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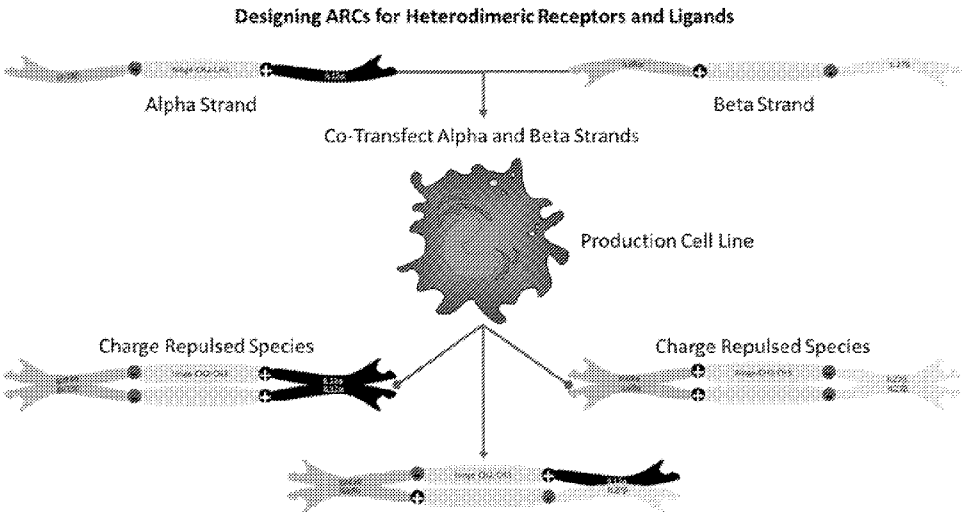
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(54) Title: HETERODIMERIC PROTEINS AND USES THEREOF

FIG. 1



(57) Abstract: The present invention relates, inter alia, to compositions and methods, including heterodimeric proteins that find use in the treatment of disease, such as immunotherapies for cancer and autoimmunity.

WO 2019/246508 A1

[Continued on next page]

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HETERODIMERIC PROTEINS AND USES THEREOF

TECHNICAL FIELD

The present invention relates to heterodimeric proteins that find use in the treatment of diseases, such as immunotherapies for cancer and autoimmunity.

PRIORITY

This application claims the benefit of U.S. Provisional Application No. 62/688,167, filed June 21, 2018 and U.S. Provisional Application No. 62/703,248, filed July 25, 2018. The contents of each of which is incorporated herein by reference in its entirety.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

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: SHK-004PC-Sequence_Listing_ST25; date created on: June 21, 2019; file size: 112 KB).

BACKGROUND

Protein-protein interactions are critical for the normal physiological functions of cells and multicellular organisms. For example, cytokines act as ligands which bind to their cognate receptors so as to regulate essential biological processes such as inflammation and immunity. In this regard, many natural cytokines, cytokine receptors, integrins, and other proteins exist or function as multimeric protein complexes. Some multimers, such as those within the tumor necrosis factor superfamily function as homotrimers, other ligands including the IL-12 family of cytokines (*e.g.*, IL12, IL23, IL27, or IL-35) form heterodimers. Similarly, cytokine receptors may also function as heterodimeric complexes. For example, many interleukin receptors form heterodimers for signal transduction.

The modulation of protein-protein interactions is a useful mechanism for therapeutic intervention in various diseases and pathologies. Soluble binding proteins which interact with ligands can potentially sequester the ligand away from the receptor, thereby reducing the activation of that particular receptor pathway. Alternatively, sequestration of the ligand may delay its elimination or degradation, thereby increasing its duration of effect and biological activity. Additionally, soluble ligands may be utilized to either activate or inhibit specific receptors. Nevertheless, the synthesis and manufacturing of soluble proteins may be hampered where it is desirable to produce heterodimeric proteins. Particularly, the efficiency of synthesis may be greatly compromised by the formation of mixtures of homodimers and heterodimers.

Accordingly, there remains a need for novel methods for the efficient synthesis and manufacturing of heterodimeric proteins for therapeutic use.

SUMMARY

In various embodiments, the present invention provides a heterodimeric protein comprising a first polypeptide chain and a second polypeptide chain, wherein the first polypeptide chain comprises a first subunit of a first protein at the amino terminus linked by a first charge polarized core domain to a first subunit of a second protein at the

carboxy terminus; and the second polypeptide chain comprises a second subunit of the first protein at the amino terminus linked by a second charge polarized core domain to a second subunit of the second protein at the carboxy terminus.

In various embodiments, the first polypeptide chain and the second polypeptide chain heterodimers through electrostatic interactions between positively charged amino acid residues and negatively charged amino acid residues on the first and second charge polarized core domains. In some embodiments, the positively charged amino acid residues may include one or more of amino acids selected from His, Lys, and Arg. In some embodiments, the negatively charged amino acid residues may include one or more amino acids selected from Asp and Glu.

Accordingly, in various embodiments, each of the first and/or second charge polarized core domains comprises peptides having positively or negatively charged amino acid residues at the amino and carboxy terminus of the core domain. In an exemplary embodiment, the first charge polarized core domain may comprise a peptide having positively charged amino acids at the amino terminus which are adjoined by a linker (*e.g.*, a stabilizing domain) to a peptide having negatively charged amino acid residues at the carboxy terminus. In such an embodiment, the second charge polarized core domain may comprise a peptide having negatively charged amino acids at the amino terminus which are adjoined by a linker (*e.g.*, a stabilizing domain) to a peptide having positively charged amino acid residues at the carboxy terminus. In another exemplary embodiment, the first charge polarized core domain may comprise a peptide having negatively charged amino acids at the amino terminus which are adjoined by a linker (*e.g.*, a stabilizing domain) to a peptide having positively charged amino acid residues at the carboxy terminus. In such an embodiment, the second charge polarized core domain may comprise peptides having positively charged amino acids at the amino terminus which are adjoined by a linker (*e.g.*, a stabilizing domain) to a peptide having negatively charged amino acid residues at the carboxy terminus.

In various embodiments, each of the first and/or second charge polarized core domains further comprise a linker (*e.g.*, a stabilizing domain) which adjoins the peptides having positively or negatively charged amino acids. In some embodiments, the linker (*e.g.*, a stabilizing domain) is optionally selected from a flexible amino acid sequence, IgG hinge region, or antibody sequence. In an embodiment, the linker (*e.g.*, a stabilizing domain) comprises the hinge-CH2-CH3 Fc domain derived from IgG1, optionally human IgG1. In another embodiment, the linker (*e.g.*, a stabilizing domain) comprises the hinge-CH2-CH3 Fc domain derived from IgG4, optionally human IgG4.

In some embodiments, the first and/or second protein is selected from a cytokine, a growth factor, and/or a hormone. In some embodiments, the first and/or second protein is selected from a receptor for a cytokine, a growth factor, and/or a hormone.

In embodiments, in a heterodimeric protein, the first protein is selected from Table 1 and/or the second protein is selected from Table 1.

In embodiments, the first and/or second protein is an interleukin. In embodiments, the first and/or second protein is IL-35 comprising the IL12α and IL27β subunits.

In embodiments, the first and/or second protein is selected from a receptor for a cytokine, a growth factor, and/or a hormone. In embodiments, the first and/or second protein is a receptor for an interleukin.

In embodiments, the first and/or second protein is IL6 receptor comprising the IL6Rα and gp130 subunits.

In embodiments, the first and/or second protein is IL21 receptor comprising the IL21r and IL2rg subunits.

In embodiments, the first and/or second protein is IL21 receptor comprising the IFNgR and IFNGR2 subunits.

In embodiments, the protein on the amino- or carboxy-terminus is natively heterodimeric, and wherein the protein on the opposite terminus is not natively heterodimeric.

Also in various aspects, the present heterodimeric protein is used in a method for treating autoimmune diseases comprising administering an effective amount of a pharmaceutical composition comprising the heterodimeric protein to a patient in need thereof. In further aspects, the present heterodimeric protein is used in a method for treating infections, including without limitation, viral infections or other intracellular pathogens. In still further aspects, the present heterodimeric protein is used in a method for treating cancers.

Any aspect or embodiment disclosed herein can be combined with any other aspect or embodiment as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 provides illustrations protein engineering embodiments of the invention, showing an exemplary heterodimeric protein of the invention comprising the IL6 receptor (which includes the IL6Rα and gp130 subunits) and the IL-35 cytokine (which includes the IL12α and IL27β subunits). The heterodimeric protein is preferentially formed through electrostatic interactions between the charge polarized core domains.

