant thereof, and

(b) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest;

wherein the IFNα1 or the variant thereof, and the one or more targeting moieties are optionally connected with one or more linkers.

2. The chimeric protein of claim 1, wherein the IFNα1 comprises an amino acid sequence having at least about 90%, or at least about 93%, or at least about 95%, or at least about 97%, or at least about 98%, or at least about 99% identity with SEQ ID NO:1.

3. The chimeric protein of claim 1 or 2, wherein the IFNα1 comprises the amino acid sequence of SEQ ID NO:1, optionally wherein the IFNα1 is pegylated, one or more targeting moieties are pegylated, or a linker is pegylated.

4. The chimeric protein of claim 1 or 2, wherein the variant IFNα1 comprises one or more mutations.

5. The chimeric protein of claim 4, wherein the one or more mutations of the variant IFNα1 confer reduced affinity for interferon-α/β receptor (IFNAR).

6. The chimeric protein of claim 5, wherein the IFNα1 exhibits reduced affinity for IFNAR1.

7. The chimeric protein of claim 5, wherein the IFNα1 exhibits reduced affinity for IFNAR2.

8. The chimeric protein of claim 5, wherein the amino acid position of the one or more mutations of the variant IFNα1 is selected from the group consisting of L15, A19, R23, S25, L30, D32, R33, H34, Q40, D115, L118, K121, R126, E133, K134, K135, R145, A146, M149, R150, S153, L154, and N157 or a combination thereof, wherein the positions are in reference to SEQ ID NO: 1, optionally selected from the group consisting of L15A, A19W, R23A, S25A, L30A, L30V, D32A, R33K, R33A, R33Q, H34A, Q40A, D115R, L118A, K121A, K121E, R126A, R126E, E133A, K134A, K135A, R145A, R145D, R145E, R145G, R145H, R145I, R145K, R145L, R145N, R145Q, R145S, R145T, R145V, R145Y, A146D, A146E, A146G, A146H, A146I, A146K, A146L, A146M, A146N, A146Q, A146R, A146S, A146T, A146V, A146Y, M149A, M149V, R150A, S153A, L154A, N157A, L30A-H58Y-E59N-Q62S, R33A-H58Y-E59N-Q62S, M149A-H58Y-E59N-Q62S, L154A-H58Y-E59N-Q62S, R145A-H58Y-E59N-Q62S, D115A-R121A, L118A-R121A, L118A-R121A-K122A, R121A-K122A, and R121E-K122E.

9. The chimeric protein of any one of claims 1-8, further comprising a mutation at position C1, C29, C86, C99, C139 in reference to SEQ ID NO: 1, optionally selected from the group consisting of C86S, C86A, and C86Y.

10. The chimeric protein of any one of claims 5-9, wherein the one or more mutations confer reduced affinity that is restorable by attachment to one or more targeting moieties or upon inclusion in the Fc-based chimeric protein complex.

11. The chimeric protein of any one of the above claims, wherein the targeting moiety is directed against a tumor cell.

12. The chimeric protein of any one of the above claims, wherein the targeting moiety comprise a recognition domain that recognizes and/or binds an antigen or receptor on a tumor cell, endothelial cell, epithelial cell, mesenchymal cell, tumor stroma or stromal cell, ECM and/or immune cell, organ cells, and/or tissue cells.

13. The chimeric protein of claim 12, wherein the immune cell is selected from a T cell, a B cell, a dendritic cell, a macrophage, a neutrophil, a mast cell, a monocyte, a red blood cell, myeloid cell, myeloid derived suppressor cell, a NKT cell, and a NK cell, or derivatives thereof.

14. The chimeric protein of any one of the above claims, wherein the targeting moiety comprises a recognition domain that is a full-length antibody or a fragment thereof, a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a Humabody, a shark heavy-chain-only antibody (VNAR), a microprotein (*e.g.* cysteine knot protein, knottin), a darpin, an anticalin, an adnectin, an aptamer, a Fv, a Fab, a Fab', a $F(ab')_2$, a peptide mimetic molecule, a natural ligand for a receptor, or a synthetic molecule.

15. The chimeric protein of any one of the above claims, wherein the recognition domain i) is a natural ligand, optionally comprising Flt3L or a truncation thereof, optionally an extracellular domain of Flt3L, optionally a single chain Flt3L; ii) recognizes CD20; iii) recognizes PD-1 or PD-L1; or iv) recognizes Clec9A.

16. The chimeric protein of any one of the above claims, wherein the recognition domain is a single-domain antibody (V_{HH}), optionally a V_{HH}, humanized V_{HH}, or camelized V_{HH}.

17. The chimeric protein of any one of the above claims, wherein the recognition domain functionally modulates the antigen or receptor of interest.

18. The chimeric protein of any one of the above claims, wherein the recognition domain binds but does not functionally modulate the antigen or receptor of interest.

19. The chimeric protein of any one of the above claims, comprising two or more targeting moieties.

20. The chimeric protein of any one of the above claims, further comprising one or more additional modified signaling agents.

21. The chimeric protein of any one of the above claims, wherein the chimeric protein comprises two signaling agents or two targeting moieties or two of both.

22. The chimeric protein of any one of the above claims, wherein the chimeric protein comprises three signaling agents or three targeting moieties or three of both.

23. The chimeric protein of claim 20, wherein the modified signaling agent comprises one or more mutations conferring reduced affinity or activity for a receptor relative to an unmutated signaling agent.

24. The chimeric protein of claim 23, wherein the one or more mutations allow for attenuation of activity.

25. The chimeric protein of claim 24, wherein agonistic or antagonistic activity is attenuated.

26. The chimeric protein of claim 24 or 25, wherein the modified signaling agent comprises one or more mutations that convert its activity from agonistic to antagonistic.

27. The chimeric protein of claim 23, wherein the one or more mutations confer reduced affinity or activity that is restorable by attachment to one or more targeting moiety or upon inclusion in the Fc-based chimeric protein complex.

28. The chimeric protein of any one of the above claims, wherein the chimeric protein is suitable for use in a patient having one or more of: cancer, infections, immune disorders, autoimmune diseases, cardiovascular diseases, wound, ischemia-related diseases, neurodegenerative diseases, and/or metabolic diseases.

29. A recombinant nucleic acid composition encoding one or more chimeric proteins of any one of the above claims.

30. A host cell comprising a nucleic acid of claim 29.

31. A method for treating cancer, comprising administering an effective amount of i) the chimeric protein of any one of the claims 1-28 to a patient in need thereof; ii) the recombinant nucleic acid of claim 29 to a patient in need thereof; or iii) the host cell of claim 30 to a patient in need thereof.

32. The method of claim 31, wherein the cancer is selected form one or more of basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.*, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma (*e.g.*, Kaposi's sarcoma); skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia as well as other carcinomas and sarcomas; post-transplant lymphoproliferative disorder (PTLD) as well as abnormal vascular proliferation associated with phakomatoses; edema (*e.g.* that associated with brain tumors); and Meigs' syndrome.

33. The method of claim 32, wherein the cancer is hairy cell leukemia.

34. The method of claim 32, wherein the cancer is melanoma.

35. The method of claim 32, wherein the cancer is Kaposi's sarcoma.

36. The chimeric protein of any one of claims 1-28 for use as a medicament.

37. The chimeric protein of any one of claims 1-28 for use in the treatment of cancer, autoimmune diseases, inflammatory diseases, metabolic diseases, cardiovascular diseases, infectious disease, degenerative and neurodegenerative diseases.

38. Use of a chimeric protein of any one of claims 1-28 in the manufacture of a medicament.

39. A Fc-based chimeric protein complex comprising:

(a) an interferon alpha 1 (IFNα1) or a variant thereof, and

(b) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest; and

(c) a Fc domain, the Fc domain optionally having one or more mutations that reduces or eliminates one or more effector functions of the Fc domain, promotes Fc chain pairing in the Fc domain, and/or stabilizes a hinge region in the Fc domain.

40. The Fc-based chimeric protein complex of claim 39, wherein the IFNα1 comprises an amino acid sequence having at least about 90%, or at least about 93%, or at least about 95%, or at least about 97%, or at least about 98%, or at least about 99% identity with SEQ ID NO:1.

41. The Fc-based chimeric protein complex of claim 39 or 40, wherein the IFNα1 comprises the amino acid sequence of SEQ ID NO:1, optionally wherein the IFNα1 is pegylated, one or more targeting moieties are pegylated, and/or the Fc domain is pegylated.

42. The Fc-based chimeric protein complex of claim 39, wherein the variant IFNα1 comprises one or more mutations.

43. The Fc-based chimeric protein complex of claim 42, wherein the one or more mutations of the variant IFNα1 confer reduced affinity for interferon-α/β receptor (IFNAR).

44. The Fc-based chimeric protein complex of claim 43, wherein the IFNα1 exhibits reduced affinity for IFNAR1.

45. The Fc-based chimeric protein complex of claim 43, wherein the IFNα1 exhibits reduced affinity for IFNAR2.

46. The Fc-based chimeric protein complex of claim 42, wherein the amino acid position of the one or more mutations of the variant IFNα1 is selected from the group consisting of L15, A19, R23, S25, L30, D32, R33, H34, Q40, D115, L118, K121, R126, E133, K134, K135, R145, A146, M149, R150, S153, L154, and N157 or a combination thereof, wherein the positions are in reference to SEQ ID NO: 1, optionally selected from the group consisting of L15A, A19W, R23A, S25A, L30A, L30V, D32A, R33K, R33A, R33Q, H34A, Q40A, D115R, L118A, K121A, K121E, R126A, R126E, E133A, K134A, K135A, R145A, R145D, R145E, R145G, R145H, R145I, R145K, R145L, R145N, R145Q, R145S, R145T, R145V, R145Y, A146D, A146E, A146G, A146H, A146I, A146K, A146L, A146M, A146N, A146Q, A146R, A146S, A146T, A146V, A146Y, M149A, M149V, R150A, S153A, L154A, N157A, L30A-H58Y-E59N-Q62S, R33A-H58Y-E59N-Q62S, M149A-H58Y-E59N-Q62S, L154A-H58Y-E59N-Q62S,

R145A-H58Y-E59N-Q62S, D115A-R121A, L118A-R121A, L118A-R121A-K122A, R121A-K122A, and R121E-K122E.

47. The chimeric protein of any one of claims 39-46, further comprising a mutation at position C1, C29, C86, C99, C139 in reference to SEQ ID NO: 1, optionally selected from the group consisting of C86S, C86A, and C86Y.

48. The Fc-based chimeric protein complex of any one of claims 39-47, wherein the one or more mutations confer reduced affinity that is restorable by attachment to one or more targeting moieties or upon inclusion in the Fc-based chimeric protein complex.

49. The Fc-based chimeric protein complex of any one of 39-48, wherein the targeting moiety is directed against a tumor cell.

50. The Fc-based chimeric protein complex of any one of claims 39-49, wherein the targeting moiety is directed against an immune cell.

51. The Fc-based chimeric protein complex of claim 50, wherein the immune cell is selected from T cell, a B cell, a dendritic cell, a macrophage, a neutrophil, a mast cell, a monocyte, a red blood cell, myeloid cell, myeloid derived suppressor cell, a NKT cell, and a NK cell, or derivatives thereof.

52. The Fc-based chimeric protein complex of any one of claims 39-51, wherein the targeting moiety comprises a recognition domain that is a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a shark heavy-chain-only antibody (VNAR), a microprotein (*e.g.* cysteine knot protein, knottin), a darpin, an anticalin, an adnectin, an aptamer, a Fv, a Fab, a Fab', a $F(ab')_2$, a peptide mimetic molecule, a natural ligand for a receptor, or a synthetic molecule.

53. The Fc-based chimeric protein complex of any one of claims 39-52, wherein the recognition domain i) is a natural ligand, optionally comprising Flt3L or a truncation thereof, optionally an extracellular domain of Flt3L; ii) recognizes CD20; iii) recognizes PD-1 or PD-L1; or iv) recognizes Clec9A.

54. The Fc-based chimeric protein complex of any one of claims 39-53, wherein the recognition domain is a single-domain antibody (V_{HH}), optionally a V_{HH}, humanized V_{HH}, or camelized V_{HH}.

55. The Fc-based chimeric protein complex of any one of claims 39-54, wherein the recognition domain functionally modulates the antigen or receptor of interest.

56. The Fc-based chimeric protein complex of any one of claims 39-55, wherein the recognition domain binds but does not functionally modulate the antigen or receptor of interest.

57. The Fc-based chimeric protein complex of any one of claims 39-56, comprising two or more targeting moieties.

58. The Fc-based chimeric protein complex of any one of claims 39-57, further comprising one or more additional modified signaling agents.

59. The Fc-based chimeric protein complex of any one of claims 39-58, wherein the Fc-based chimeric protein complex comprises two signaling agents or two targeting moieties or two of both.

60. The Fc-based chimeric protein complex of any one of claims 39-59, wherein the Fc-based chimeric protein complex comprises three signaling agents or three targeting moieties or three of both.

61. The Fc-based chimeric protein complex of claim 58, wherein the modified signaling agent comprises one or more mutations conferring reduced affinity or activity for a receptor relative to an unmutated signaling agent.

62. The Fc-based chimeric protein complex of claim 61, wherein the one or more mutations allow for attenuation of activity.

63. The Fc-based chimeric protein complex of claim 62, wherein agonistic or antagonistic activity is attenuated.

64. The Fc-based chimeric protein complex of claim 61 or 62, wherein the modified signaling agent comprises one or more mutations which convert its activity from agonistic to antagonistic.

65. The Fc-based chimeric protein complex of claim 39, wherein the one or more mutations confer reduced affinity or activity that is restorable by attachment to one or more targeting moiety or upon inclusion in the Fc-based chimeric protein complex.

66. The Fc-based chimeric protein complex of any one of the claims 39-65, wherein the Fc-based chimeric protein complex is suitable for use in a patient having one or more of: cancer, infections, immune disorders, autoimmune diseases, cardiovascular diseases, wound, ischemia-related diseases, neurodegenerative diseases, and/or metabolic diseases.

67. A recombinant nucleic acid composition encoding one or Fc-based chimeric protein complexes of any one of claims 39-66, or a constituent polypeptide thereof.

68. A host cell comprising a nucleic acid of claim 67.

69. A method for treating cancer, comprising administering an effective amount of i) the Fc-based chimeric protein of any one of the claims 39-66 to a patient in need thereof; ii) the recombinant nucleic acid of claim 67 to a patient in need thereof; or iii) the host cell of claim 68 to a patient in need thereof.

70. The method of claim 69, wherein the cancer is selected form one or more of basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.*, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma (*e.g.*, Kaposi's sarcoma); skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma

(NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (*e.g.* that associated with brain tumors), and Meigs' syndrome.

71. The method of claim 70, wherein the cancer is hairy cell leukemia.

72. The method of claim 70, wherein the cancer is melanoma.

73. The method of claim 70, wherein the cancer is Kaposi's sarcoma.

74. The Fc-based chimeric protein complex of any one of claims 39-66 for use as a medicament.

75. The Fc-based chimeric protein complex of any one of claims 39-66 for use in the treatment of cancer, autoimmune diseases, inflammatory diseases, metabolic diseases, cardiovascular diseases, infectious disease, degenerative and neurodegenerative diseases.

76. Use of a Fc-based chimeric protein complex of any one of claims 39-66 in the manufacture of a medicament.

77. The Fc-based chimeric protein complex of any one of claims 39-66, wherein the Fc domain is from IgG, IgA, IgD, IgM or IgE.

78. The Fc-based chimeric protein complex of claim 77, wherein the IgG is selected from IgG1, IgG2, IgG3, or IgG4.

79. The Fc-based chimeric protein complex of any one of claims 39-66, wherein the Fc domain is from human IgG, IgA, IgD, IgM or IgE.

80. The Fc-based chimeric protein complex of claim 79, wherein the human IgG is selected from human IgG1, IgG2, IgG3, or IgG4.

81. The Fc-based chimeric protein complex of any one of claims 39-66 and 74-80, wherein the Fc chain pairing is promoted by ionic pairing and/or a knob-in-hole pairing.

82. The Fc-based chimeric protein complex of any one of claims 39-66 and 74-81, wherein the one or more mutations to the Fc domain results in an ionic pairing between the Fc chains in the Fc domain.

83. The Fc-based chimeric protein complex of any one of claims 39-66 and 74-82, wherein the one or more mutations to the Fc domain results in a knob-in-hole pairing in the Fc domain.

84. The Fc-based chimeric protein complex of any one of claims 39-66 and 74-83, wherein the one or more mutations to the Fc domain results in the reduction or elimination of an effector function of the Fc domain.