FIG. 2 provides Western blot analysis of a production run of gp130-Fc-IL12a and IL6RA-Fc-IL27β from a transient transfection culture. The secreted protein was captured using affinity chromatography and eluted from the column to obtain a protein which contains a domain recognized by a human Fc specific antibody. Because the protein was run under denaturing conditions, individual bands are visualized for the alpha and beta strands.

FIG. 3 provides Western blot analysis of the purified gp130-Fc-IL12a and IL6RA-Fc-IL27β heterodimeric protein. The SDS-PAGE gels (left two gels) indicated the presence of a single band at approximately 300 kDa under non-denaturing conditions (left lane beside the molecular weight ladder in each blot). This band could be separated to the constituent alpha and beta strands following incubation with beta-mercaptoethanol (middle lane in each gel), which ran at an apparent molecular weight which was higher than predicted, and potentially indicative of post-translational modifications including glycosylation. This was confirmed in the right-most lane in each gel, which indicated that the molecular weight of the alpha and beta strands decreased to the predicted molecular weight following removal of N- and O-linked glycosylations. For the gel on the right, the native PAGE gel was used to further investigate the proportion of the purified protein which existed in the alpha/beta heterodimer form as compared to the alpha/alpha or beta/beta homodimer form. The gel indicated an enrichment of the alpha/beta

heterodimer to approximately 60% of the total protein in the preparation as compared to approximately 30% of the alpha/alpha homodimer and 10% of the beta/beta homodimer.

FIG. 4 depicts quantification of captured heterodimeric IL-6R-Fc-IL-35 protein using spectrophotometry.

FIG. 5 provides a size-exclusion chromatography (SEC) chromatogram of the IL-6R-Fc-IL-35 construct following dual transfection of the gp130-alpha-IL12A and IL6RA-beta-IL27B constructs in CHO cells followed by purification of the secreted protein using protein A.

FIG. 6 provides a schematic of an ELISA assay that was developed to demonstrate that the IL-6R-Fc-IL-35 protein was capable of binding to immobilized human IL-6. Only the intended species (shown in the upper middle diagram) was expected to bind IL-6 in this assay, which could be specifically detected with an antibody against the IL-27a (EBI3) domain of the heterodimer.

FIG. 7 provides another schematic of an ELISA assay that was developed to demonstrate that the IL-6R-Fc-IL-35 protein was capable of binding to immobilized human IL-6. The bound protein was detected using the IL-6RA domain.

FIG. 8 provides a schematic of an ELISA assay that was developed which specifically captured an exemplary heterodimeric protein of the invention using an anti-human gp130 antibody and detected the bound protein with an anti-human IL-27a (EBI3) antibody.

FIG. 9 provides a schematic of an ELISA assay that was developed which specifically captured an exemplary heterodimeric protein of the invention using an anti-human gp130 antibody and detected the bound protein using the IL-6RA domain.

FIG. 10 provides a schematic of an ELISA assay that was developed which specifically captured an exemplary heterodimeric protein of the invention using the IL-6RA domain and detected the bound protein with an anti-human IL-27a (EBI3) antibody.

FIG. 11 provides a schematic of an ELISA assay that was developed which specifically captured an exemplary heterodimeric protein of the invention using the IL-6RA domain and detected the bound protein using the IL-6RA domain.

FIG. 12 provides a schematic of an ELISA assay that was developed which specifically captured an exemplary heterodimeric protein of the invention using the IL-12a p35 and detected the bound protein with an anti-human IL-27a (EBI3) antibody.

FIG. 13 provides a schematic of an ELISA assay that was developed which specifically captured an exemplary heterodimeric protein of the invention using the IL-12a p35 domain and detected the bound protein with the IL-6RA domain.

FIG. 14 provides a schematic of an ELISA assay that was developed which specifically captured an exemplary heterodimeric protein of the invention using an anti-human IL-27a (EBI3) antibody and detected the bound protein with the anti-human IL-27a (EBI3) antibody.

FIG. 15 provides a schematic of an ELISA assay that was developed which specifically captured an exemplary heterodimeric protein of the invention using an anti-human IL-27a (EBI3) antibody and detected the bound protein with the IL-6RA domain.

FIG. 16A and FIG. 16B provide size-exclusion chromatography (SEC) chromatogram of the IL-6R-Fc-IL-35 heterodimeric proteinIL-6R-Fc-IL-35. In FIG. 16A, the absorbance wavelength was 210 nm and in FIG. 16B, the absorbance wavelength was 280 nm.

FIG. 17 is a graph showing the results of an IL-6 SINK Assay using the IL-6R-Fc-IL-35 heterodimeric protein.

FIG. 18 includes graph showing the ability of the IL-6R-Fc-IL-35 heterodimeric protein (identified as HdA '00) to induce at least IL-35. The condition "Halycon" refers to treatments with a control chimeric protein.

FIG. 19A shows a schematic of the IL-21R-Fc-IL-35 heterodimeric protein comprising a IL-21r-Fc(alpha)-IL12a chain and a IL2rg-Fc(beta)-IL27B chain. FIG. 19B shows an SDS-PAGE gel indicating the presence of two single bands at approximately 84.4 kDa and 78.1kDa under reduced deglycosylated conditions (right-most lane).

FIG. 20 provides a size-exclusion chromatography (SEC) chromatogram of the IL-21R-Fc-IL-35 IFNγR-Fc-IL-35 heterodimeric protein following dual transfection of the IL-21r-Fc(alpha)-IL12a and IL2rg-Fc(beta)-IL27B constructs in CHO cells followed by purification of the secreted protein using protein A.

FIG. 21 are western blot analyses of the IFNγR-Fc-IL-35 heterodimeric protein comprising the IFNgR-Alpha-IL12a chain and the IFNGR2-Beta-IL27B chain probed with an antibody indicated below each blot. The proteins were run under non-denaturing conditions (left lane beside the molecular weight ladder in each blot), denaturing conditions with beta-mercaptoethanol treatment (middle lane in each gel), and both denaturing and de-glycosylation treatments.

FIG. 22 provides a size-exclusion chromatography (SEC) chromatogram of the IFNγR-Fc-IL-35 heterodimeric protein following dual transfection of the IFNgR-Alpha-IL12a and IFNGR2-Beta-IL27B chain constructs in CHO cells followed by purification of the secreted protein using protein A.

DETAILED DESCRIPTION

The present invention is directed to a protein engineering platform for synthesizing and manufacturing heterodimeric proteins. The method of the invention allows for the efficient production of heterodimeric proteins for use in modulating immune signals for the treatment of various diseases, including, without limitation, autoimmune diseases.

Charge Polarized Core Domains

In one aspect, the present invention relates to heterodimeric proteins. In various embodiments, the heterodimeric protein of the invention comprises two polypeptide chains. The first polypeptide chain comprises a first subunit of a first protein at the amino terminus linked by a first charge polarized core domain to a first subunit of a second protein at the carboxy terminus. The second polypeptide chain comprises a second subunit of the first protein at the amino terminus linked by a second charge polarized core domain to a second subunit of the second protein at the carboxy terminus. In various embodiments, the first polypeptide chain and the second polypeptide chain forms a heterodimer through electrostatic interactions between positively charged amino acid residues and negatively charged amino acid residues on the first and second polarized core domains.

In various embodiments, each of the first and second charge polarized core domains comprises peptides having positively or negatively charged amino acid residues at the amino and carboxy terminus of the core domain. In an exemplary embodiment, the first charge polarized core domain may comprise a peptide having positively charged amino acids at the amino terminus which are adjoined by a linker (*e.g.*, a stabilizing domain) to a peptide having negatively charged amino acid residues at the carboxy terminus. The second charge polarized core domain may comprise a peptide having negatively charged amino acids at the amino terminus which are adjoined by a linker (*e.g.*, a stabilizing domain) to a peptide having positively charged amino acid residues at the carboxy terminus.

In another exemplary embodiment, the first charge polarized core domain may comprise a peptide having negatively charged amino acids at the amino terminus which are adjoined by a linker (*e.g.*, a stabilizing domain) to a peptide having positively charged amino acid residues at the carboxy terminus. The second charge polarized core domain may comprise peptides having positively charged amino acids at the amino terminus which are adjoined by a linker (*e.g.*, a stabilizing domain) to a peptide having negatively charged amino acid residues at the carboxy terminus.

In various embodiments, formation of heterodimeric proteins is driven by electrostatic interactions between the positively charged and negatively charged amino acid residues located at the amino and carboxy termini of the first and second charge polarized core domains. Further, formation of homodimeric proteins is prevented by the repulsion between the positively charged amino acid residues or negatively charged amino acid residues located at the amino and carboxy termini of the first and second charge polarized core domains.