85. The Fc based chimeric protein complex of any one of claims 39-66 and 74-84, wherein the Fc-based chimeric protein complex is a heterodimer and has a trans orientation/configuration, as relates to any targeting

moiety and signaling agent, relative to each other, or any targeting moieties relative to each other, or any signaling agents relative to each other.

86. The Fc based chimeric protein complex of any one of claims 39-66 and 74-85, wherein the Fc-based chimeric protein complex is a heterodimer and has a cis orientation, as relates to any targeting moiety and signaling agent, relative to each other, or any targeting moieties relative to each other, or any signaling agents relative to each other.

87. The Fc based chimeric protein complex of any one of claims 39-66 and 74-86, wherein the Fc comprises L234A, L235A, and K322Q substitutions in human IgG1 (according to EU numbering).

88. The Fc-based chimeric protein complex of any one of claims 39-66 and 74-87, wherein the Fc is human IgG1, and optionally contains one or more mutations of L234, L235, K322, D265, P329, and P331 (according to EU numbering).

89. The Fc-based chimeric protein complex of any one of claims 39-66 and 74-88, wherein the Fc-based chimeric protein complex has an orientation and/or configuration of any one of **FIGs. 1A-F**, **2A-H, 3A-H**, **4A-D**, **5A-F**, **6A-J**, **7A-D**, **8A-F**, **9A-J**, **10A-F, 11A-L**, **12A-L**, **13A-F**, **14A-L**, **15A-L**, **16A-J**, **17A-J**, **18A-F,** and **19A-F**.

90. The Fc-based chimeric protein complex of any one of claims 39-66 and 74-89, wherein the Fc-based chimeric protein complex comprises a polypeptide having an amino acid sequence having at least 95%, or at least 98%, or at least 99% identity with any one of SEQ ID NOs: 290, 291, 293-303.

91. The Fc-based chimeric protein complex of any one of claims 39-66 and 74-89 wherein the Fc-based chimeric protein complex comprises a polypeptide having an amino acid sequence selected from SEQ ID NOs: 290, 291, 293-303 and less than 10 mutations to the amino acid sequence.

92. The Fc-based chimeric protein complex of claim 91, wherein the Fc-based chimeric protein complex comprises a polypeptide having an amino acid sequence selected from SEQ ID NOs: 290, 291, 293-303, and less than 5 mutations to the amino acid sequence.

93. The Fc-based chimeric protein complex of claim 91, wherein the Fc-based chimeric protein complex comprises a polypeptide having an amino acid sequence selected from SEQ ID NOs: 290, 291, 293-303.

94. The Fc-based chimeric protein complex of claim 90, wherein the Fc-based chimeric protein complex comprises a first amino acid sequence having at least 95%, or at least 98%, or at least 99% identity to SEQ ID NO: 290 and a second amino acid sequence having at least 95%, or at least 98%, or at least 99% identity to SEQ ID NO: 291.

95. The Fc-based chimeric protein complex of claim 90, wherein the Fc-based chimeric protein complex comprises a first amino acid sequence having at least 95%, or at least 98%, or at least 99% identity to SEQ ID NO: 293 and a second amino acid sequence having at least 95%, or at least 98%, or at least 99% identity to any one of SEQ ID NO: 294, 295, 296, 297, 298, or 299.

96. The Fc-based chimeric protein complex of claim 90, wherein the Fc-based chimeric protein complex comprises a first amino acid sequence having at least 95%, or at least 98%, or at least 99% identity to any one of

SEQ ID NO: 300, 301, 302, 303, 303 and a second amino acid sequence having at least 95%, or at least 98%, or at least 99% identity to SEQ ID NO: 294.

97. A chimeric protein complex comprising:

(a) an interferon alpha 1 (IFNα1) or a variant thereof,

(b) one or more targeting moieties, said targeting moieties comprising recognition domains which specifically bind to an antigen or receptor of interest, and

(c) a complexation domain.

98. The chimeric protein complex of claim 97, wherein the complexation domain comprises a leucine zipper.

99. The chimeric protein complex of claim 97 or 98, wherein the IFNα1 comprises an amino acid sequence having at least about 90%, or at least about 93%, or at least about 95%, or at least about 97%, or at least about 98%, or at least about 99% identity with SEQ ID NO: 1, optionally wherein the IFNα1 is pegylated, one or more targeting moieties are pegylated, or a linker is pegylated. is pegylated.

100. The chimeric protein complex of any one of claims 97-99, wherein the variant IFNα1 comprises one or more mutations.

101. The chimeric protein complex of claim 100, wherein the one or more mutations of the variant IFNα1 confer reduced affinity for interferon-α/β receptor (IFNAR).

102. The chimeric protein complex of claim 101, wherein the IFNα1 exhibits reduced affinity for IFNAR1.

103. The chimeric protein complex of claim 101, wherein the IFNα1 exhibits reduced affinity for IFNAR2.

104. The chimeric protein complex of claim 100, wherein the amino acid position of the one or more mutations of the variant IFNα1 is selected from the group consisting of L15, A19, R23, S25, L30, D32, R33, H34, Q40, D115, L118, K121, R126, E133, K134, K135, R145, A146, M149, R150, S153, L154, and N157 or a combination thereof, wherein the positions are in reference to SEQ ID NO: 1, optionally selected from the group consisting of L15A, A19W, R23A, S25A, L30A, L30V, D32A, R33K, R33A, R33Q, H34A, Q40A, D115R, L118A, K121A, K121E, R126A, R126E, E133A, K134A, K135A, R145A, R145D, R145E, R145G, R145H, R145I, R145K, R145L, R145N, R145Q, R145S, R145T, R145V, R145Y, A146D, A146E, A146G, A146H, A146I, A146K, A146L, A146M, A146N, A146Q, A146R, A146S, A146T, A146V, A146Y, M149A, M149V, R150A, S153A, L154A, N157A, L30A-H58Y-E59N-Q62S, R33A-H58Y-E59N-Q62S, M149A-H58Y-E59N-Q62S, L154A-H58Y-E59N-Q62S, R145A-H58Y-E59N-Q62S, D115A-R121A, L118A-R121A, L118A-R121A-K122A, R121A-K122A, and R121E-K122E.

105. The chimeric protein of any one of claims 97-104, further comprising a mutation at position C1, C29, C86, C99, C139 in reference to SEQ ID NO: 1, optionally selected from the group consisting of C86S, C86A, and C86Y.

106. The chimeric protein complex of any one of claims 97-105, wherein the one or more mutations confer reduced affinity that is restorable by attachment to one or more targeting moieties or upon inclusion in the chimeric protein complex.

107. The chimeric protein complex of any one of 97-106, wherein the targeting moiety is directed against a tumor cell, endothelial cell, epithelial cell, mesenchymal cell, tumor stroma or stromal cell, ECM and/or immune cell, organ cells, and/or tissue cells.

108. The chimeric protein complex of any one of claims 97-107, wherein the targeting moiety is directed against an immune cell.

109. The chimeric protein complex of claim 108, wherein the immune cell is selected from T cell, a B cell, a dendritic cell, a macrophage, a neutrophil, a mast cell, a monocyte, a red blood cell, myeloid cell, myeloid derived suppressor cell, a NKT cell, and a NK cell, or derivatives thereof.

110. The chimeric protein complex of any one of claims 97-109, wherein the targeting moiety comprises a recognition domain that is a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a shark heavy-chain-only antibody (VNAR), a microprotein (*e.g.* cysteine knot protein, knottin), a darpin, an anticalin, an adnectin, an aptamer, a Fv, a Fab, a Fab', a $F(ab')_2$, a peptide mimetic molecule, a natural ligand for a receptor, or a synthetic molecule.

111. The chimeric protein complex of any one of claims 97-110, wherein the recognition domain i) is a natural ligand, optionally comprising Flt3L or a truncation thereof, optionally an extracellular domain of Flt3L; optionally a single chain Flt3L; ii) recognizes CD20; iii) recognizes PD-1 or PD-L1; or iv) recognizes Clec9A.

112. The chimeric protein complex of any one of claims 97-111, wherein the recognition domain is a single-domain antibody (V_{HH}), optionally a V_{HH}, humanized V_{HH}, or camelized V_{HH}.

113. The chimeric protein complex of any one of claims 97-112, wherein the recognition domain functionally modulates the antigen or receptor of interest.

114. The chimeric protein complex of any one of claims 97-113, wherein the recognition domain binds but does not functionally modulate the antigen or receptor of interest.

115. The chimeric protein complex of any one of claims 97-114, comprising two or more targeting moieties.

116. The chimeric protein complex of any one of claims 97-115, further comprising one or more additional modified signaling agents.

117. The chimeric protein complex of any one of claims 97-116, wherein the chimeric protein complex comprises two signaling agents or two targeting moieties or two of both.

118. The chimeric protein complex of any one of claims 97-117, wherein the chimeric protein complex comprises three signaling agents or three targeting moieties or three of both.

119. The chimeric protein complex of claim 116, wherein the modified signaling agent comprises one or more mutations conferring reduced affinity or activity for a receptor relative to an unmutated signaling agent.

120. The chimeric protein complex of claim 119, wherein the one or more mutations allow for attenuation of activity.

121. The chimeric protein complex of claim 120, wherein agonistic or antagonistic activity is attenuated.

122. The chimeric protein complex of claim 119 or 120, wherein the modified signaling agent comprises one or more mutations which convert its activity from agonistic to antagonistic.

123. The chimeric protein complex of claim 97, wherein the one or more mutations confer reduced affinity or activity that is restorable by attachment to one or more targeting moiety or upon inclusion in the chimeric protein complex.

124. The chimeric protein complex of any one of the claims 97-123, wherein the chimeric protein complex is suitable for use in a patient having one or more of: cancer, infections, immune disorders, autoimmune diseases, cardiovascular diseases, wound, ischemia-related diseases, neurodegenerative diseases, and/or metabolic diseases.

125. A recombinant nucleic acid composition encoding one or chimeric protein complexes of any one of claims 97-124, or a constituent polypeptide thereof.

126. A host cell comprising a nucleic acid of claim 125.

127. A method for treating cancer, comprising administering an effective amount of i) the chimeric protein of any one of the claims 97-124 to a patient in need thereof; ii) the recombinant nucleic acid of claim 125 to a patient in need thereof; or iii) the host cell of claim 126 to a patient in need thereof.

128. The method of claim 127, wherein the cancer is selected form one or more of basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.*, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma (*e.g.*, Kaposi's sarcoma); skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (*e.g.* that associated with brain tumors), and Meigs' syndrome.

129. The method of claim 128, wherein the cancer is hairy cell leukemia.

130. The method of claim 128, wherein the cancer is melanoma.

131. The method of claim 128, wherein the cancer is Kaposi's sarcoma.

132. The chimeric protein complex of any one of claims 97-124 for use as a medicament.

133. The chimeric protein complex of any one of claims 97-124 for use in the treatment of cancer, autoimmune diseases, inflammatory diseases, metabolic diseases, cardiovascular diseases, infectious disease, degenerative and neurodegenerative diseases.

134. Use of a chimeric protein complex of any one of claims 97-124 in the manufacture of a medicament.

FIG. 1A

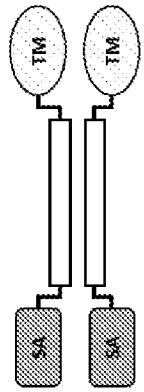


FIG. 1B

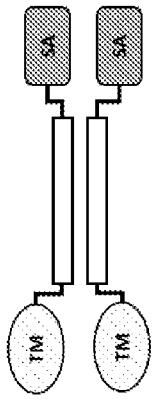


FIG. 1C

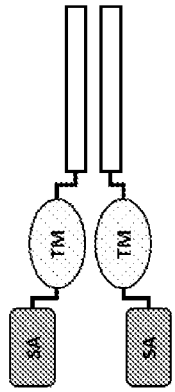


FIG. 1D

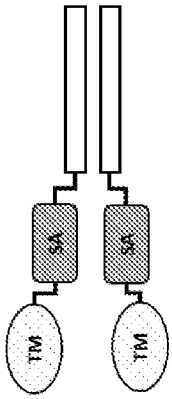


FIG. 1E

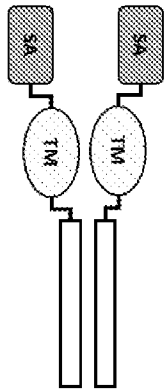


FIG. 1F

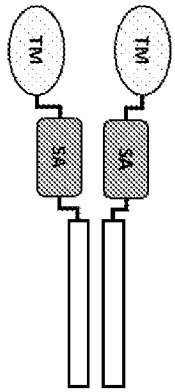


FIG. 2A FIG. 2B FIG. 2C FIG. 2D

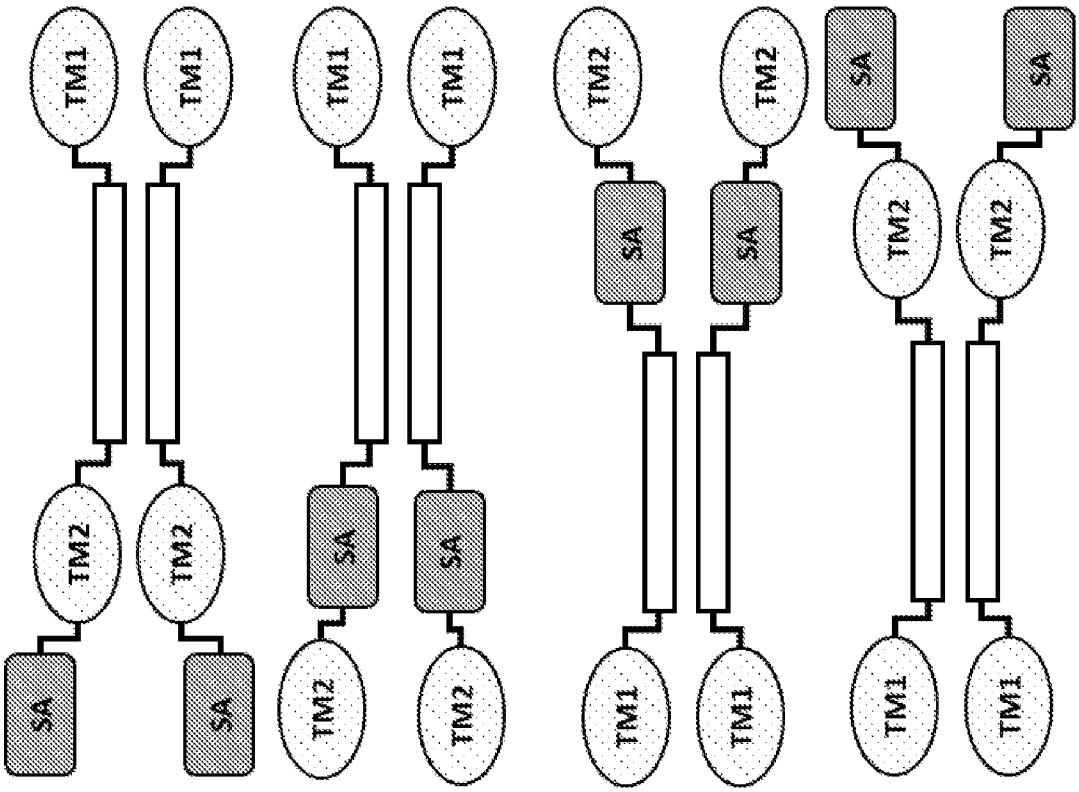


FIG. 2E

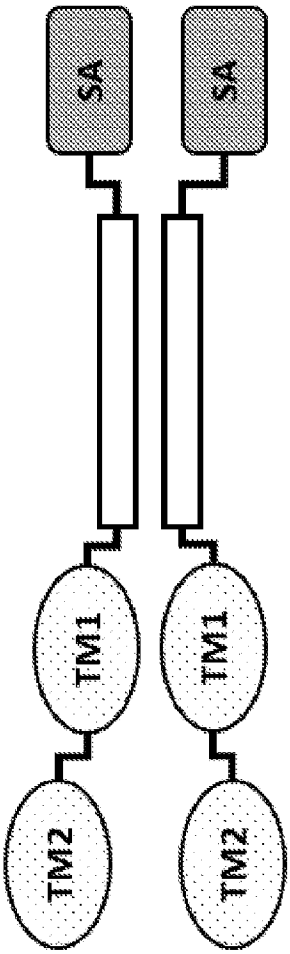


FIG. 2F

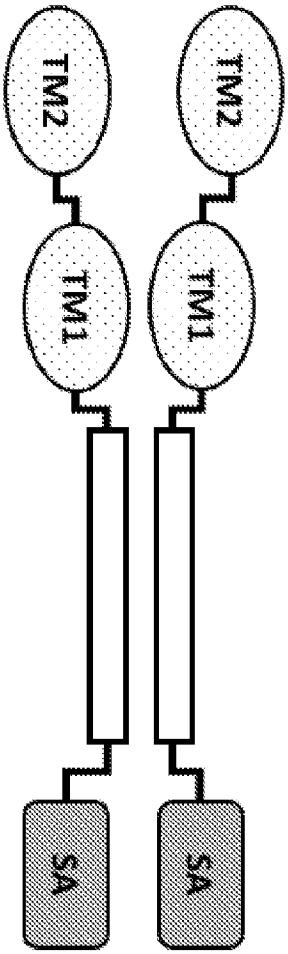


FIG. 2G

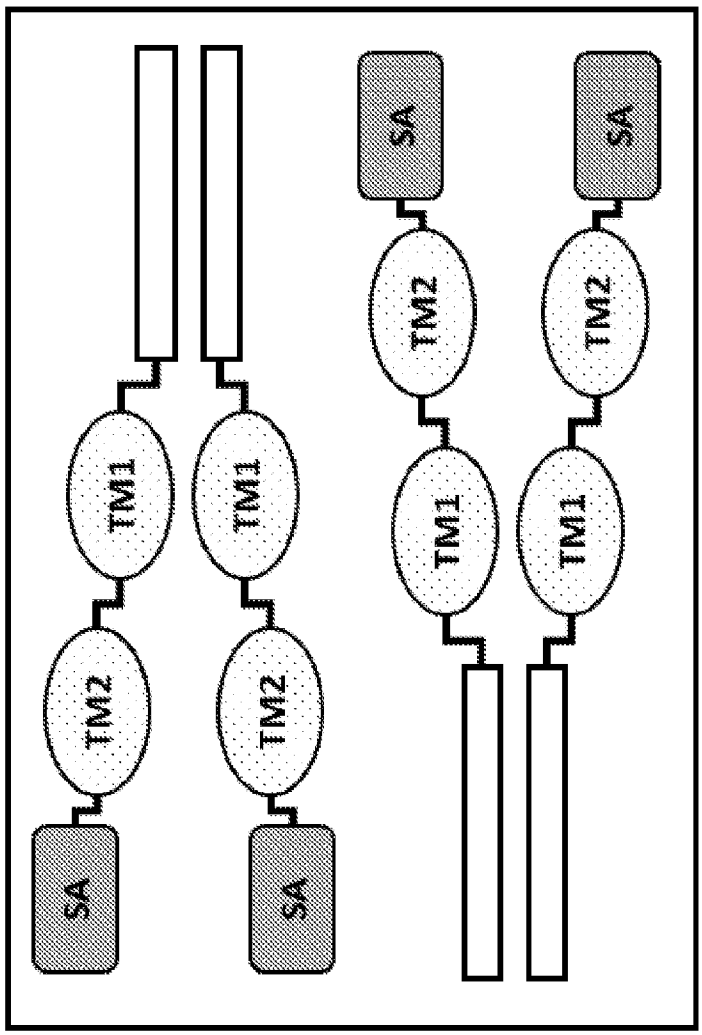


FIG. 2H

FIG. 3A

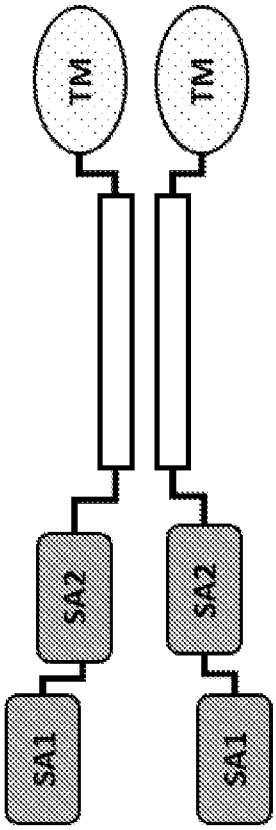


FIG. 3B

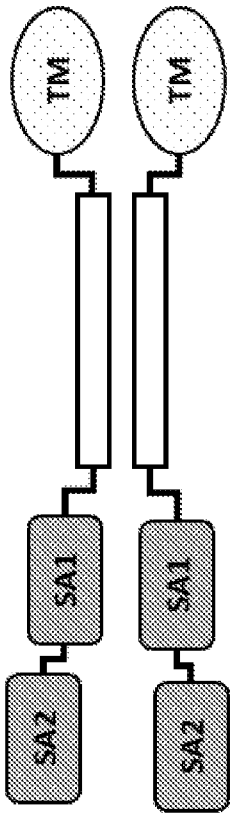


FIG. 3C

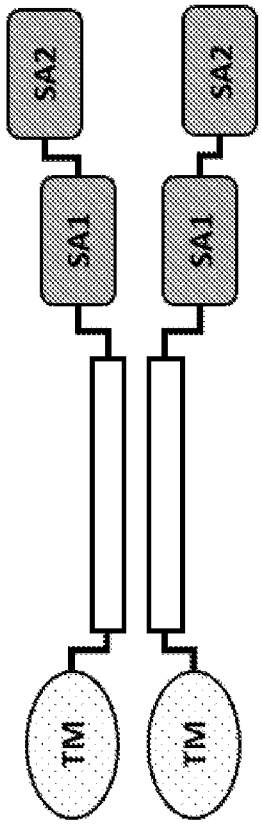


FIG. 3D

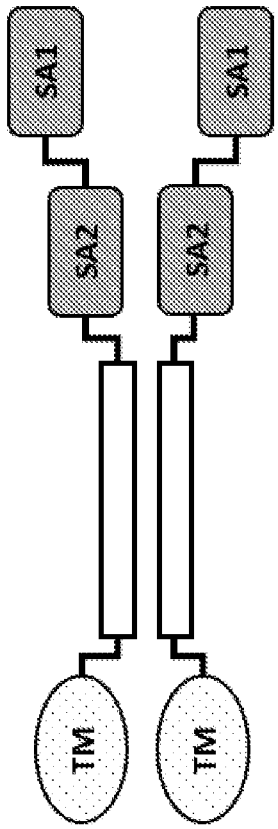


FIG. 3E

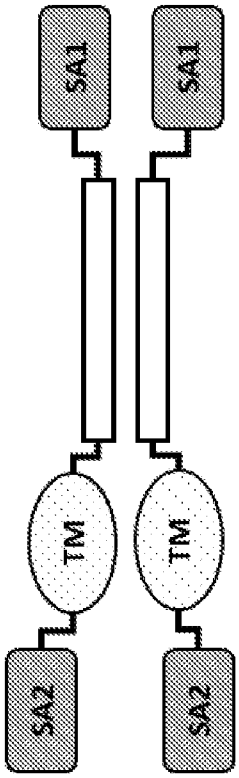


FIG. 3F

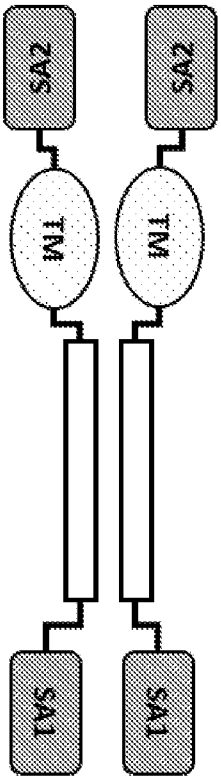