In various embodiments, the peptide comprising positively and/or negatively charged amino acid residues at the amino or carboxy terminus of the charge polarized core domains is about 2 to about 50 amino acids long. For example, the peptide comprising positively and/or negatively charged amino acid residues at either terminus of the charge polarized core domain may be 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 various embodiments, the peptide comprising positively charged amino acid residues may include one or more of amino acids selected from His, Lys, and Arg. In various embodiments, the peptide comprising negatively charged amino acid residues may include one or more amino acids selected from Asp and Glu.

In various embodiments, each of the first and/or second charge polarized core domains may comprise a peptide comprising an amino acid sequence as provided in the Table below or an amino acid sequence having at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identity thereto.

SEQ ID NO.	Sequence
1	$Y_nX_nY_nX_nY_n$ (where X is a positively charged amino acid such as arginine, histidine or lysine and Y is a spacer amino acid such as serine or glycine)
2	$Y_nZ_nY_nZ_nY_n$ (where Z is a negatively charged amino acid such as aspartic acid or glutamic acid and Y is a spacer amino acid such as serine or glycine)
3	$YY_nXX_nYY_nXX_nYY_n$ (where X is a positively charged amino acid such as arginine, histidine or lysine and Y is a spacer amino acid such as serine or glycine)
4	$YY_nZZ_nYY_nZZ_nYY_n$ (where Z is a negatively charged amino acid such as aspartic acid or glutamic acid and Y is a spacer amino acid such as serine or glycine)
5	$Y_nX_nCY_nX_nY_n$ (where X is a positively charged amino acid such as arginine, histidine or lysine and Y is a spacer amino acid such as serine or glycine)
6	$Y_nZ_nCY_nZ_nY_n$ (where Z is a negatively charged amino acid such as aspartic acid or glutamic acid and Y is a spacer amino acid such as serine or glycine)
7	GSGSRKGGKRGS
8	GSGSRKCGKRGS
9	GSGSDEGGEDGS
10	GSGSDECGEDGS

For example, in an embodiment, each of the first and second charge polarized core domains may comprise a peptide comprising the sequence $YY_nXX_nYY_nXX_nYY_n$ (where X is a positively charged amino acid such as arginine, histidine or lysine and Y is a spacer amino acid such as serine or glycine; SEQ ID NO: 3). Exemplary peptide sequences include, but are not limited to, RKGGKR (SEQ ID NO: 11) or GSGSRKGGKRGS (SEQ ID NO: 12).

In another exemplary embodiment, each of the first and second charge polarized core domains may comprise a peptide comprising the sequence $YY_nZZ_nYY_nZZ_nYY_n$ (where Z is a negatively charged amino acid such as aspartic acid or glutamic acid and Y is a spacer amino acid such as serine or glycine). Exemplary peptide sequences include, but are not limited to, DEGGED (SEQ ID NO: 13) or GSGSDEGGEDGS (SEQ ID NO: 14).

In embodiments, a charge polarized core domain (negative – positive, also referred herein as an “alpha core domain”) is provided below:

GSGSRKGGKRGSKYGPPCPPCPAPEFLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSQEDPEVQF
NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIEKTISNATGQP
REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT
VDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLSLGKDEGGEDGSGS (SEQ ID NO: 16)

In embodiments, a heterodimeric protein comprises a variant alpha core domain. As examples, the variant may have 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 SEQ ID NO: 16.

In embodiments, a charge polarized core domain (positive - negative, also referred herein as a "beta core domain") is provided below:

GSGSDEGGEDGSKYGPPCPPCPAPEFLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSQEDPEVQF
NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIEKTISNATGQP
REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT
VDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLSLGKRKGGKRGSGS (SEQ ID NO: 17)

In embodiments, a heterodimeric protein comprises a variant beta core domain. As examples, the variant may have 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 SEQ ID NO: 17.

In various embodiments, the peptide comprising the charged amino acid residues may further comprise one or more cysteine residues to facilitate disulfide bonding between the electrostatically charged core domains as an additional method to stabilize the heterodimer.

In various embodiments, each of the first and second charge polarized core domains comprises a linker sequence which may optionally function as a stabilizing domain. 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 some embodiments, the linker (*e.g.*, a stabilizing domain) is a synthetic linker such as PEG.

In other embodiments, the linker (*e.g.*, a stabilizing domain) is a polypeptide. In some embodiments, the linker (*e.g.*, a stabilizing domain) is less than about 500 amino acids long, about 450 amino acids long, about 400 amino

acids long, about 350 amino acids long, about 300 amino acids long, about 250 amino acids long, about 200 amino acids long, about 150 amino acids long, or about 100 amino acids long. For example, the linker (*e.g.*, a stabilizing domain) 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 various embodiments, the linker (*e.g.*, a stabilizing domain) 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).

In various embodiments, the linker (*e.g.*, a stabilizing domain) 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. In other embodiments, the linker may be derived from human IgG4 and contain one or more mutations to enhance dimerization (including S228P) or FcRn binding.

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 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 (*e.g.*, a stabilizing domain) 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 (*e.g.*, a stabilizing domain) of the present invention comprises one or more glycosylation sites.

In various embodiments, the linker (*e.g.*, a stabilizing domain) comprises an Fc domain 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 (*e.g.*, a stabilizing domain) comprises a hinge-CH2-CH3 Fc domain derived from a human IgG4 antibody. In various embodiments, the linker (*e.g.*, a stabilizing domain) comprises a hinge-CH2-CH3 Fc domain derived from a human IgG1 antibody. In some embodiments, the Fc domain exhibits increased affinity for and enhanced binding to the neonatal Fc receptor (FcRn). In some embodiments, the Fc domain includes one or more mutations that increases the affinity and enhances binding to FcRn. Without wishing to be bound by theory, it is believed that increased affinity and enhanced binding to FcRn increases the *in vivo* half-life of the present heterodimeric proteins.

In some embodiments, the Fc domain contains one or more amino acid substitutions at amino acid residue 250, 252, 254, 256, 308, 309, 311, 428, 433 or 434 (in accordance with Kabat numbering), or equivalents thereof. In an embodiment, the amino acid substitution at amino acid residue 250 is a substitution with glutamine. In an embodiment, the amino acid substitution at amino acid residue 252 is a substitution with tyrosine, phenylalanine, tryptophan or threonine. In an embodiment, the amino acid substitution at amino acid residue 254 is a substitution with threonine. In an embodiment, the amino acid substitution at amino acid residue 256 is a substitution with serine, arginine, glutamine, glutamic acid, aspartic acid, or threonine. In an embodiment, the amino acid substitution at amino acid residue 308 is a substitution with threonine. In an embodiment, the amino acid substitution at amino acid residue 309 is a substitution with proline. In an embodiment, the amino acid substitution at amino acid residue 311 is a substitution with serine. In an embodiment, the amino acid substitution at amino acid residue 385 is a substitution with arginine, aspartic acid, serine, threonine, histidine, lysine, alanine or glycine. In an embodiment, the amino acid substitution at amino acid residue 386 is a substitution with threonine, proline, aspartic acid, serine, lysine, arginine, isoleucine, or methionine. In an embodiment, the amino acid substitution at amino acid residue 387 is a substitution with arginine, proline, histidine, serine, threonine, or alanine. In an embodiment, the amino acid substitution at amino acid residue 389 is a substitution with proline, serine or asparagine. In an embodiment, the amino acid substitution at amino acid residue 428 is a substitution with leucine. In an embodiment, the amino acid substitution at amino acid residue 433 is a substitution with arginine, serine, isoleucine, proline, or glutamine. In an embodiment, the amino acid substitution at amino acid residue 434 is a substitution with histidine, phenylalanine, or tyrosine.

In some embodiments, the Fc domain (*e.g.*, comprising an IgG constant region) comprises one or more mutations such as substitutions at amino acid residue 252, 254, 256, 433, 434, or 436 (in accordance with Kabat numbering). In an embodiment, the IgG constant region includes a triple M252Y/S254T/T256E mutation or YTE mutation. In another embodiment, the IgG constant region includes a triple H433K/N434F/Y436H mutation or KFH mutation. In a further embodiment, the IgG constant region includes an YTE and KFH mutation in combination.