FIG. 3G

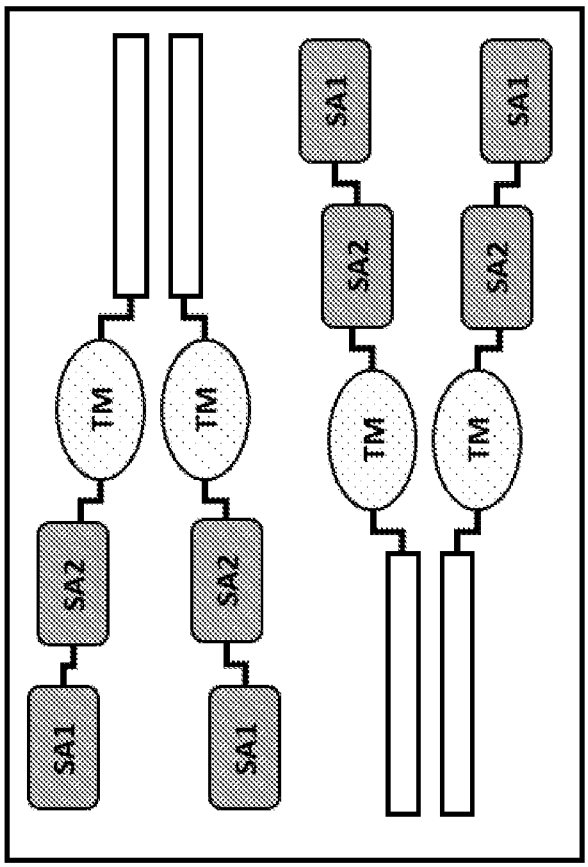


FIG. 3H

FIG. 4A

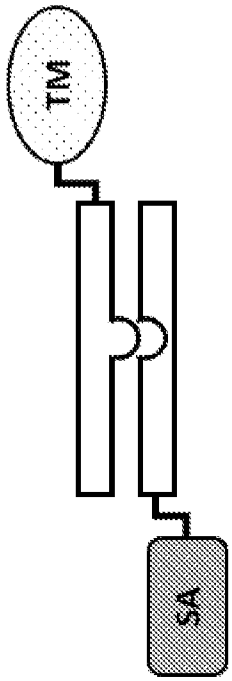


FIG. 4B

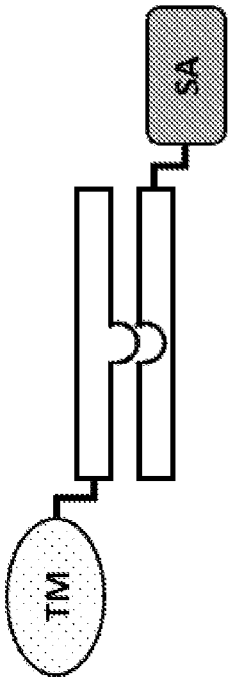


FIG. 4C

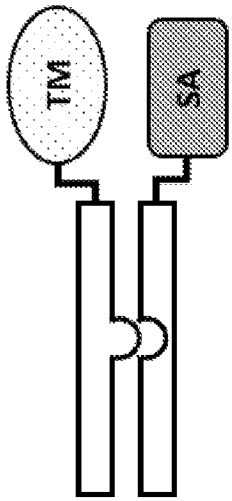


FIG. 4D

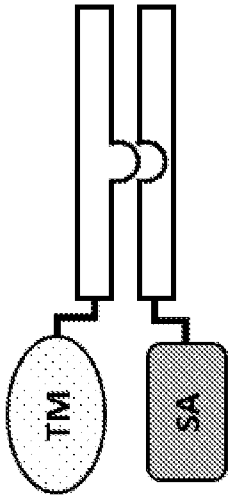


FIG. 5A FIG. 5B FIG. 5C FIG. 5D FIG. 5E FIG. 5F

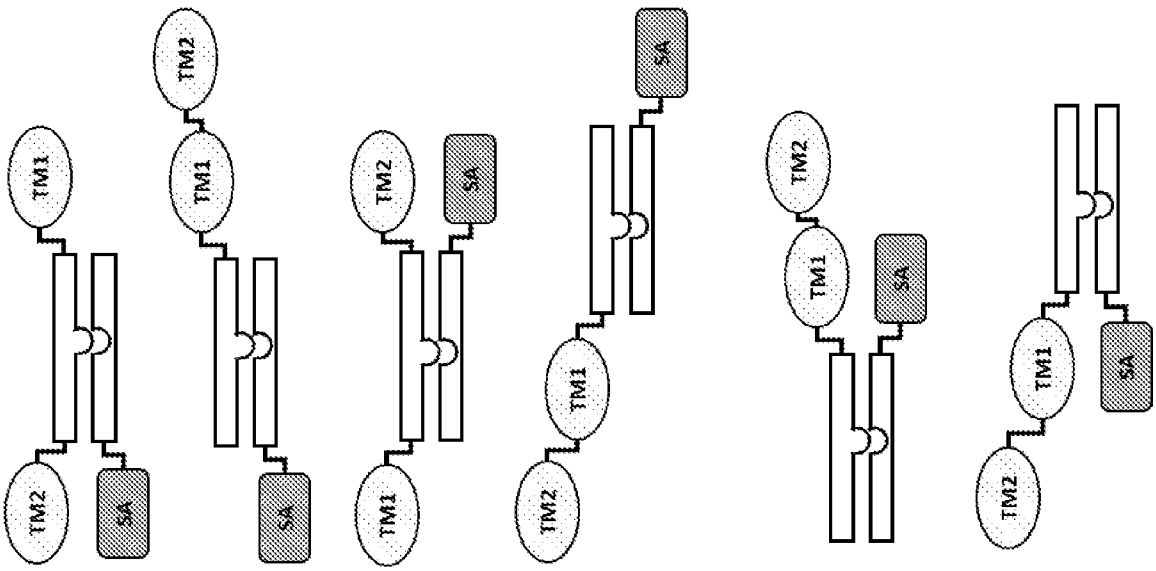


FIG. 6A FIG. 6B FIG. 6C FIG. 6D FIG. 6E FIG. 6F

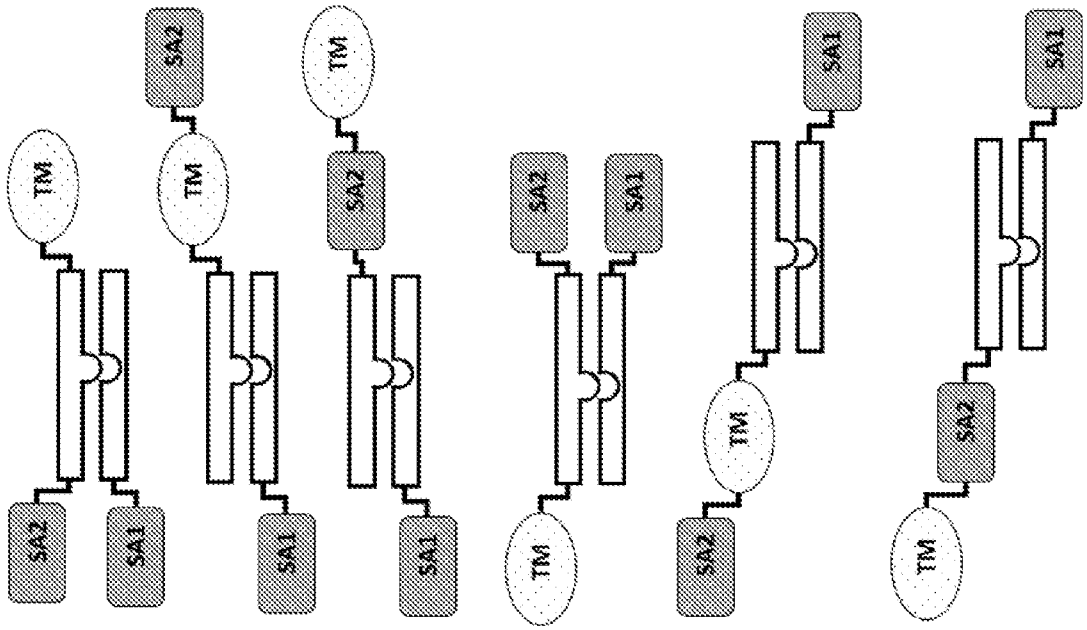


FIG. 6G

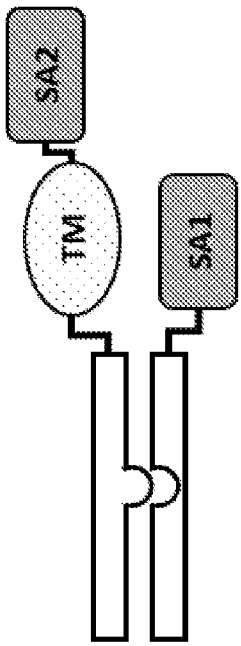


FIG. 6H

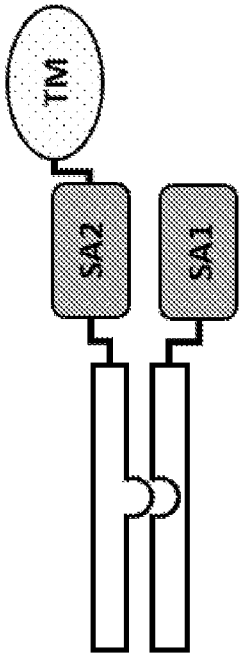


FIG. 6I

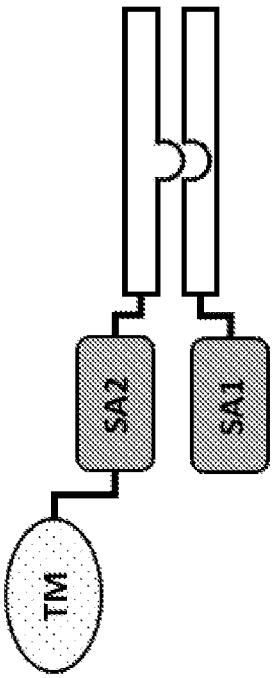


FIG. 6J

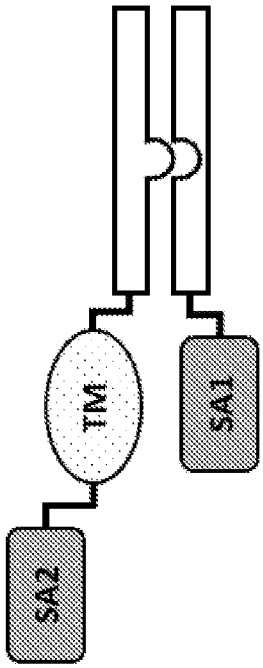


FIG. 7A

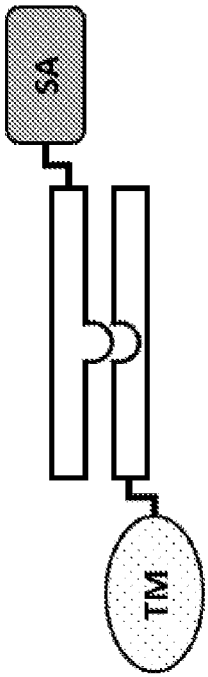


FIG. 7B

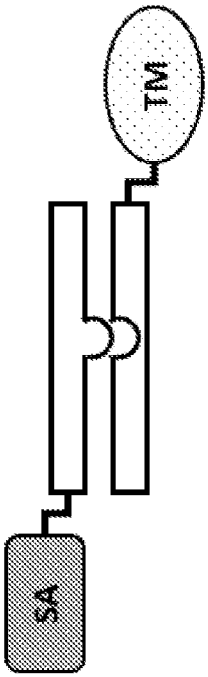


FIG. 7C

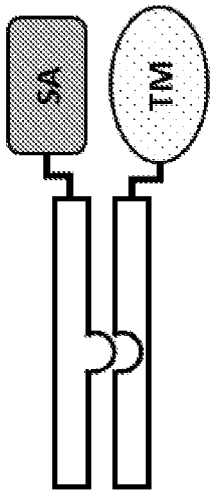


FIG. 7D

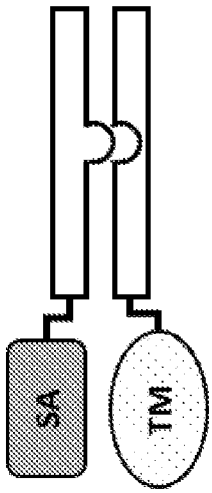


FIG. 8A FIG. 8B FIG. 8C FIG. 8D FIG. 8E FIG. 8F

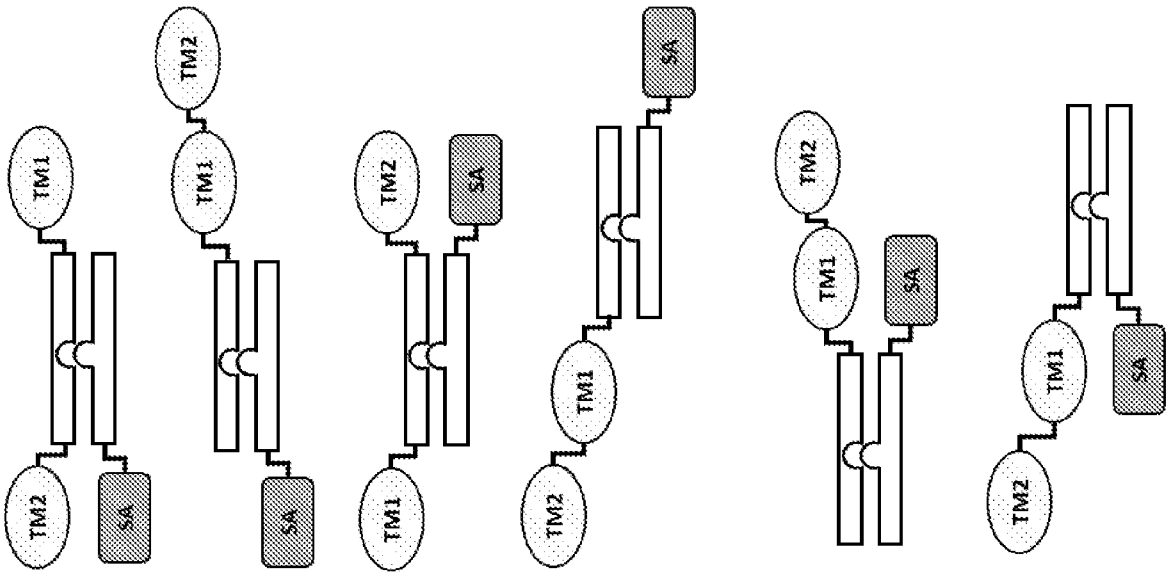


FIG. 9A FIG. 9B FIG. 9C FIG. 9D FIG. 9E

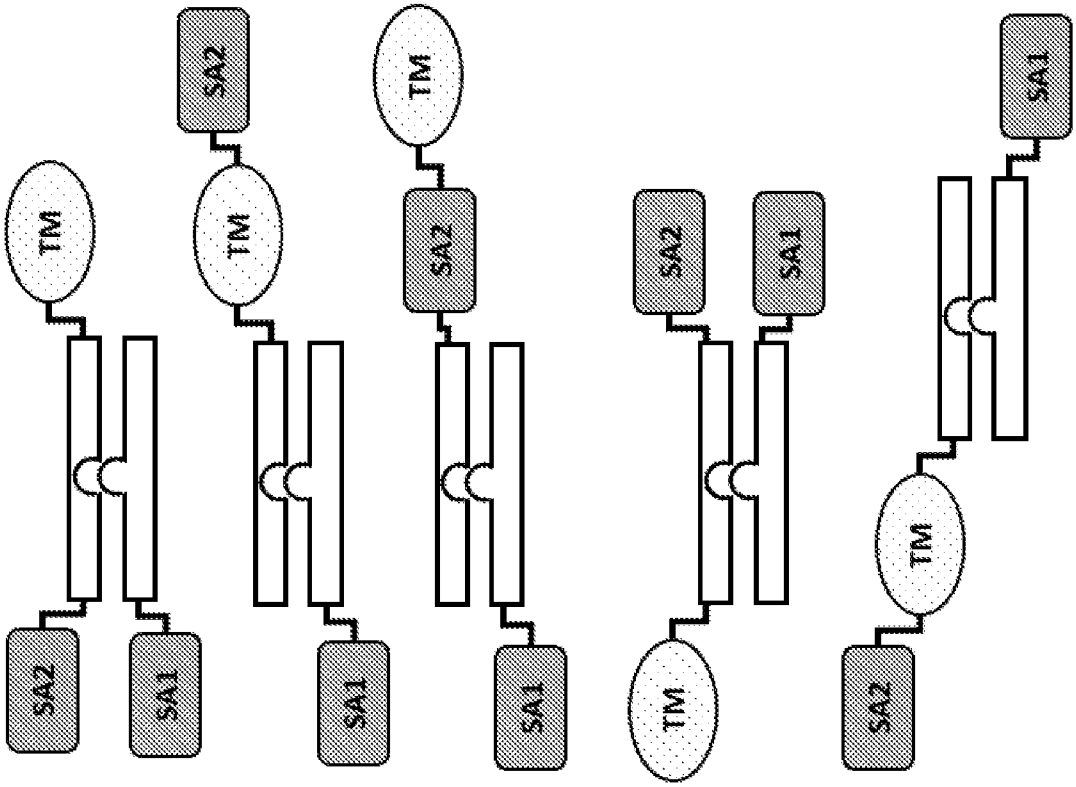


FIG. 9F FIG. 9G FIG. 9H FIG. 9I FIG. 9J

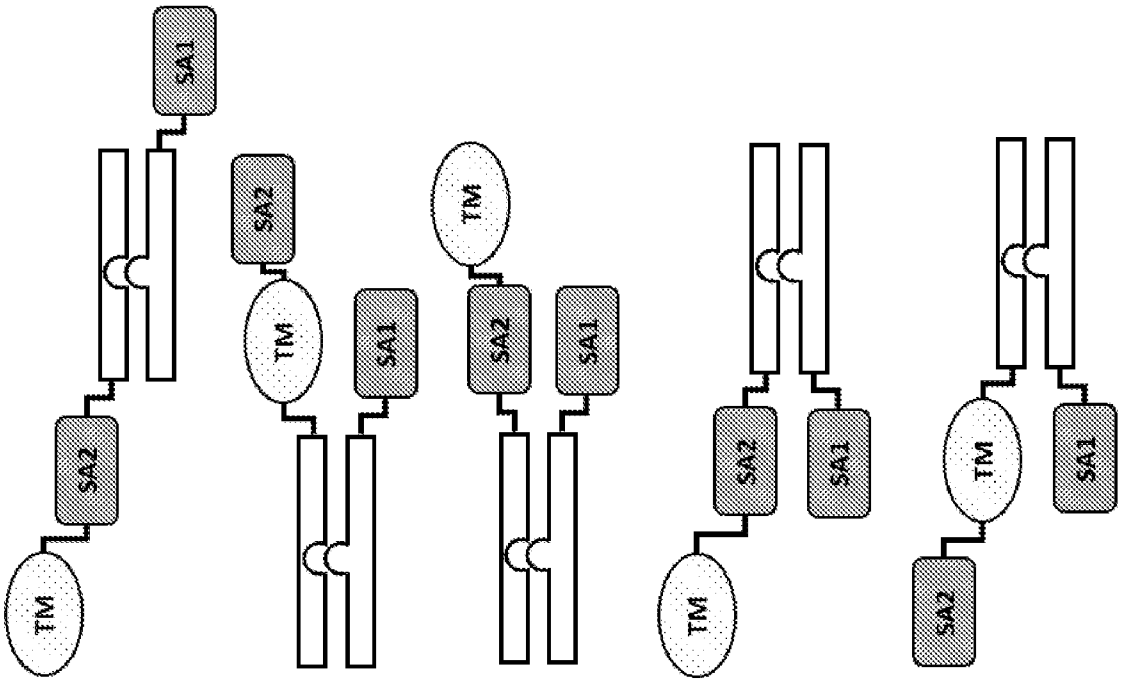


FIG. 10A FIG. 10B FIG. 10C FIG. 10D FIG. 10E FIG. 10F

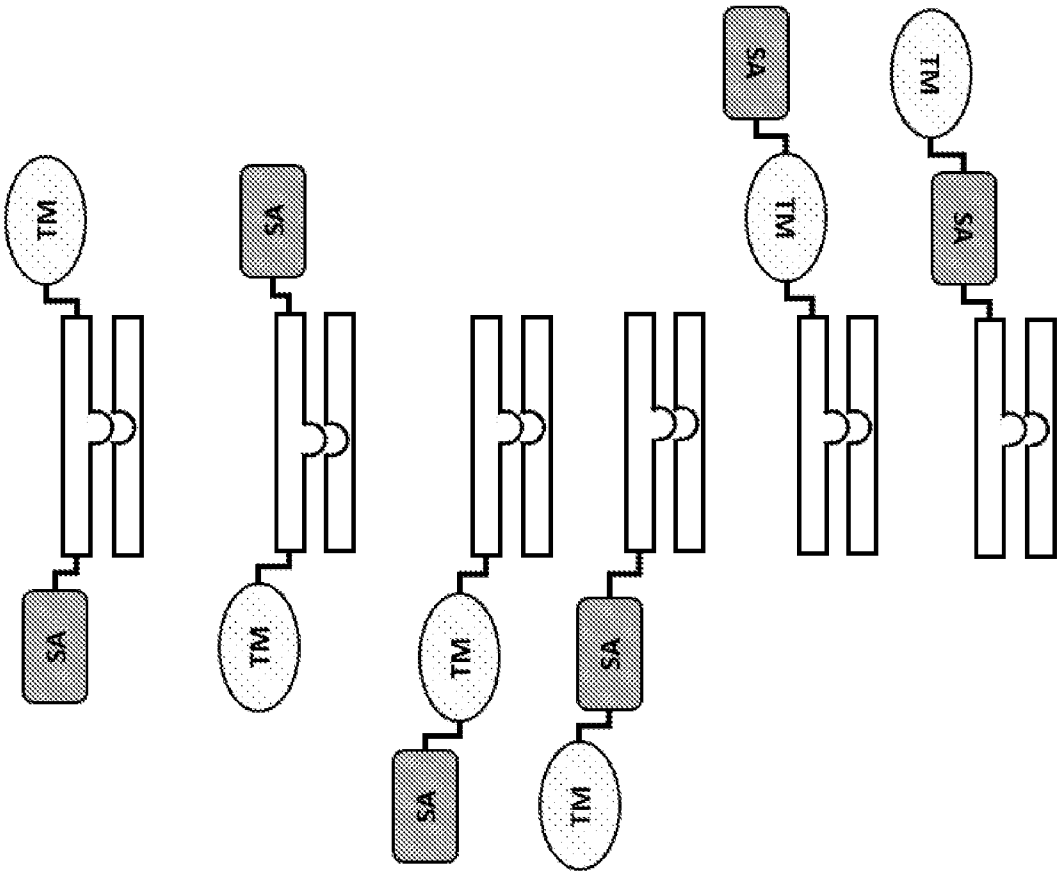


FIG. 11A FIG. 11B FIG. 11C FIG. 11D FIG. 11E

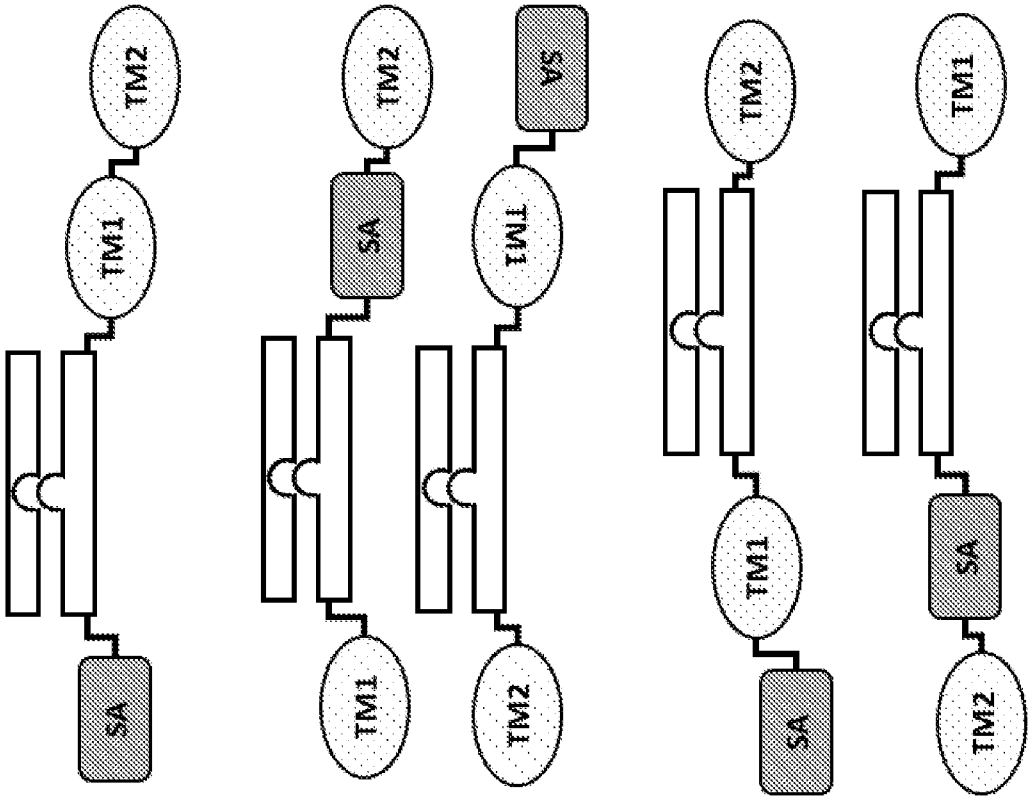


FIG. 11F FIG. 11G FIG. 11H FIG.11I FIG. 11J FIG. 11K FIG. 11L

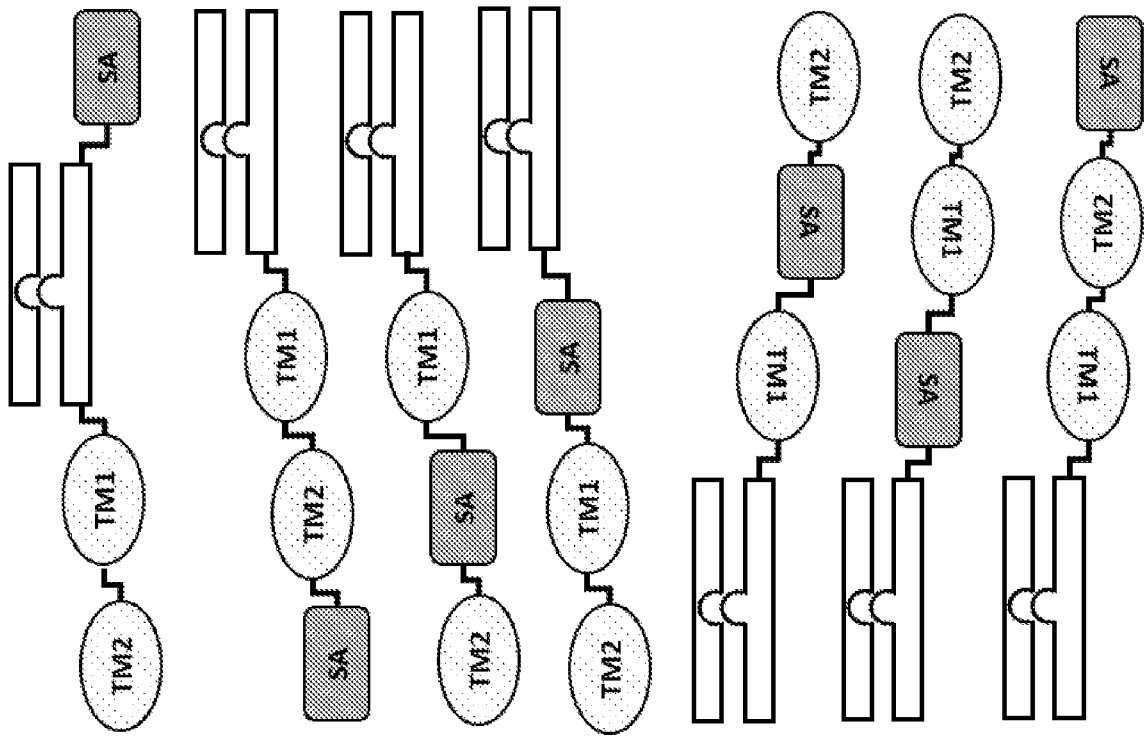


FIG. 12A FIG. 12B FIG. 12C FIG. 12D FIG. 12E FIG. 12F

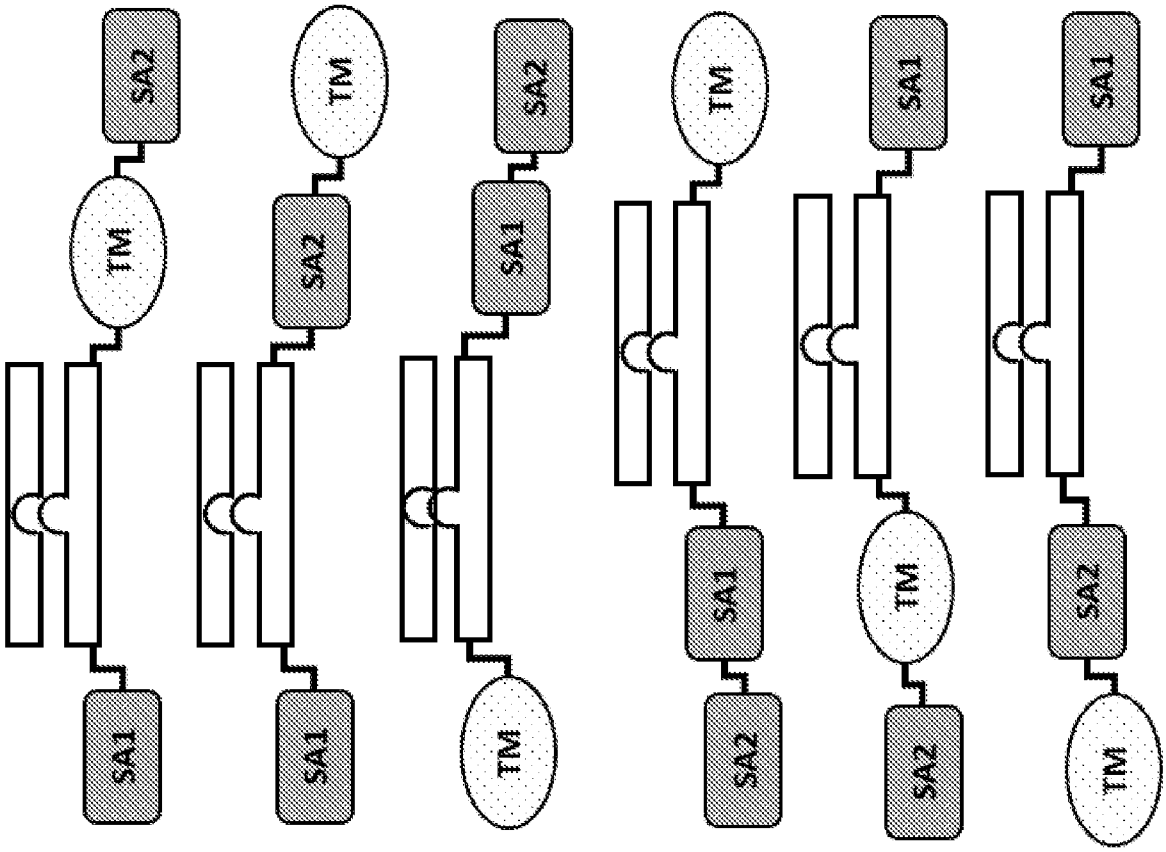


FIG. 12G FIG. 12H FIG. 12I FIG. 12J FIG. 12K FIG. 12L

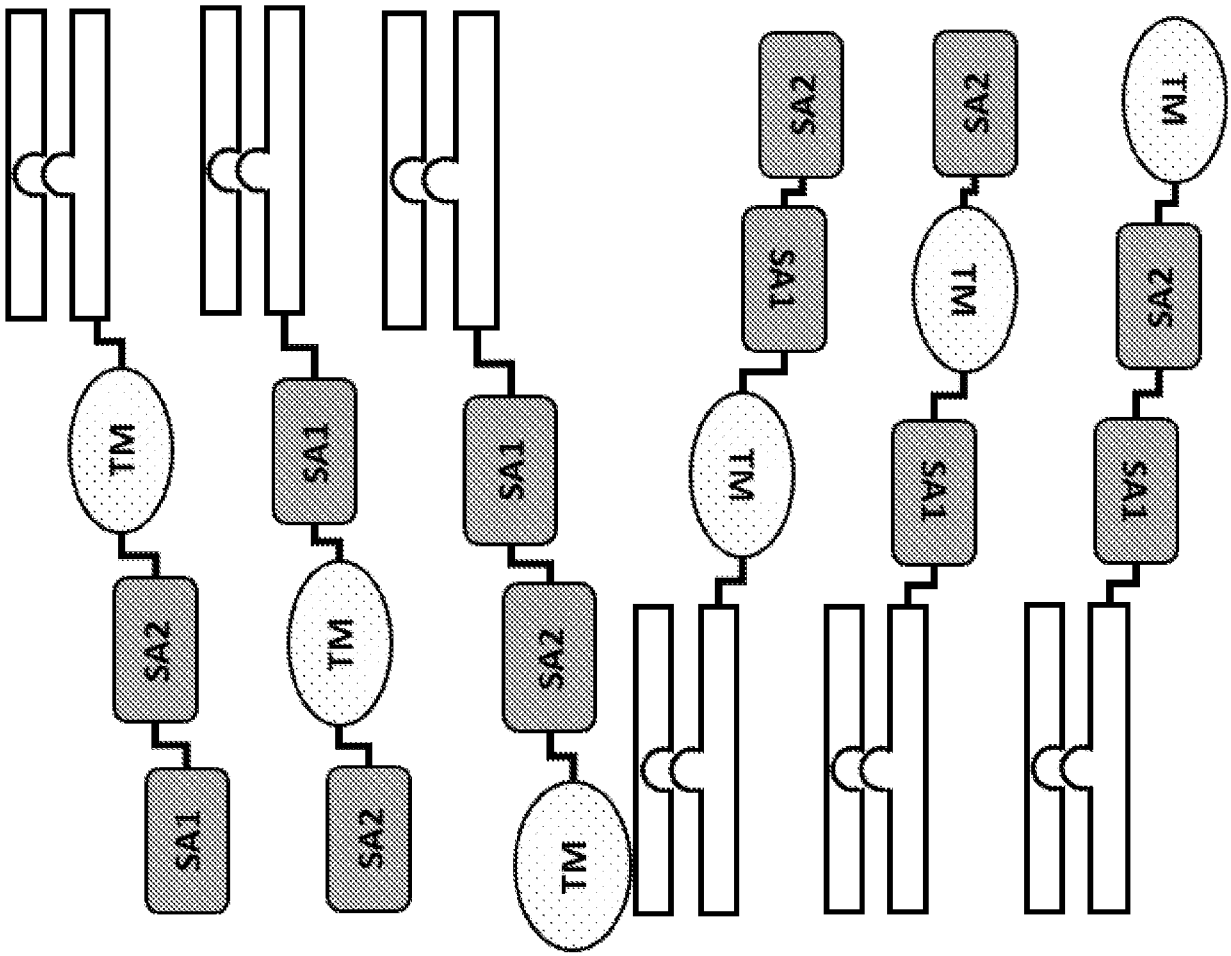


FIG. 13A FIG. 13B FIG. 13C FIG. 13D FIG. 13E FIG. 13F

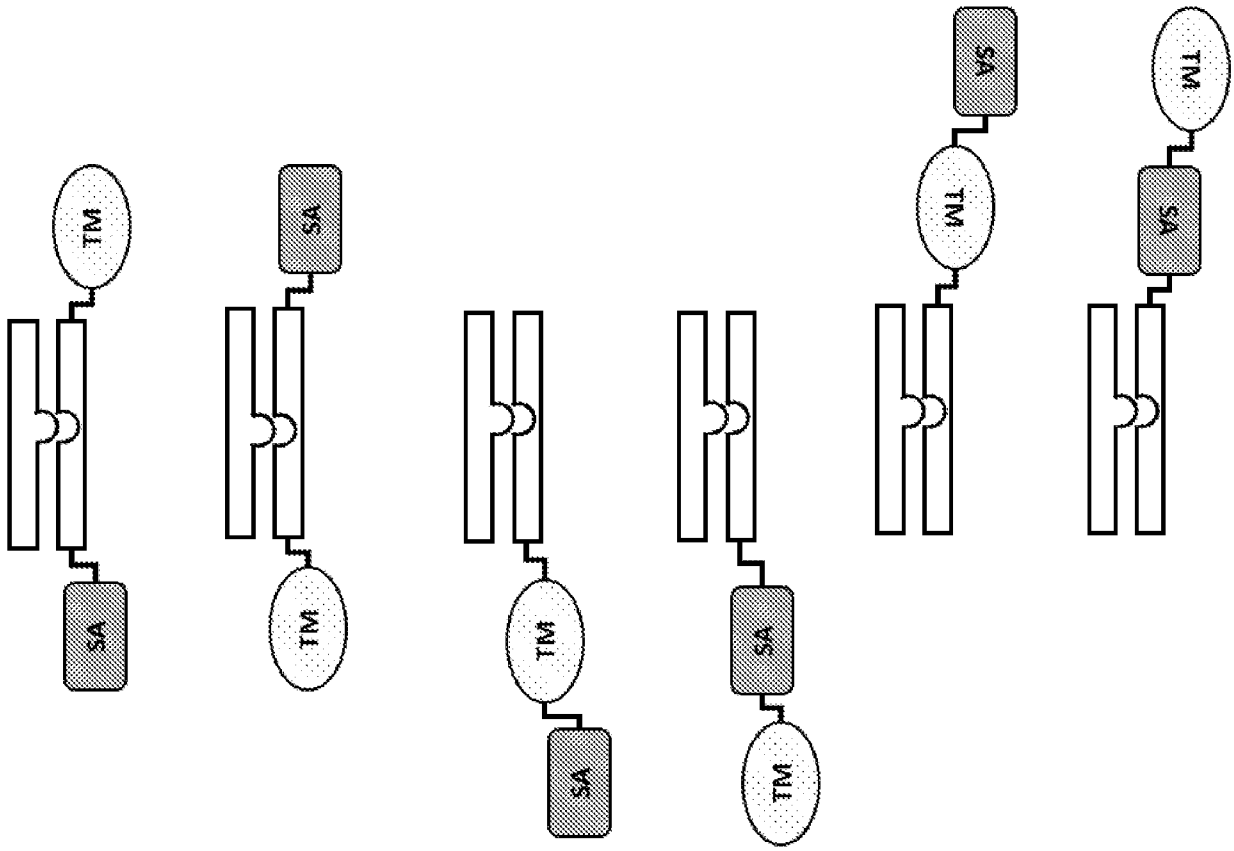


FIG. 14A FIG. 14B FIG. 14C FIG. 14D FIG. 14E FIG. 14F

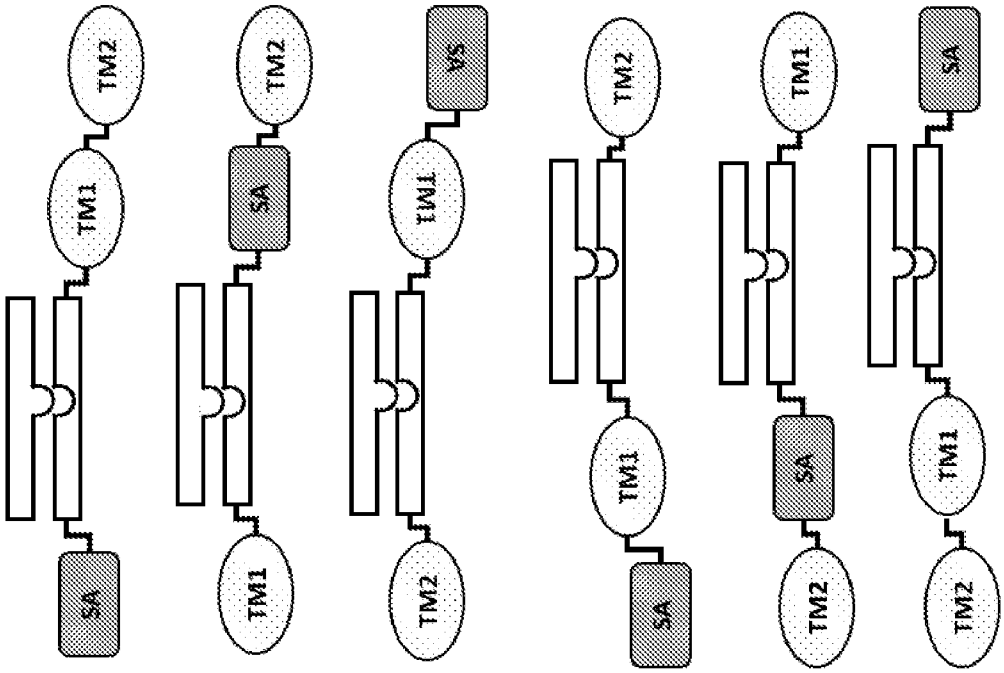


FIG. 14G FIG. 14H FIG. 14I FIG. 14J FIG. 14K FIG. 14L

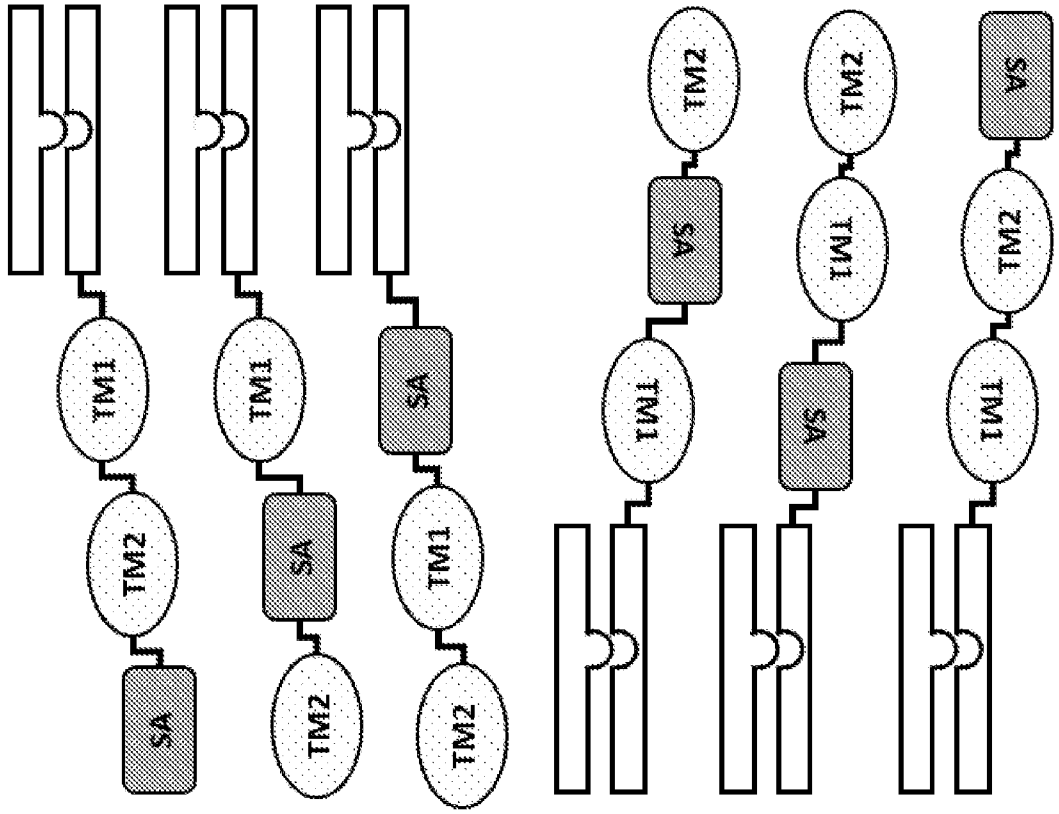