In some embodiments, the modified humanized antibodies of the invention comprise an IgG constant region that contains one or more mutations at amino acid residues 250, 253, 307, 310, 380, 428, 433, 434, and 435. Illustrative mutations include T250Q, M428L, T307A, E380A, I253A, H310A, M428L, H433K, N434A, N434F, N434S, and H435A. In an embodiment, the IgG constant region comprises a M428L/N434S mutation or LS mutation. In another embodiment, the IgG constant region comprises a T250Q/M428L mutation or QL mutation. In another embodiment, the IgG constant region comprises an N434A mutation. In another embodiment, the IgG constant region comprises a T307A/E380A/N434A mutation or AAA mutation. In another embodiment, the IgG constant region comprises an I253A/H310A/H435A mutation or IHH mutation. In another embodiment, the IgG constant region comprises a H433K/N434F mutation. In another embodiment, the IgG constant region comprises a M252Y/S254T/T256E and a H433K/N434F mutation in combination.

In various embodiments, mutations are introduced to increase stability and/or half-life of the Fc domain. An illustrative Fc stabilizing mutant is S228P. Additional illustrative Fc half-life extending mutants are T250Q, M428L, V308T, L309P, and Q311S and the present linkers (*e.g.*, stabilizing domains) may comprise 1, or 2, or 3, or 4, or 5 of these mutants.

In embodiments, a core domain, which lacks charge polarization, has the following sequence:

SKYGPPCPPCPAPEFLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIEKTISNATGQPREPQVYTLPPSQ
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNV
FSCSVLHEALHNHYTQKSLSLSLGKIEGRMD (SEQ ID NO: 15)

Additional exemplary mutations in the IgG constant region are described, for example, in Robbie, *et al.*, Antimicrobial Agents and Chemotherapy (2013), 57(12):6147-6153, Dall'Acqua *et al.*, JBC (2006), 281(33):23514-24, Dall'Acqua *et al.*, Journal of Immunology (2002), 169:5171-80, Ko *et al.* Nature (2014) 514:642-645, Grevys *et al*. Journal of Immunology. (2015), 194(11):5497-508, and U.S. Patent No. 7,083,784, the entire contents of which are hereby incorporated by reference.

In various embodiments, the linker may be flexible, including without limitation highly flexible. In various embodiments, the linker may be rigid, including without limitation a rigid alpha helix.

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 heterodimeric protein. In another example, the linker may function to target the heterodimeric protein to a particular cell type or location.

In embodiments, a core domian comprises one or more “Knobs-into-holes” amino acid changes. A “Knobs-into-holes” amino acid change is a rational design strategy previously used in antibody engineering for the heterodimerization of their heavy chains. See, e.g., Ridgway, J. B. et al. “'Knobs-into-holes' engineering of antibody CH3 domains for heavy chain heterodimerization,” *Protein Eng*. 9(7):617-2 (1996) and Carter, “Bispecific human IgG by design.” *Immunol. Methods*, 248(1-2):7-15 (2001), the contents of each of which is incorporated herein by reference in its entirety. Here, amino acid changes are engineered in order to create a “knob” in the CH3 domain of an “alpha” heavy chain and a “hole” in the CH3 of the “beta” heavy chain; alternately, Here, amino acid changes are engineered in order to create a “knob” in the CH3 domain of an “beta” heavy chain and a “hole” in the CH3 of the “alpha” heavy chain. In one example, the “knob” is represented by a tyrosine (Y) that belongs to the “very large” IMGT volume class of amino acids, whereas the “hole” is represented by a threonine (T) that belongs to the “small” IMGT volume class. Characterizations of the IMGT classes of amino acids is described at Pommié, C. et al., “IMGT standardized criteria for statistical analysis of immunoglobulin V-REGION amino acid properties.” *J. Mol. Recognit*., 17, 17-32 (2004), the contents of which is incorporated herein by reference in its entirety. In the interface between two CH3 domains on separate heavy chains, the threonine (T) T22 in the beta heavy chain is within hydrogen-bonding distance of tyrosine (Y) Y86 in the alpha heavy chain. The Y86 is the principal interdomain contact of T22 and these amino acids are involved in a hydrogen bond. However, Y86 also makes numerous van der Waals contacts with Y86 and with Lysine (K) K88 on its opposite heavy chain.

Below are illustrative hinge-CH2-CH3 comprising “knobs-into-holes” amino acid changes and useful in the present invention. The below illustrative sequences are based on IgG1 and further comprise additional effector and complement silencing substitutions: L234A and L235A (LALA) and optionally, P329G; and half-life extension mutations: M252Y, S254T, T256E.

An illustrative human IGHG1 Knob In Hole “alpha core domain” (T22Y) is shown below:

EPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSRDELTKNQVSLYCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 24)

An illustrative human IGHG1 Knob In Hole “beta core domain” (Y86T)

EPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 25)

Any core domain useful in the present invention may comprise one or more “knob in holes” mutation.

Protein Subunits

In various embodiments, the heterodimeric protein of the invention comprises two polypeptide chains. In various embodiments, each polypeptide chain comprises a subunit of a first protein linked by a charge polarized core domain to a subunit of a second protein. Upon electrostatic interactions between the charge polarized core

domains, the subunits are heterodimerized to form a functional dimeric first protein and a functional dimeric second protein. In some embodiments, the polypeptide chains form a functional two-sided heterodimeric protein linked *via* the charge polarized core domains, which optionally include a linker (*e.g.*, a stabilizing domain) such as an Fc region.

In various embodiments, the first and second proteins may be any multimeric protein having two or more subunits. In some embodiments, the first protein and second protein are selected from cytokines, growth factors, and/or hormones. Illustrative examples of such cytokines, growth factors, and hormones include, but are not limited to, lymphokines, monokines, traditional polypeptide hormones, including, but not limited to, colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as, without limitation, IL-18, IL-27, and IL-35; interleukin receptors such as, without limitation, IL-2R, IL-3R, IL-4R, IL-5R, IL-6R, IL-7R, IL-9R, IL-10R, IL-11R, IL-12R, IL-13R, IL-15R, IL-17R, IL-18R, IL-20R, IL-21R, IL-22R, IL-23R, IL-27R, IL-35R; and other polypeptide factors including, without limitation, EGFR, integrins, neuropilins, and somatostatin receptors. 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 first and/or second protein is an immune-modulating agent, *e.g.,* one or more of an interleukin and interferon.

In some embodiments, the first and/or second protein is an interleukin, including for example IL-18, IL-27, and IL-35, 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 Interferons: 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 first and/ or second protein is a hormone such as somatostatin.

In various embodiments, the first and/or second protein is a receptor for a cytokine, growth factor, and/or hormone. In some embodiments, the first and/or second protein is a type I cytokine receptor, a type II cytokine receptor, a chemokine receptor, TGF-beta Receptors, a receptor in the immunoglobulin (Ig) superfamily, and/or a receptor in the tyrosine kinase superfamily.

In some embodiments, the first and/or second protein 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 some embodiments, the first and/or second protein 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 some embodiments, the first and/or second protein 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). Exemplary 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 some embodiments, the first and/or second protein 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 some embodiments, the first and/or second protein 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 some embodiments, the first and/or second protein is a B7 superfamily member. Members of the B7 superfamily share structural homology with one another. Members of this family include, but are not limited to, CD28, CD80, CD86, ICOS, ICOSL, B7-H3, B7-H4, PD-1, PD-L1, PD-L2, etc.

In some embodiments, the first and/or second protein 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 exemplary embodiment, the first and/or second protein is an IFN-α/β receptor (IFNAR) comprising IFNAR1 and/or IFNAR2 subunits.

In an exemplary embodiment, the first and/or second protein is an interferon-gamma receptor (IFNGR) comprising IFNGR1 (also known as IFNgR) and IFNGR2 subunits.

In an exemplary embodiment, the first and/or second protein is a VEGF receptor including VEGFR-1, VEGFR-2, and VEGFR -3.

In any heterodimeric protein disclosed herein, the amino- or carboxy-terminus is natively heterodimeric, and wherein the protein on the opposite terminus is not natively heterodimeric.

In an exemplary embodiment, the first and/or second protein is a receptor for IL-1 such as IL-1R1 and/or IL-1RAcP.

In an exemplary embodiment, the first and/or second protein is a receptor for IL-2 such as IL-2Rα or IL-2Rβ or IL-2Rγ.

In an exemplary embodiment, the first and/or second protein is an IL-3 receptor, which is a heterodimer with a unique alpha chain paired with the common beta (beta c or CD131) subunit.

In an exemplary embodiment, the first and/or second protein is a receptor for IL-4 such as a type 1 or type 2 IL-4 receptor.

In an exemplary embodiment, the first and/or second protein is a receptor for IL-6, which is a cell-surface type I cytokine receptor complex including the ligand-binding IL-6R chain (CD126 or IL-6Rα) and the signal-transducing component gp130.

In an exemplary embodiment, the first and/or second protein is a receptor for IL-10, such as IL-10 receptor-1 and IL-10 receptor-2.