FIG. 15A FIG. 15B FIG. 15C FIG. 15D FIG. 15E FIG. 15F

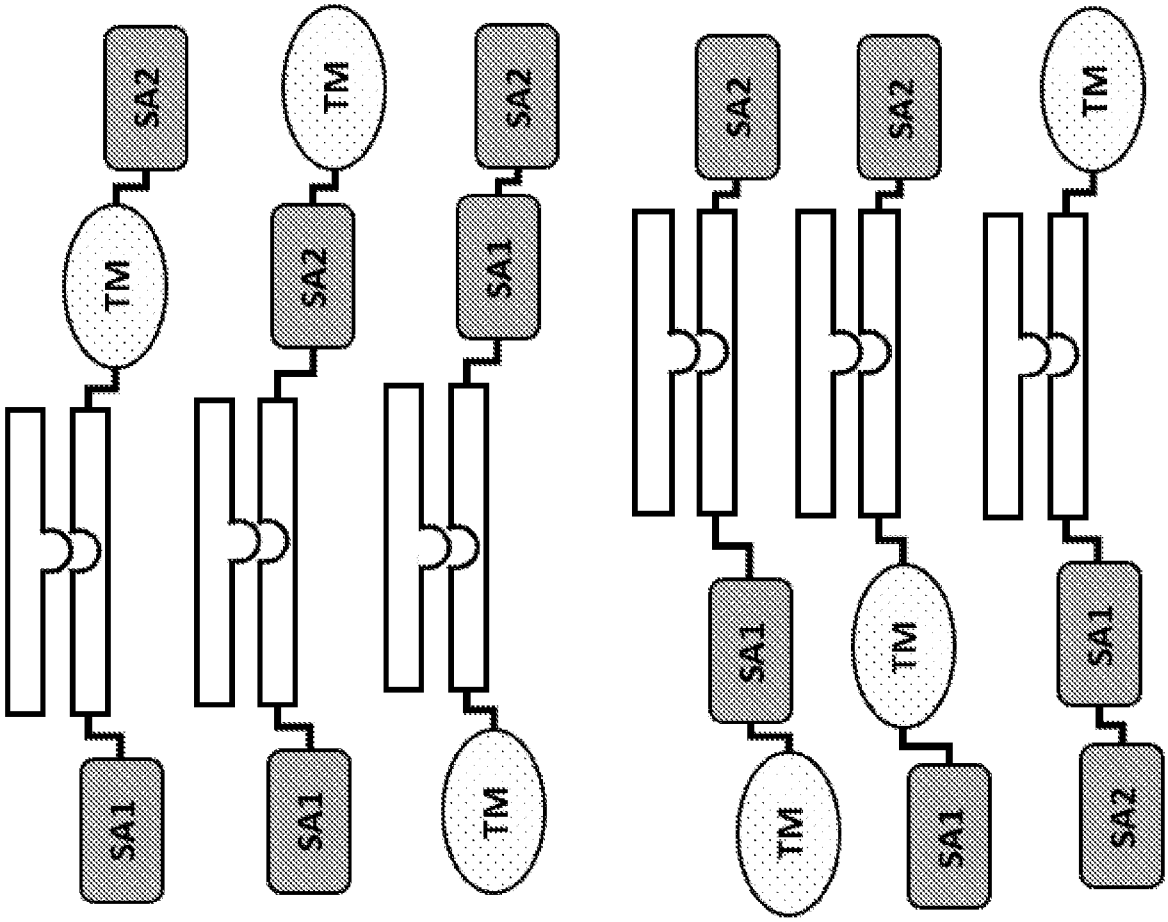


FIG. 15G FIG. 15H FIG. 15I FIG. 15J FIG. 15K FIG. 15L

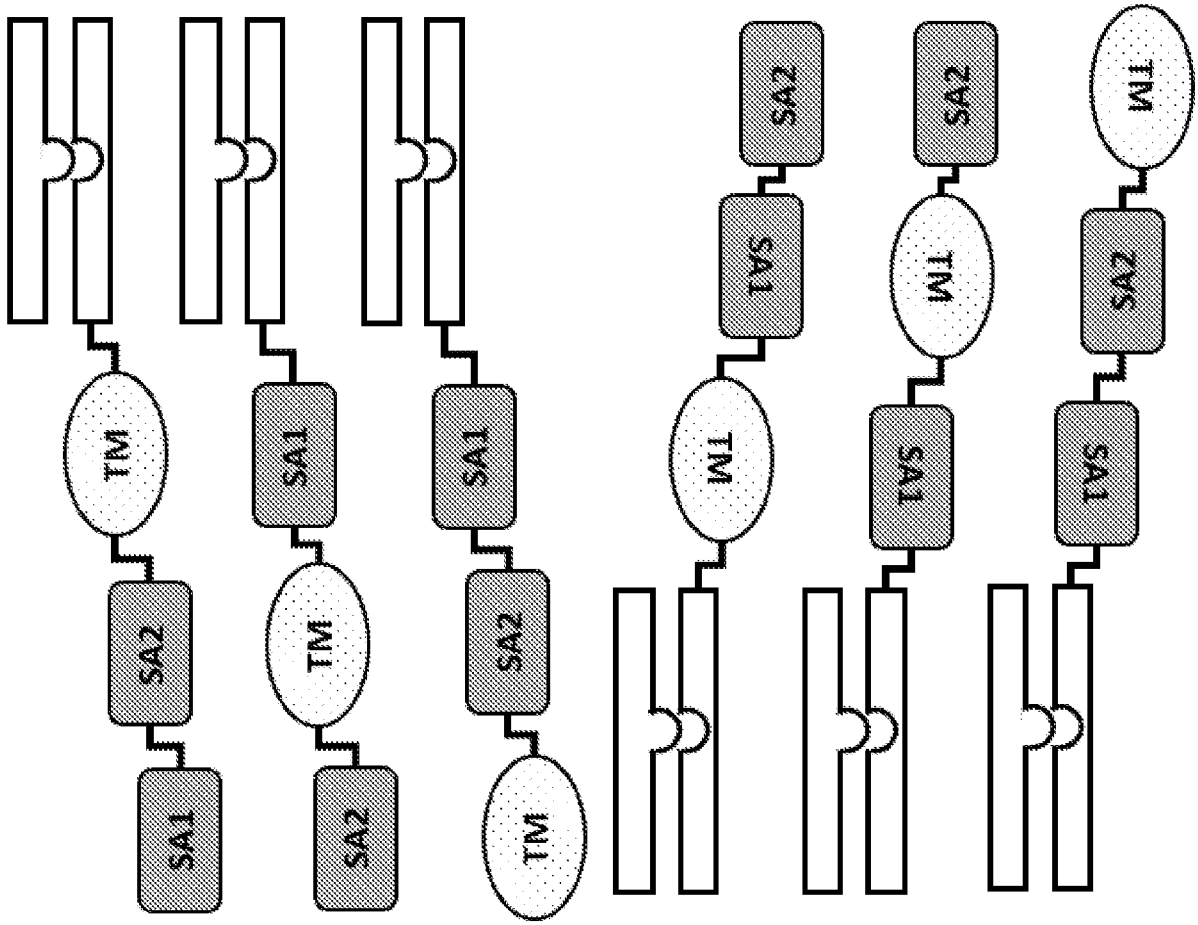


FIG. 16A FIG. 16B FIG. 16C FIG. 16D FIG. 16E

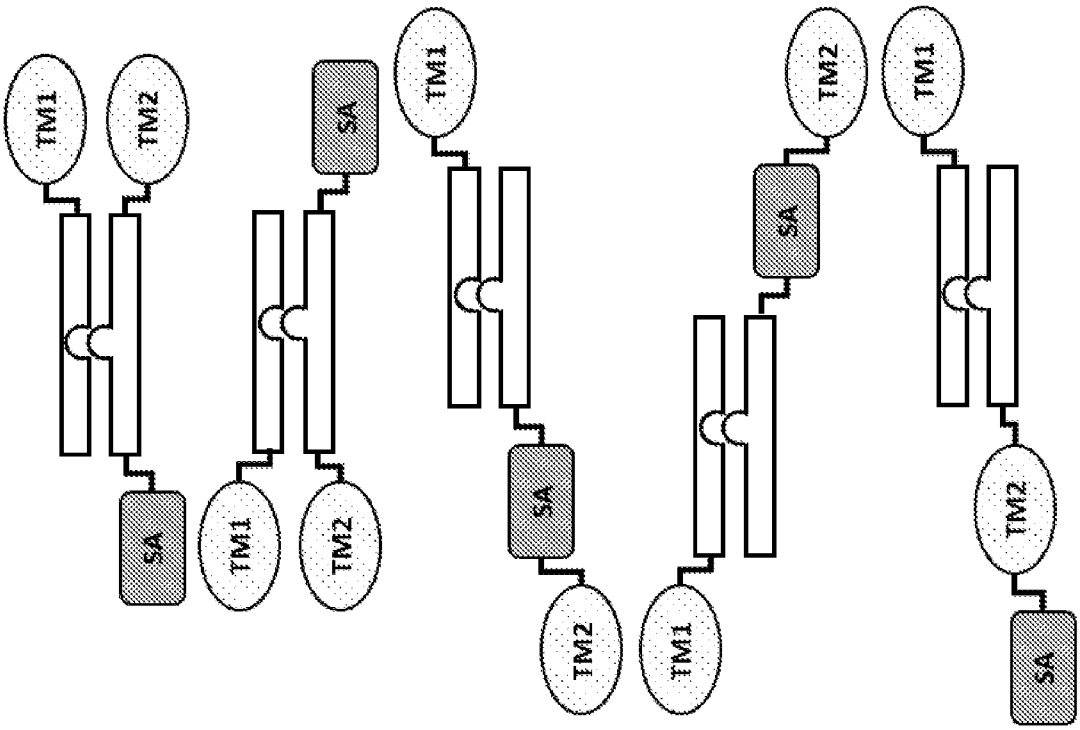


FIG. 16F FIG. 16G FIG. 16H FIG. 16I FIG. 16J

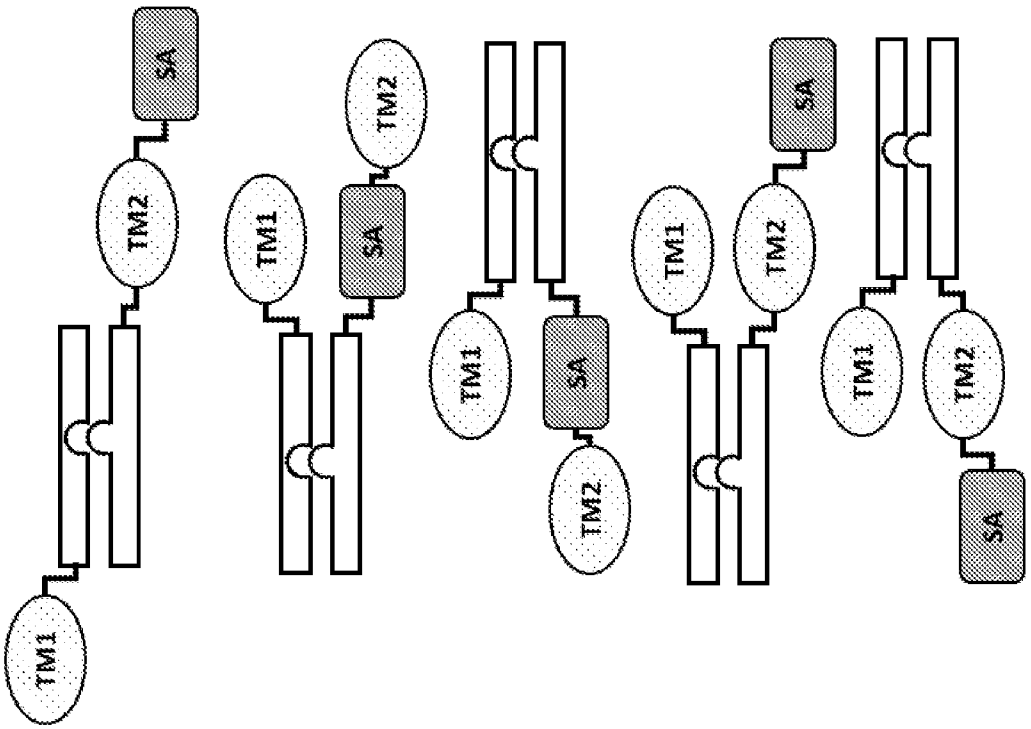


FIG. 17A FIG. 17B FIG. 17C FIG. 17D FIG. 17E

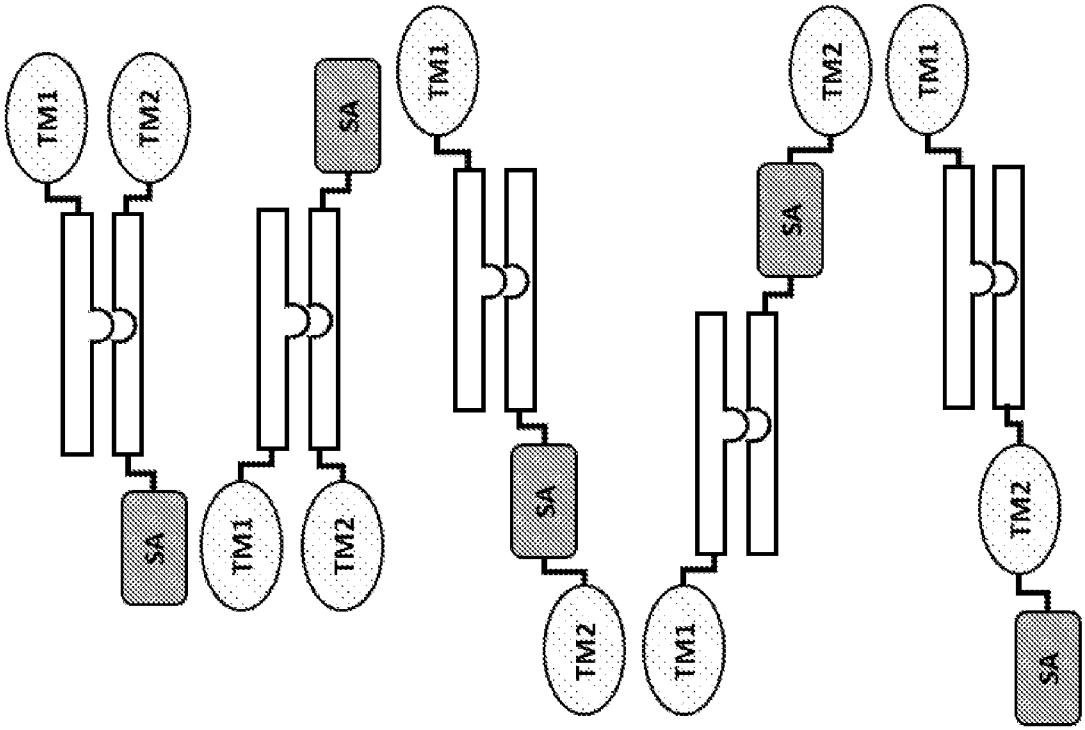


FIG. 17F FIG. 17G FIG. 17H FIG. 17I FIG. 17J

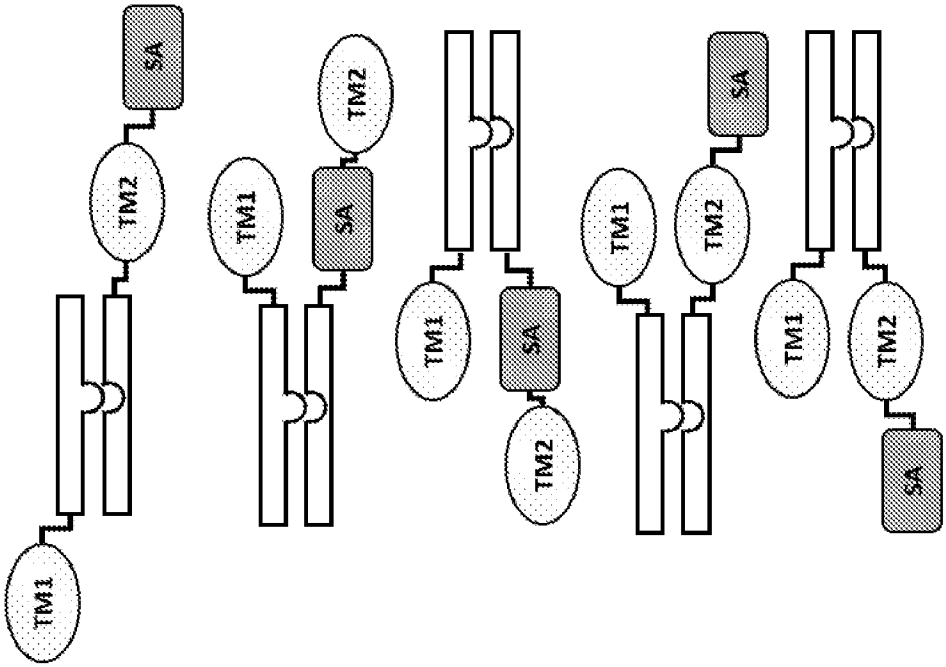


FIG. 18A FIG. 18B FIG. 18C FIG. 18D FIG. 18E FIG. 18F

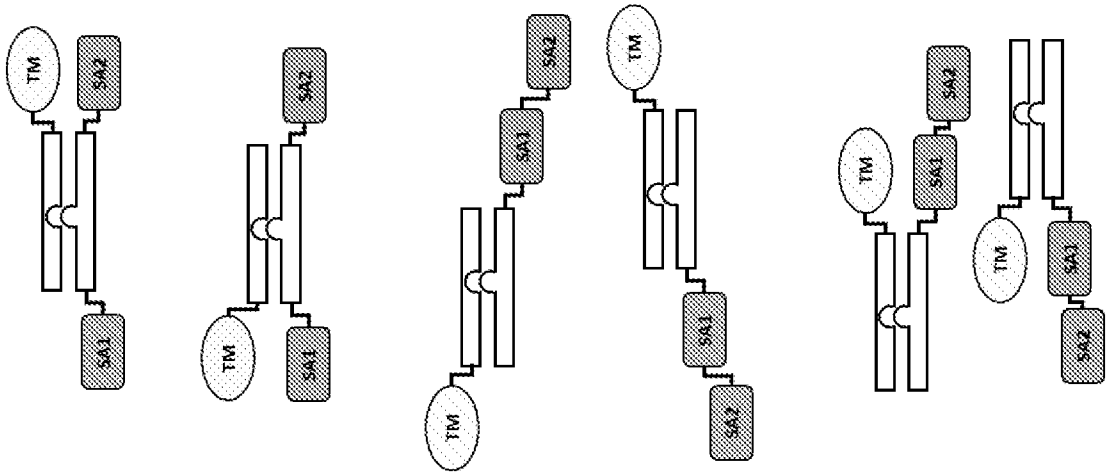


FIG. 19A

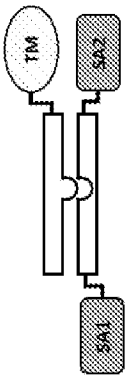


FIG. 19B

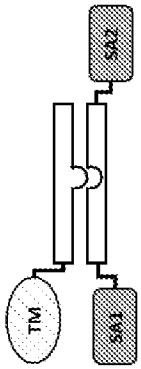


FIG. 19C

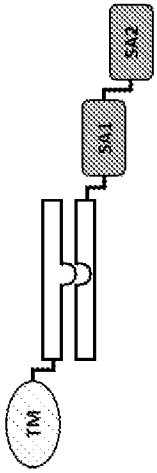


FIG. 19D

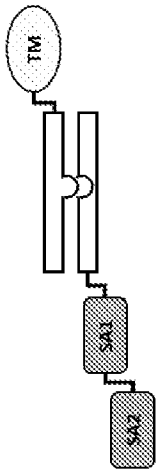


FIG. 19E

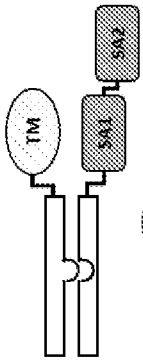


FIG. 19F

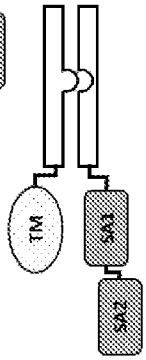


FIG. 20

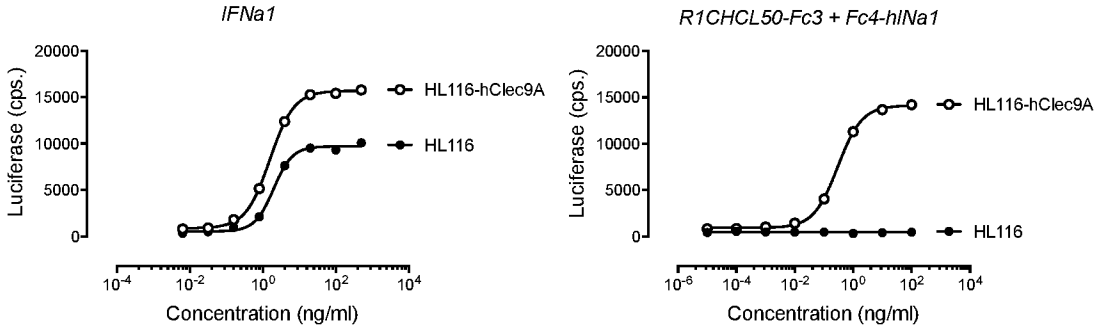

IFNa1
Luciferase (cps.)
20000
15000
10000
5000
0
HL116-hClec9A
HL116
10^{-4} 10^{-2} 10^{0} 10^{2} 10^{4}
Concentration (ng/ml)
R1CHCL50-Fc3 + Fc4-hINa1
Luciferase (cps.)
20000
15000
10000
5000
0
HL116-hClec9A
HL116
10^{-6} 10^{-4} 10^{-2} 10^{0} 10^{2} 10^{4}
Concentration (ng/ml)

FIG. 21A

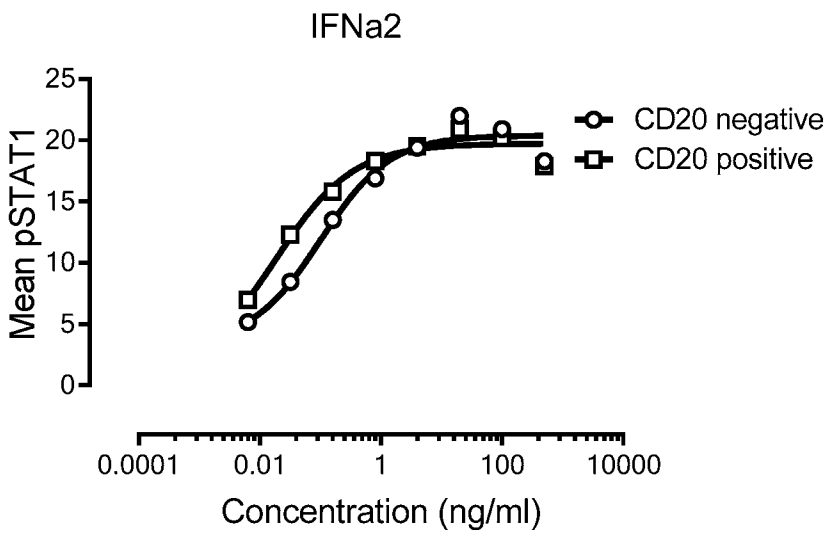

IFNa2
Mean pSTAT1
25
20
15
10
5
0
0.0001
0.01
1
100
10000
Concentration (ng/ml)
CD20 negative
CD20 positive

FIG. 21B

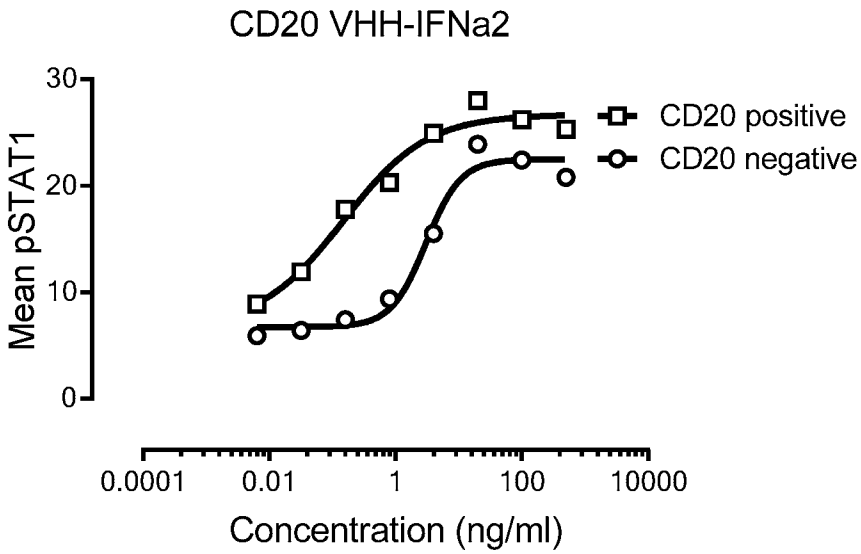


FIG. 21C

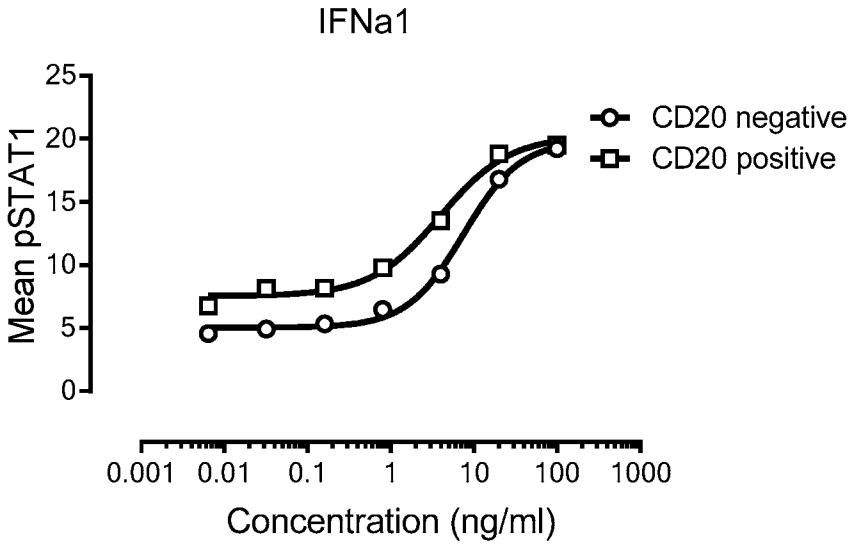


FIG. 21D

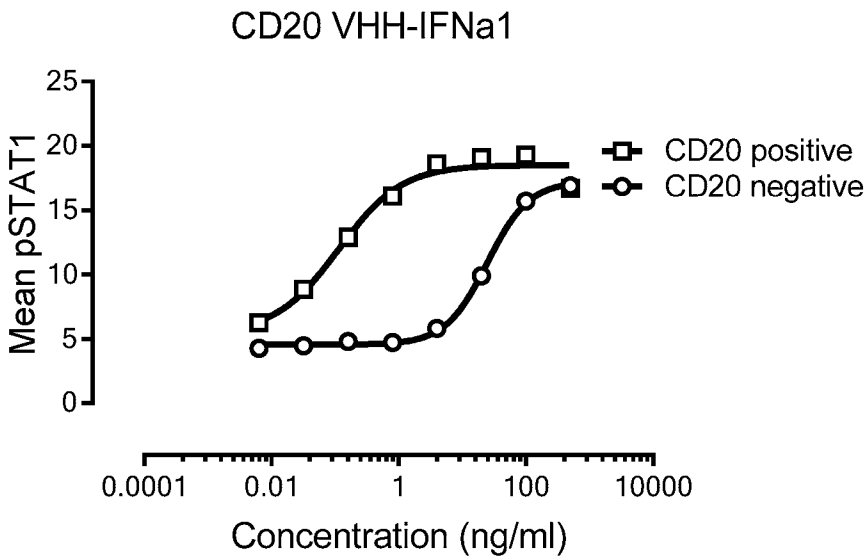

CD20 VHH-IFNa1
Mean pSTAT1
25
20
15
10
5
0
CD20 positive
CD20 negative
0.0001
0.01
1
100
10000
Concentration (ng/ml)

FIG. 22A

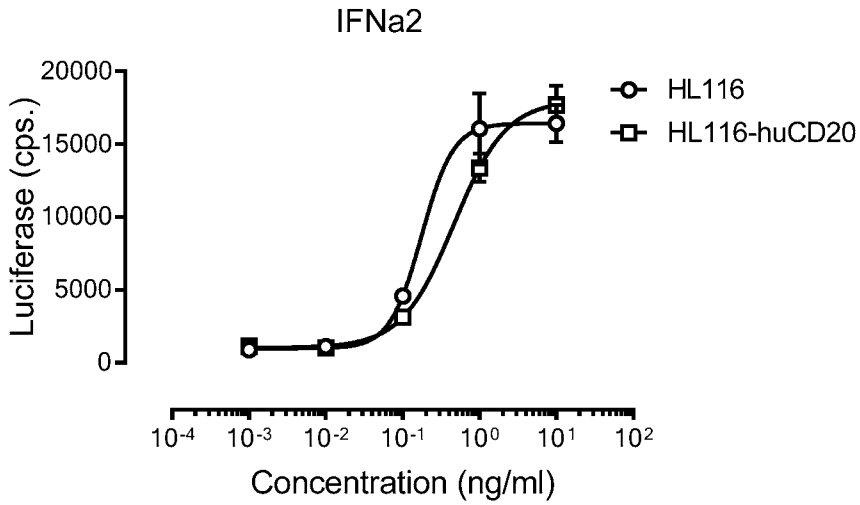

IFNa2
Luciferase (cps.)
20000
15000
10000
5000
0
HL116
HL116-huCD20
10-4
10-3
10-2
10-1
100
101
102
Concentration (ng/ml)