In an exemplary embodiment, the first and/or second protein is a receptor for IL-11, such as IL-11Rα or IL-11Rβ or gp130.

In an exemplary embodiment, the first and/or second protein is a receptor for IL-12, such as IL-12Rβ1 and/or IL-12Rβ2.

In an exemplary embodiment, the first and/or second protein is a receptor for IL-13, such as the IL-4 receptor (IL-4Rα) or IL-13Rα1.

In an exemplary embodiment, the first and/or second protein is IL-18. In another exemplary embodiment, the first and/or second protein is a receptor for IL-18, such as IL-18Rα and/or IL-18Rβ.

In an exemplary embodiment, the first and/or second protein is a receptor for IL-21, which is a cell-surface type I cytokine receptor complex including the ligand-binding IL-21R chain comprising IL-21r and IL-2rg.

In an exemplary embodiment, the first and/or second protein is a receptor for IL-33, such as the ST-2 receptor or IL-1RAcP.

In an exemplary embodiment, the first and/or second protein is IL-35 (*e.g.*, comprising the IL12α and IL27β subunits). In another exemplary embodiment, the first and/or second protein is a receptor for IL-35, such as an IL-35 receptor comprising IL6Rα and gp130 subunits.

In an exemplary embodiment, the first and/or second protein is a receptor for EGP such as EGFR (ErbB1), ErbB2, ErbB3 and ErbB4.

In an exemplary embodiment, the first and/or second protein is a receptor for insulin or an insulin analog such as the insulin receptor and/or IGF1 or IGF2 receptor.

In an exemplary embodiment, the first and/or second protein is a receptor for EPO such as the EPO receptor (EPOR) receptor and/or the ephrin receptor (EphR)

In various embodiments, the first and second proteins may comprise a domain of a soluble (*e.g.*, non-membrane associated) protein. In various embodiments, the first and second proteins may comprise a fragment of the soluble protein which is involved in signaling (*e.g.,* a portion of the soluble protein which interacts with a receptor).

In various embodiments, the first and second proteins may comprise the extracellular domain of a transmembrane protein. In various embodiments, one of the extracellular domains transduces an immune inhibitory signal and one of the extracellular domains transduces an immune stimulatory signal.

In some embodiments, an extracellular domain refers to a portion of a transmembrane protein which is capable of interacting with the extracellular environment. In various embodiments, an extracellular domain refers to a portion of a transmembrane protein which is sufficient to bind to a ligand or receptor and effective transmit a signal to a cell. In various embodiments, an extracellular domain is the entire amino acid sequence of a transmembrane protein which is external of a cell or the cell membrane. In various embodiments, an extracellular domain is the that portion of an amino acid sequence of a transmembrane protein which is external of a cell or the cell membrane and is needed for signal transduction and/or ligand binding as may be assayed using methods know in the art (*e.g., in vitro* ligand binding and/or cellular activation assays).

In some embodiments, an immune inhibitory signal refers to a signal that diminishes or eliminates an immune response. For example, in the context of oncology, such signals may diminish or eliminate antitumor immunity. Under normal physiological conditions, inhibitory signal are useful in the maintenance of self-tolerance (*e.g.,* prevention of autoimmunity) and also to protect tissues from damage when the immune system is responding to pathogenic infection. For instance, without limitation, immune inhibitory signal may be identified by detecting an increase in cellular proliferation, cytokine production, cell killing activity or phagocytic activity when such an inhibitory signal is blocked.

In some embodiments, an immune stimulatory signal refers to a signal that enhances an immune response. For example, in the context of oncology, such signals may enhance antitumor immunity. For instance, without limitation, immune stimulatory signal may be identified by directly stimulating proliferation, cytokine production, killing activity or phagocytic activity of leukocytes. Specific examples include direct stimulation of cytokine receptors such as IL-2R, IL-7R, IL-15R, IL-17R or IL-21R using fusion proteins encoding the ligands for such receptors (IL-2, IL-7, IL-15, IL-17 or IL-21, respectively). Stimulation from any one of these receptors may directly stimulate the proliferation and cytokine production of individual T cell subsets.

In some embodiments, the extracellular domain may be used to produce a soluble protein to competitively inhibit signaling by that receptor's ligand. For instance, without limitation, competitive inhibition of PD-L1 or PD-L2 could be achieved using PD-1, or competitive inhibition of PVR could be achieved using TIGIT. In some embodiments, the extracellular domain may be used to provide artificial signaling.

In some embodiments, the present heterodimeric proteins deliver or mask an immune inhibitory signal. In some embodiments, the present heterodimeric proteins deliver or mask an immune stimulatory signal.

In various embodiments, the present heterodimeric proteins comprise two independent binding domains, each from one subunit of a heterodimeric human protein. Exemplary proteins that may be formed as part of the heterodimeric protein of the invention are provided in Table 1. In various embodiments, the present heterodimeric proteins have one of the exemplary proteins provided in Table 1. In various embodiments, the present heterodimeric proteins have two of the exemplary proteins provided in Table 1.

TABLE 1

Illustrative proteins which may be incorporated into the present compositions and methods include the following (as used herein, "Entry" refers to the protein entry in the Uniprot database and "Entry name" refers to the protein entry in the Uniprot database):

Entry	Entry name	Protein names	Gene names
P00533	EGFR _HUMAN	Epidermal growth factor receptor (EC 2.7.10.1) (Proto-oncogene c-ErbB-1) (Receptor tyrosine-protein kinase erbB-1)	EGFR ERBB ERBB1 HER1
P49768	PSN1 _HUMAN	Presenilin-1 (PS-1) (EC 3.4.23.-) (Protein S182) [Cleaved into: Presenilin-1 NTF subunit; Presenilin-1 CTF subunit; Presenilin-1 CTF12 (PS1-CTF12)]	PSEN1 AD3 PS1 PSNL1
P13569	CFTR _HUMAN	Cystic fibrosis transmembrane conductance regulator (CFTR) (ATP-binding cassette sub-family C member 7) (Channel conductance-controlling ATPase) (EC 3.6.3.49) (cAMP-dependent chloride channel)	CFTR ABCC7
P04626	ERBB2 _HUMAN	Receptor tyrosine-protein kinase erbB-2 (EC 2.7.10.1) (Metastatic lymph node gene 19 protein) (MLN 19) (Proto-oncogene Neu) (Proto-oncogene c-ErbB-2) (Tyrosine kinase-type cell surface receptor HER2) (p185erbB2) (CD antigen CD340)	ERBB2 HER2 MLN19 NEU NGL
P08581	MET _HUMAN	Hepatocyte growth factor receptor (HGF receptor) (EC 2.7.10.1) (HGF/SF receptor) (Proto-oncogene c-Met) (Scatter factor receptor) (SF receptor) (Tyrosine-protein kinase Met)	MET
P17861	XBP1 _HUMAN	X-box-binding protein 1 (XBP-1) (Tax-responsive element-binding protein 5) (TREB-5) [Cleaved into: X-box-binding protein 1, cytoplasmic form; X-box-binding protein 1, luminal form]	XBP1 TREB5 XBP2
P05106	ITB3 _HUMAN	Integrin beta-3 (Platelet membrane glycoprotein IIIa) (GPIIIa) (CD antigen CD61)	ITGB3 GP3A
P05556	ITB1 _HUMAN	Integrin beta-1 (Fibronectin receptor subunit beta) (Glycoprotein IIa) (GPIIA) (VLA-4 subunit beta) (CD antigen CD29)	ITGB1 FNRB MDF2 MSK12
P46531	NOTC1 _HUMAN	Neurogenic locus notch homolog protein 1 (Notch 1) (hN1) (Translocation-associated notch protein TAN-1) [Cleaved into: Notch 1 extracellular truncation (NEXT); Notch 1 intracellular domain (NICD)]	NOTCH1 TAN1
P16671	CD36 _HUMAN	Platelet glycoprotein 4 (Fatty acid translocase) (FAT) (Glycoprotein IIIb) (GPIIIB) (Leukocyte differentiation antigen CD36) (PAS IV) (PAS-4) (Platelet collagen receptor) (Platelet glycoprotein IV) (GPIV) (Thrombospondin receptor) (CD antigen CD36)	CD36 GP3B GP4
Q15303	ERBB4 _HUMAN	Receptor tyrosine-protein kinase erbB-4 (EC 2.7.10.1) (Proto-oncogene-like protein c-ErbB-4) (Tyrosine kinase-type cell surface receptor HER4) (p180erbB4) [Cleaved into: ERBB4 intracellular domain (4ICD) (E4ICD) (s80HER4)]	ERBB4 HER4
Q02763	TIE2 _HUMAN	Angiopoietin-1 receptor (EC 2.7.10.1) (Endothelial tyrosine kinase) (Tunica interna endothelial cell kinase) (Tyrosine kinase with Ig and EGF homology domains-2) (Tyrosine-protein kinase receptor TEK) (Tyrosine-protein kinase receptor TIE-2) (hTIE2) (p140 TEK) (CD antigen CD202b)	TEK TIE2 VMCM VMCM1