FIG. 22B

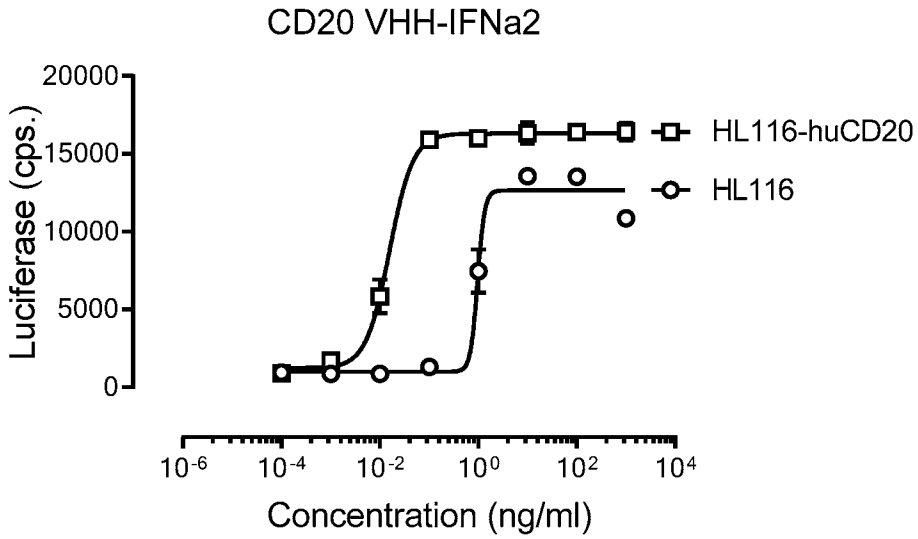


FIG. 22C

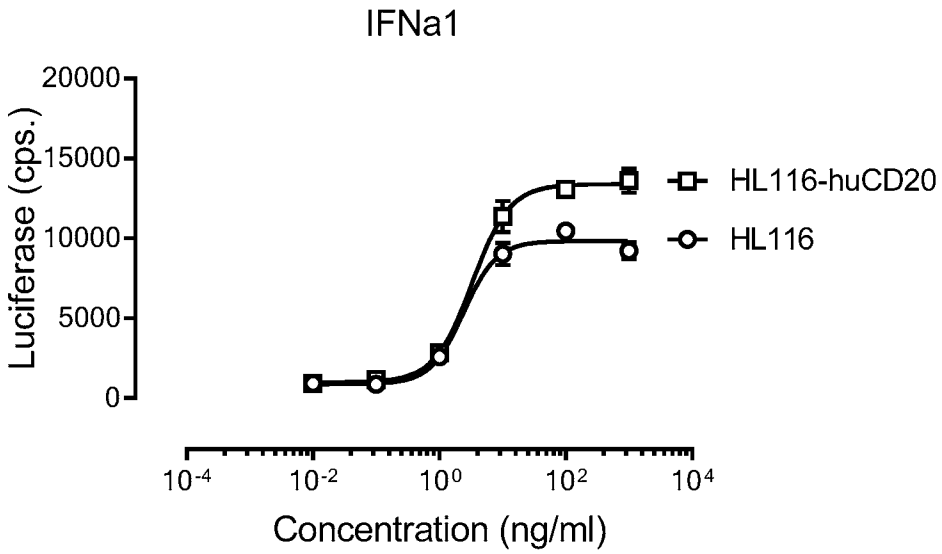


FIG. 22D

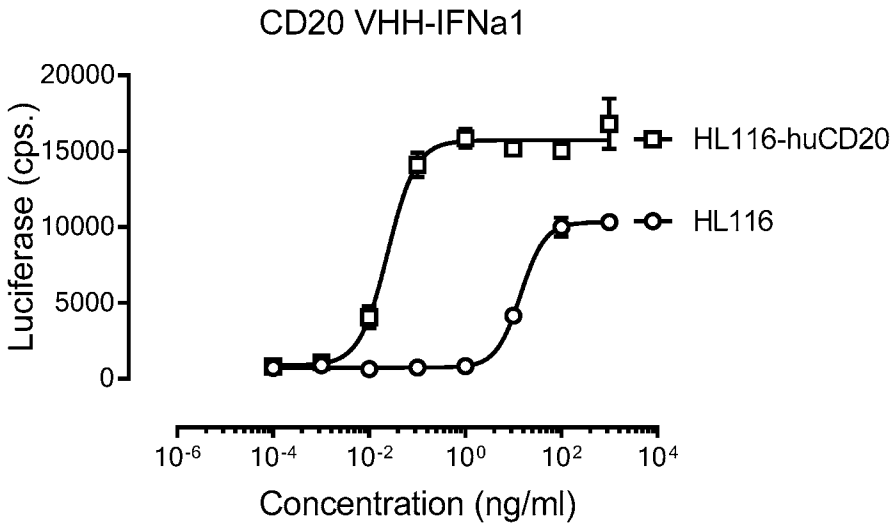


FIG. 23

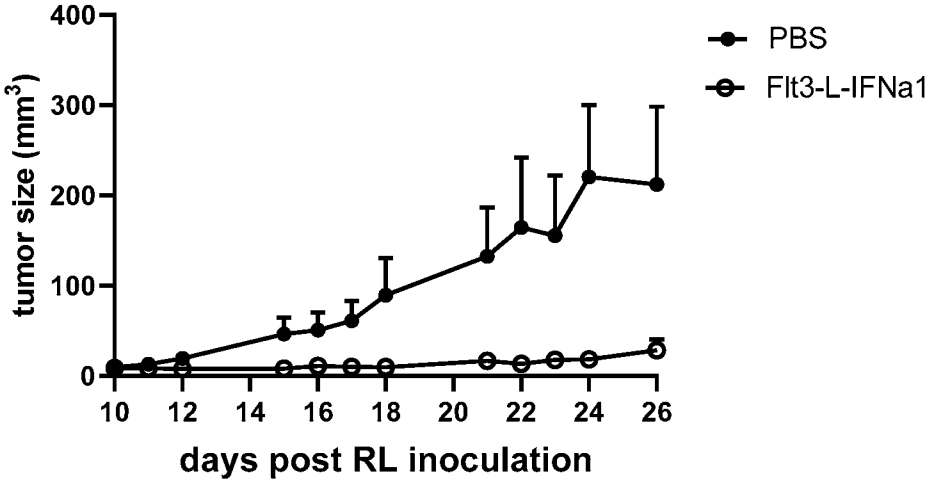


FIG. 24A

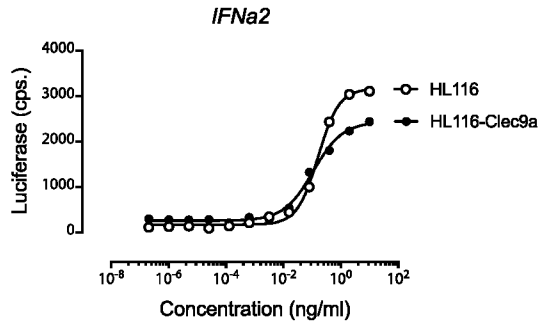


FIG. 24B

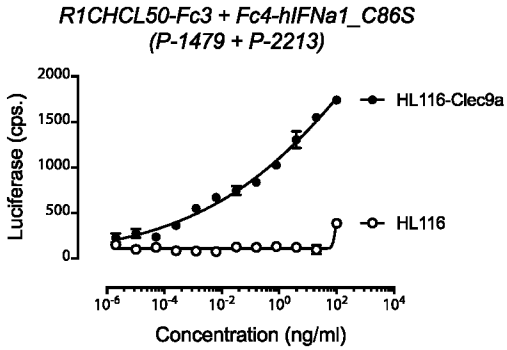


FIG. 24C

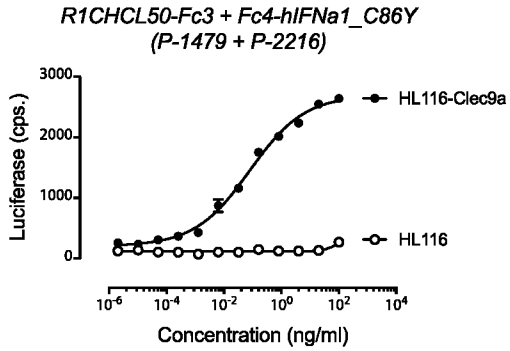


FIG. 24D

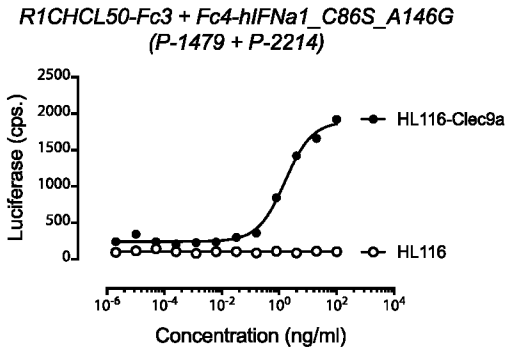


FIG. 24E

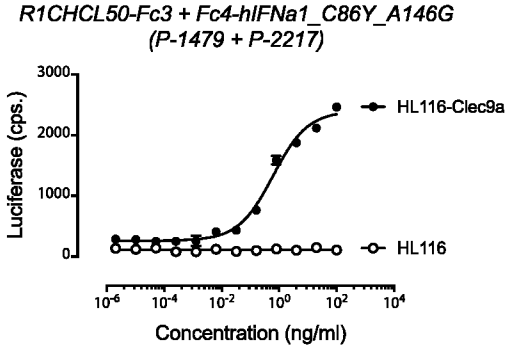


FIG. 24F

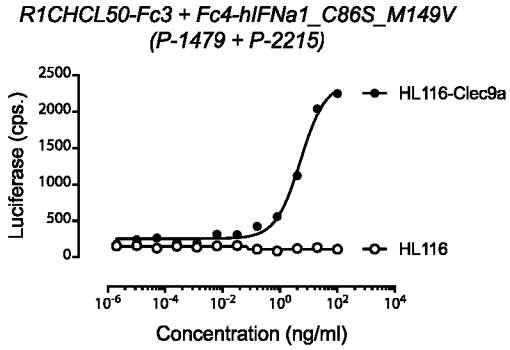


FIG. 24G

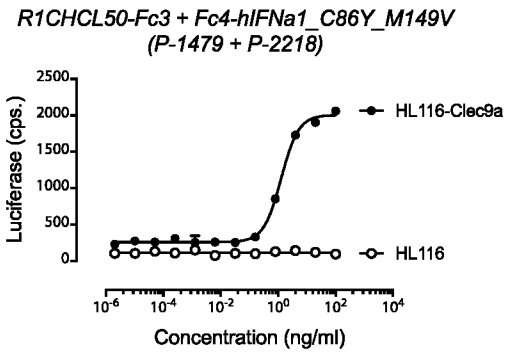


FIG. 25A

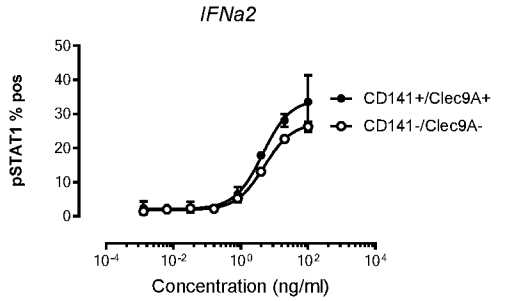


FIG. 25B

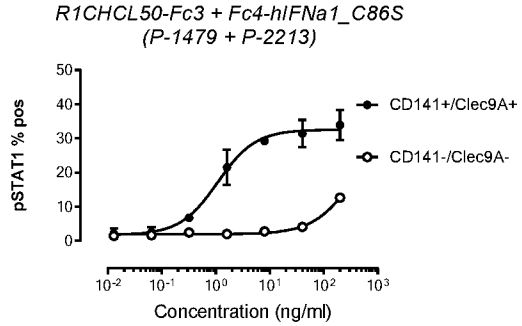


FIG. 25C

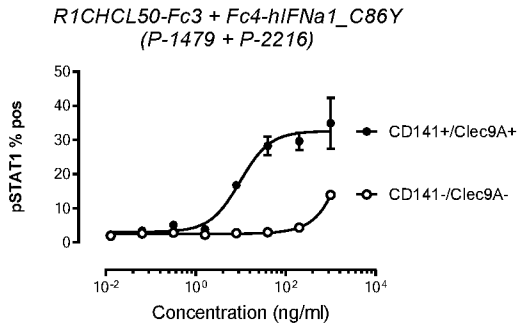


FIG. 25D

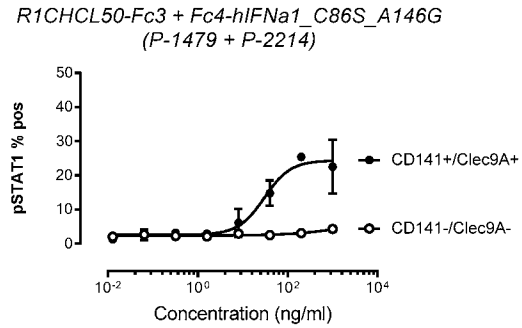


FIG. 25E

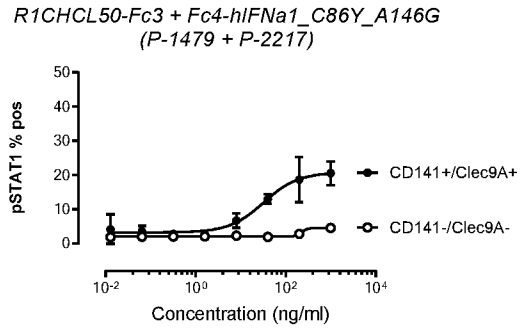

R1CHCL50-Fc3 + Fc4-hIFNa1_C86Y_A146G
(P-1479 + P-2217)
pSTAT1 % pos
50
40
30
20
10
0
CD141+/Clec9A+
CD141-/Clec9A-
10-2
100
102
104
Concentration (ng/ml)

FIG. 26A

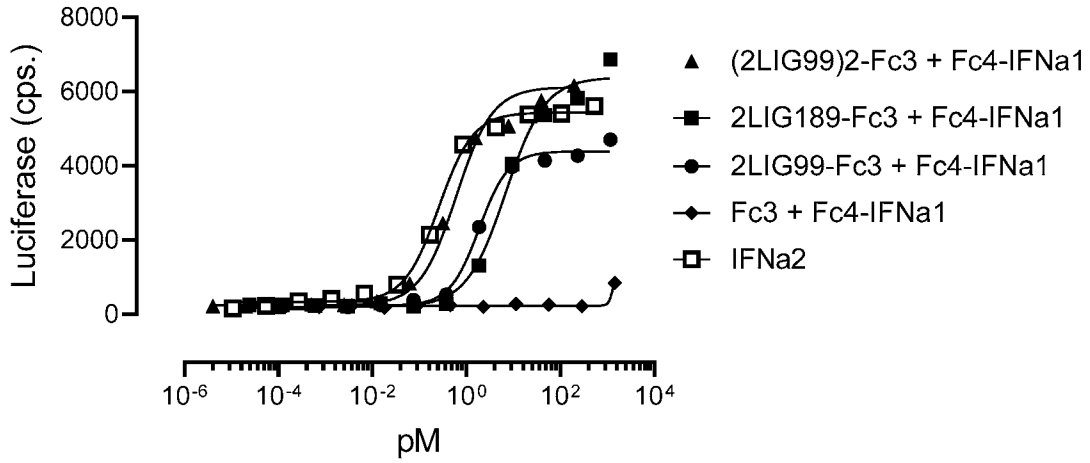
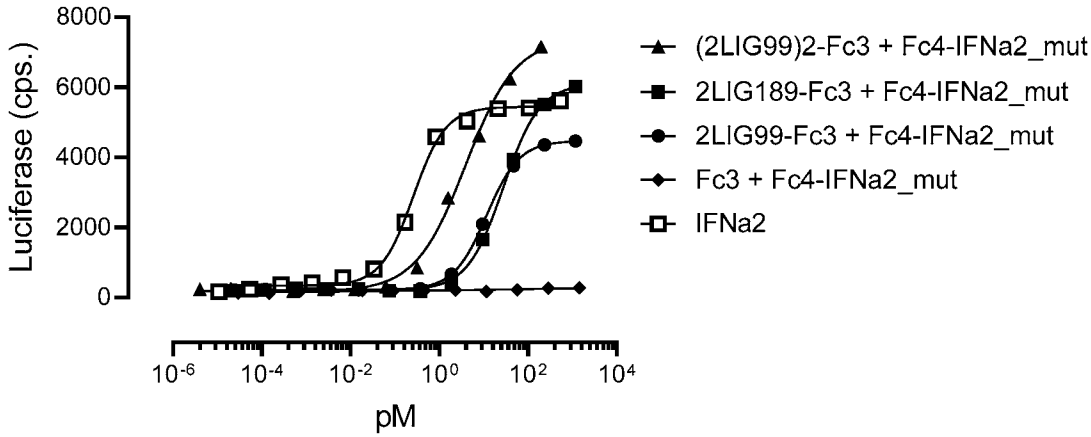


FIG. 26B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/025411

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/00; A61K 38/21; A61K 39/00; A61K 39/395; A61K 45/06; A61K 47/48 (2020.01)

CPC - A61K 38/00; A61K 38/212; A61K 39/39558; A61K 45/06; A61K 2039/505; A61P 35/00; A61P 35/02; C07K 14/56; C07K 16/18; C07K 16/2803; C07K 16/2827; C07K 16/2851; C07K 16/2863; C07K 16/2887; C07K 16/2896; C07K 16/30; C07K 16/32; C07K 2317/73; C07K 2317/76; C07K 2317/90; C07K 2319/00; C07K 2319/30; C07K 2319/33 (2020.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2014/0248238 A1 (TEVA PHARMACEUTICALS AUSTRALIA PTY LTD) 04 September 2014 (04.09.2014) entire document	1-8, 39-46, 65 ---------- 99
X -- Y	US 2013/0230517 A1 (IMMUNGENE INC.) 05 September 2013 (05.09.2013) entire document	97, 98 --------- 99
A	US 2010/0297076 A1 (MORRISON et al) 25 November 2010 (25.11.2010) entire document	1-8, 39-46, 65, 97-99
A	US 2011/0020273 A1 (CHANG et al) 27 January 2011 (27.01.2011) entire document	1-8, 39-46, 65, 97-99
A	CN 103319608 B (ZONHON BIOPHARMA INST INC et al) 27 August 2014 (27.08.2014) entire document; see machine translation	1-8, 39-46, 65, 97-99

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 June 2020	09 JUL 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/025411

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NO:1 was searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/025411

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 9-38, 47-64, 66-96, 100-134
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.