O00206	TLR4 _HUMAN	Toll-like receptor 4 (hToll) (CD antigen CD284)	TLR4
Q14118	DAG1 _HUMAN	Dystroglycan (Dystrophin-associated glycoprotein 1) [Cleaved into: Alpha-dystroglycan (Alpha-DG); Beta-dystroglycan (Beta-DG)]	DAG1
P16473	TSHR _HUMAN	Thyrotropin receptor (Thyroid-stimulating hormone receptor) (TSH-R)	TSHR LGR3
Q99527	GPER1 _HUMAN	G-protein coupled estrogen receptor 1 (Chemoattractant receptor-like 2) (Flow-induced endothelial G-protein coupled receptor 1) (FEG-1) (G protein-coupled estrogen receptor 1) (G-protein coupled receptor 30) (GPCR-Br) (IL8-related receptor DRY12) (Lymphocyte-derived G-protein coupled receptor) (LYGPR) (Membrane estrogen receptor) (mER)	GPER1 CEPR CMKRL2 DRY12 GPER GPR30
P17948	VGFR1 _HUMAN	Vascular endothelial growth factor receptor 1 (VEGFR-1) (EC 2.7.10.1) (Fms-like tyrosine kinase 1) (FLT-1) (Tyrosine-protein kinase FRT) (Tyrosine-protein kinase receptor FLT) (FLT) (Vascular permeability factor receptor)	FLT1 FLT FRT VEGFR1
Q9UM47	NOTC3 _HUMAN	Neurogenic locus notch homolog protein 3 (Notch 3) [Cleaved into: Notch 3 extracellular truncation; Notch 3 intracellular domain]	NOTCH3
P21860	ERBB3 _HUMAN	Receptor tyrosine-protein kinase erbB-3 (EC 2.7.10.1) (Proto-oncogene-like protein c-ErbB-3) (Tyrosine kinase-type cell surface receptor HER3)	ERBB3 HER3
P01920	DQB1 _HUMAN	HLA class II histocompatibility antigen, DQ beta 1 chain (MHC class II antigen DQB1)	HLA-DQB1 HLA-DQB
P06756	ITAV _HUMAN	Integrin alpha-V (Vitronectin receptor) (Vitronectin receptor subunit alpha) (CD antigen CD51) [Cleaved into: Integrin alpha-V heavy chain; Integrin alpha-V light chain]	ITGAV MSK8 VNRA VTNR
Q9H251	CAD23 _HUMAN	Cadherin-23 (Otocadherin)	CDH23 KIAA1774 KIAA1812 UNQ1894/ PRO4340
Q07954	LRP1 _HUMAN	Prolow-density lipoprotein receptor-related protein 1 (LRP-1) (Alpha-2-macroglobulin receptor) (A2MR) (Apolipoprotein E receptor) (APOER) (CD antigen CD91) [Cleaved into: Low-density lipoprotein receptor-related protein 1 85 kDa subunit (LRP-85); Low-density lipoprotein receptor-related protein 1 515 kDa subunit (LRP-515); Low-density lipoprotein receptor-related protein 1 intracellular domain (LRPICD)]	LRP1 A2MR APR
O60603	TLR2 _HUMAN	Toll-like receptor 2 (Toll/interleukin-1 receptor-like protein 4) (CD antigen CD282)	TLR2 TIL4
P04839	CY24B _HUMAN	Cytochrome b-245 heavy chain (EC 1.-.-.-) (CGD91-phox) (Cytochrome b(558) subunit beta) (Cytochrome b558 subunit beta) (Heme-binding membrane glycoprotein gp91phox) (NADPH oxidase 2) (Neutrophil cytochrome b 91 kDa polypeptide) (Superoxide-generating NADPH oxidase heavy chain subunit) (gp91-1) (gp91-phox) (p22 phagocyte B-cytochrome)	CYBB NOX2
P04233	HG2A _HUMAN	HLA class II histocompatibility antigen gamma chain (HLA-DR antigens-associated invariant chain) (Ia antigen-associated invariant chain) (Ii) (p33) (CD antigen CD74)	CD74 DHLAG
P13746	1A11 _HUMAN	HLA class I histocompatibility antigen, A-11 alpha chain (MHC class I antigen A*11)	HLA-A HLAA
P18462	1A25 _HUMAN	HLA class I histocompatibility antigen, A-25 alpha chain (HLA class I histocompatibility antigen, A-10 alpha chain) (MHC class I antigen A*25)	HLA-A HLAA
P16188	1A30 _HUMAN	HLA class I histocompatibility antigen, A-30 alpha chain (MHC class I antigen A*30)	HLA-A HLAA
P30457	1A66 _HUMAN	HLA class I histocompatibility antigen, A-66 alpha chain (Aw-66) (HLA class I histocompatibility antigen, A-10 alpha chain) (MHC class I antigen A*66)	HLA-A HLAA
P10316	1A69 _HUMAN	HLA class I histocompatibility antigen, A-69 alpha chain (Aw-69) (HLA class I histocompatibility antigen, A-28 alpha chain) (MHC class I antigen A*69)	HLA-A HLAA
Q09160	1A80 _HUMAN	HLA class I histocompatibility antigen, A-80 alpha chain (Aw-80) (HLA class I histocompatibility antigen, A-1 alpha chain) (MHC class I antigen A*80)	HLA-A HLAA

P30460	1B08 _HUMAN	HLA class I histocompatibility antigen, B-8 alpha chain (MHC class I antigen B*8)	HLA-B HLAB
P30685	1B35 _HUMAN	HLA class I histocompatibility antigen, B-35 alpha chain (MHC class I antigen B*35)	HLA-B HLAB
P08195	4F2 _HUMAN	4F2 cell-surface antigen heavy chain (4F2hc) (4F2 heavy chain antigen) (Lymphocyte activation antigen 4F2 large subunit) (Solute carrier family 3 member 2) (CD antigen CD98)	SLC3A2 MDU1
P28222	5HT1B _HUMAN	5-hydroxytryptamine receptor 1B (5-HT-1B) (5-HT1B) (S12) (Serotonin 1D beta receptor) (5-HT-1D-beta) (Serotonin receptor 1B)	HTR1B HTR1DB
P28221	5HT1D _HUMAN	5-hydroxytryptamine receptor 1D (5-HT-1D) (5-HT1D) (Serotonin 1D alpha receptor) (5-HT-1D-alpha) (Serotonin receptor 1D)	HTR1D HTR1DA HTRL
O14678	ABCD4 _HUMAN	ATP-binding cassette sub-family D member 4 (PMP70-related protein) (P70R) (Peroxisomal membrane protein 1-like) (PXMP1-L) (Peroxisomal membrane protein 69) (PMP69)	ABCD4 PXMP1L
P30462	1B14 _HUMAN	HLA class I histocompatibility antigen, B-14 alpha chain (MHC class I antigen B*14)	HLA-B HLAB
P30466	1B18 _HUMAN	HLA class I histocompatibility antigen, B-18 alpha chain (MHC class I antigen B*18)	HLA-B HLAB
Q04826	1B40 _HUMAN	HLA class I histocompatibility antigen, B-40 alpha chain (Bw-60) (MHC class I antigen B*40)	HLA-B HLAB
P30480	1B42 _HUMAN	HLA class I histocompatibility antigen, B-42 alpha chain (MHC class I antigen B*42)	HLA-B HLAB
P30484	1B46 _HUMAN	HLA class I histocompatibility antigen, B-46 alpha chain (Bw-46) (MHC class I antigen B*46)	HLA-B HLAB
P30487	1B49 _HUMAN	HLA class I histocompatibility antigen, B-49 alpha chain (HLA class I histocompatibility antigen, B-21 alpha chain) (MHC class I antigen B*49)	HLA-B HLAB
P18464	1B51 _HUMAN	HLA class I histocompatibility antigen, B-51 alpha chain (MHC class I antigen B*51)	HLA-B HLAB
P30495	1B56 _HUMAN	HLA class I histocompatibility antigen, B-56 alpha chain (Bw-22) (Bw-56) (MHC class I antigen B*56)	HLA-B HLAB
P30498	1B78 _HUMAN	HLA class I histocompatibility antigen, B-78 alpha chain (MHC class I antigen B*78)	HLA-B HLAB
Q29718	1B82 _HUMAN	HLA class I histocompatibility antigen, B-82 alpha chain (MHC class I antigen B*82)	HLA-B HLAB
P30501	1C02 _HUMAN	HLA class I histocompatibility antigen, Cw-2 alpha chain (MHC class I antigen Cw*2)	HLA-C HLAC
P30504	1C04 _HUMAN	HLA class I histocompatibility antigen, Cw-4 alpha chain (MHC class I antigen Cw*4)	HLA-C HLAC
P01912	2B13 _HUMAN	HLA class II histocompatibility antigen, DRB1-3 chain (Clone P2-beta-3) (MHC class II antigen DRB1*3)	HLA-DRB1
P13760	2B14 _HUMAN	HLA class II histocompatibility antigen, DRB1-4 beta chain (MHC class II antigen DRB1*4) (DR-4) (DR4)	HLA-DRB1
P01911	2B1F _HUMAN	HLA class II histocompatibility antigen, DRB1-15 beta chain (DW2.2/DR2.2) (MHC class II antigen DRB1*15)	HLA-DRB1 HLA-DRB2
P01892	1A02 _HUMAN	HLA class I histocompatibility antigen, A-2 alpha chain (MHC class I antigen A*2)	HLA-A HLAA
P03989	1B27 _HUMAN	HLA class I histocompatibility antigen, B-27 alpha chain (MHC class I antigen B*27)	HLA-B HLAB
P13761	2B17 _HUMAN	HLA class II histocompatibility antigen, DRB1-7 beta chain (MHC class II antigen DRB1*7) (DR-7) (DR7)	HLA-DRB1
Q9TQE0	2B19 _HUMAN	HLA class II histocompatibility antigen, DRB1-9 beta chain (MHC class II antigen DRB1*9) (DR-9) (DR9)	HLA-DRB1
P20039	2B1B _HUMAN	HLA class II histocompatibility antigen, DRB1-11 beta chain (DR-5) (DR5) (DRw11) (MHC class II antigen DRB1*11)	HLA-DRB1
O75027	ABCB7 _HUMAN	ATP-binding cassette sub-family B member 7, mitochondrial (ATP-binding cassette transporter 7) (ABC transporter 7 protein)	ABCB7 ABC7
P16189	1A31 _HUMAN	HLA class I histocompatibility antigen, A-31 alpha chain (MHC class I antigen A*31)	HLA-A HLAA

P30456	1A43 _HUMAN	HLA class I histocompatibility antigen, A-43 alpha chain (Aw-43) (MHC class I antigen A*43)	HLA-A HLAA
P30459	1A74 _HUMAN	HLA class I histocompatibility antigen, A-74 alpha chain (Aw-19) (Aw-74) (MHC class I antigen A*74)	HLA-A HLAA
P18463	1B37 _HUMAN	HLA class I histocompatibility antigen, B-37 alpha chain (MHC class I antigen B*37)	HLA-B HLAB
P30483	1B45 _HUMAN	HLA class I histocompatibility antigen, B-45 alpha chain (Bw-45) (MHC class I antigen B*45)	HLA-B HLAB
P30485	1B47 _HUMAN	HLA class I histocompatibility antigen, B-47 alpha chain (Bw-47) (MHC class I antigen B*47)	HLA-B HLAB
P30486	1B48 _HUMAN	HLA class I histocompatibility antigen, B-48 alpha chain (Bw-48) (MHC class I antigen B*48)	HLA-B HLAB
P30491	1B53 _HUMAN	HLA class I histocompatibility antigen, B-53 alpha chain (Bw-53) (MHC class I antigen B*53)	HLA-B HLAB
Q29940	1B59 _HUMAN	HLA class I histocompatibility antigen, B-59 alpha chain (MHC class I antigen B*59)	HLA-B HLAB
Q31612	1B73 _HUMAN	HLA class I histocompatibility antigen, B-73 alpha chain (MHC class I antigen B*73)	HLA-B HLAB
Q31610	1B81 _HUMAN	HLA class I histocompatibility antigen, B-81 alpha chain (B'DT) (MHC class I antigen B*81)	HLA-B HLAB
P30499	1C01 _HUMAN	HLA class I histocompatibility antigen, Cw-1 alpha chain (MHC class I antigen Cw*1)	HLA-C HLAC
Q9TNN7	1C05 _HUMAN	HLA class I histocompatibility antigen, Cw-5 alpha chain (MHC class I antigen Cw*5)	HLA-C HLAC
P30508	1C12 _HUMAN	HLA class I histocompatibility antigen, Cw-12 alpha chain (MHC class I antigen Cw*12)	HLA-C HLAC
Q29865	1C18 _HUMAN	HLA class I histocompatibility antigen, Cw-18 alpha chain (MHC class I antigen Cw*18)	HLA-C HLAC
Q5Y7A7	2B1D _HUMAN	HLA class II histocompatibility antigen, DRB1-13 beta chain (MHC class II antigen DRB1*13) (DR-13) (DR13)	HLA-DRB1
Q29974	2B1G _HUMAN	HLA class II histocompatibility antigen, DRB1-16 beta chain (MHC class II antigen DRB1*16) (DR-16) (DR16)	HLA-DRB1
Q9NS82	AAA1 _HUMAN	Asc-type amino acid transporter 1 (Asc-1) (Solute carrier family 7 member 10)	SLC7A10 ASC1
P30447	1A23 _HUMAN	HLA class I histocompatibility antigen, A-23 alpha chain (HLA class I histocompatibility antigen, A-9 alpha chain) (MHC class I antigen A*23)	HLA-A HLAA
P30450	1A26 _HUMAN	HLA class I histocompatibility antigen, A-26 alpha chain (MHC class I antigen A*26)	HLA-A HLAA
P10314	1A32 _HUMAN	HLA class I histocompatibility antigen, A-32 alpha chain (MHC class I antigen A*32)	HLA-A HLAA
P30455	1A36 _HUMAN	HLA class I histocompatibility antigen, A-36 alpha chain (Aw-36) (MHC class I antigen A*36)	HLA-A HLAA
P30461	1B13 _HUMAN	HLA class I histocompatibility antigen, B-13 alpha chain (MHC class I antigen B*13)	HLA-B HLAB
P30464	1B15 _HUMAN	HLA class I histocompatibility antigen, B-15 alpha chain (MHC class I antigen B*15)	HLA-B HLAB
P30475	1B39 _HUMAN	HLA class I histocompatibility antigen, B-39 alpha chain (MHC class I antigen B*39)	HLA-B HLAB
P30479	1B41 _HUMAN	HLA class I histocompatibility antigen, B-41 alpha chain (Bw-41) (MHC class I antigen B*41)	HLA-B HLAB
P30481	1B44 _HUMAN	HLA class I histocompatibility antigen, B-44 alpha chain (Bw-44) (MHC class I antigen B*44)	HLA-B HLAB
P30488	1B50 _HUMAN	HLA class I histocompatibility antigen, B-50 alpha chain (Bw-50) (HLA class I histocompatibility antigen, B-21 alpha chain) (MHC class I antigen B*50)	HLA-B HLAB
P30490	1B52 _HUMAN	HLA class I histocompatibility antigen, B-52 alpha chain (Bw-52) (HLA class I histocompatibility antigen, B-5 alpha chain) (MHC class I antigen B*52)	HLA-B HLAB
P30493	1B55 _HUMAN	HLA class I histocompatibility antigen, B-55 alpha chain (Bw-55) (HLA class I histocompatibility antigen, B-12 alpha chain) (MHC class I antigen B*55)	HLA-B HLAB

			CDABP0067
P18465	1B57_HUMAN	HLA class I histocompatibility antigen, B-57 alpha chain (Bw-57) (MHC class I antigen B*57)	HLA-B HLAB
Q29836	1B67_HUMAN	HLA class I histocompatibility antigen, B-67 alpha chain (MHC class I antigen B*67)	HLA-B HLAB
P10319	1B58_HUMAN	HLA class I histocompatibility antigen, B-58 alpha chain (Bw-58) (MHC class I antigen B*58)	HLA-B HLAB
P04222	1C03_HUMAN	HLA class I histocompatibility antigen, Cw-3 alpha chain (MHC class I antigen Cw*3)	HLA-C HLAC
Q29963	1C06_HUMAN	HLA class I histocompatibility antigen, Cw-6 alpha chain (MHC class I antigen Cw*6)	HLA-C HLAC
P30505	1C08_HUMAN	HLA class I histocompatibility antigen, Cw-8 alpha chain (MHC class I antigen Cw*8)	HLA-C HLAC
Q07000	1C15_HUMAN	HLA class I histocompatibility antigen, Cw-15 alpha chain (MHC class I antigen Cw*15)	HLA-C HLAC
Q95604	1C17_HUMAN	HLA class I histocompatibility antigen, Cw-17 alpha chain (MHC class I antigen Cw*17)	HLA-C D6S204 HLA-JY3 HLAC
Q95365	1B38_HUMAN	HLA class I histocompatibility antigen, B-38 alpha chain (Bw-4) (MHC class I antigen B*38)	HLA-B HLAB
P30492	1B54_HUMAN	HLA class I histocompatibility antigen, B-54 alpha chain (Bw-22) (Bw-54) (MHC class I antigen B*54)	HLA-B HLAB
P10321	1C07_HUMAN	HLA class I histocompatibility antigen, Cw-7 alpha chain (MHC class I antigen Cw*7)	HLA-C HLAC
P30510	1C14_HUMAN	HLA class I histocompatibility antigen, Cw-14 alpha chain (MHC class I antigen Cw*14)	HLA-C HLAC
Q29960	1C16_HUMAN	HLA class I histocompatibility antigen, Cw-16 alpha chain (MHC class I antigen Cw*16)	HLA-C HLAC
P04229	2B11_HUMAN	HLA class II histocompatibility antigen, DRB1-1 beta chain (MHC class II antigen DRB1*1) (DR-1) (DR1)	HLA-DRB1
Q30167	2B1A_HUMAN	HLA class II histocompatibility antigen, DRB1-10 beta chain (DRw10) (MHC class II antigen DRB1*10)	HLA-DRB1
Q9GIY3	2B1E_HUMAN	HLA class II histocompatibility antigen, DRB1-14 beta chain (MHC class II antigen DRB1*14) (DR-14) (DR14)	HLA-DRB1
P08908	5HT1A_HUMAN	5-hydroxytryptamine receptor 1A (5-HT-1A) (5-HT1A) (G-21) (Serotonin receptor 1A)	HTR1A ADRB2RL1 ADRBRL1
Q96PE1	AGRA2_HUMAN	Adhesion G protein-coupled receptor A2 (G-protein coupled receptor 124) (Tumor endothelial marker 5)	ADGRA2 GPR124 KIAA1531 TEM5
P05534	1A24_HUMAN	HLA class I histocompatibility antigen, A-24 alpha chain (Aw-24) (HLA class I histocompatibility antigen, A-9 alpha chain) (MHC class I antigen A*24)	HLA-A HLAA
P30512	1A29_HUMAN	HLA class I histocompatibility antigen, A-29 alpha chain (Aw-19) (MHC class I antigen A*29)	HLA-A HLAA
P16190	1A33_HUMAN	HLA class I histocompatibility antigen, A-33 alpha chain (Aw-19) (Aw-33) (MHC class I antigen A*33)	HLA-A HLAA
P30453	1A34_HUMAN	HLA class I histocompatibility antigen, A-34 alpha chain (Aw-34) (HLA class I histocompatibility antigen, A-10 alpha chain) (MHC class I antigen A*34)	HLA-A HLAA
P01891	1A68_HUMAN	HLA class I histocompatibility antigen, A-68 alpha chain (Aw-68) (HLA class I histocompatibility antigen, A-28 alpha chain) (MHC class I antigen A*68)	HLA-A HLAA
P01889	1B07_HUMAN	HLA class I histocompatibility antigen, B-7 alpha chain (MHC class I antigen B*7)	HLA-B HLAB
Q95IE3	2B1C_HUMAN	HLA class II histocompatibility antigen, DRB1-12 beta chain (MHC class II antigen DRB1*12) (DR-12) (DR12)	HLA-DRB1

Q99965	ADAM2_HUMAN	Disintegrin and metalloproteinase domain-containing protein 2 (ADAM 2) (Cancer/testis antigen 15) (CT15) (Fertilin subunit beta) (PH-30) (PH30) (PH30-beta)	ADAM2 FTNB
Q30134	2B18_HUMAN	HLA class II histocompatibility antigen, DRB1-8 beta chain (MHC class II antigen DRB1*8) (DR-8) (DR8) (DRw8)	HLA-DRB1
Q9UHX3	AGRE2_HUMAN	Adhesion G protein-coupled receptor E2 (EGF-like module receptor 2) (EGF-like module-containing mucin-like hormone receptor-like 2) (CD antigen CD312)	ADGRE2 EMR2
O60242	AGRB3_HUMAN	Adhesion G protein-coupled receptor B3 (Brain-specific angiogenesis inhibitor 3)	ADGRB3 BAI3 KIAA0550
Q96F25	ALG14_HUMAN	UDP-N-acetylglucosamine transferase subunit ALG14 homolog	ALG14
Q9Y653	AGRG1_HUMAN	Adhesion G-protein coupled receptor G1 (G-protein coupled receptor 56) (Protein TM7XN1) [Cleaved into: ADGRG1 N-terminal fragment (ADGRG1 NT) (GPR56 N-terminal fragment) (GPR56 NT) (GPR56(N)) (GPR56 extracellular subunit) (GPR56 subunit alpha); ADGRG1 C-terminal fragment (ADGRG1 CT) (GPR56 C-terminal fragment) (GPR56 CT) (GPR56(C)) (GPR56 seven-transmembrane subunit) (GPR56 7TM) (GPR56 subunit beta)]	ADGRG1 GPR56 TM7LN4 TM7XN1 UNQ540/PRO1083
Q8IZF2	AGRF5_HUMAN	Adhesion G protein-coupled receptor F5 (G-protein coupled receptor 116)	ADGRF5 GPR116 KIAA0758
O60241	AGRB2_HUMAN	Adhesion G protein-coupled receptor B2 (Brain-specific angiogenesis inhibitor 2)	ADGRB2 BAI2
Q86SQ3	AGRE4_HUMAN	Putative adhesion G protein-coupled receptor E4P (EGF-like module receptor 4) (EGF-like module-containing mucin-like hormone receptor-like 4) (G-protein coupled receptor 127) (G-protein coupled receptor PGR16)	ADGRE4P EMR4 EMR4P GPR127 PGR16
Q8IZP9	AGRG2_HUMAN	Adhesion G-protein coupled receptor G2 (G-protein coupled receptor 64) (Human epididymis-specific protein 6) (He6)	ADGRG2 GPR64 HE6 TM7LN2
Q9HBW9	AGRL4_HUMAN	Adhesion G protein-coupled receptor L4 (EGF, latrophilin and seven transmembrane domain-containing protein 1) (EGF-TM7-latrophilin-related protein) (ETL protein)	ADGRL4 ELTD1 ETL UNQ202/PRO228
Q16853	AOC3_HUMAN	Membrane primary amine oxidase (EC 1.4.3.21) (Copper amine oxidase) (HPAO) (Semicarbazide-sensitive amine oxidase) (SSAO) (Vascular adhesion protein 1) (VAP-1)	AOC3 VAP1
Q9BY15	AGRE3_HUMAN	Adhesion G protein-coupled receptor E3 (EGF-like module receptor 3) (EGF-like module-containing mucin-like hormone receptor-like 3)	ADGRE3 EMR3 UNQ683/PRO1562
O94910	AGRL1_HUMAN	Adhesion G protein-coupled receptor L1 (Calcium-independent alpha-latrotoxin receptor 1) (CIRL-1) (Latrophilin-1) (Lectomedin-2)	ADGRL1 KIAA0821 LEC2 LPHN1
O95490	AGRL2_HUMAN	Adhesion G protein-coupled receptor L2 (Calcium-independent alpha-latrotoxin receptor 2) (CIRL-2) (Latrophilin homolog 1) (Latrophilin-2) (Lectomedin-1)	ADGRL2 KIAA0786 LEC1 LPHH1 LPHN2
Q86WK6	AMGO1_HUMAN	Amphoterin-induced protein 1 (AMIGO-1) (Alivin-2)	AMIGO1 ALI2 AMIGO KIAA1163
P21397	AOFA_HUMAN	Amine oxidase [flavin-containing] A (EC 1.4.3.4) (Monoamine oxidase type A) (MAO-A)	MAOA

P27338	AOFB _HUMAN	Amine oxidase [flavin-containing] B (EC 1.4.3.4) (Monoamine oxidase type B) (MAO-B)	MAOB
Q99941	ATF6B _HUMAN	Cyclic AMP-dependent transcription factor ATF-6 beta (cAMP-dependent transcription factor ATF-6 beta) (Activating transcription factor 6 beta) (ATF6-beta) (Protein G13) (cAMP response element-binding protein-related protein) (Creb-rp) (cAMP-responsive element-binding protein-like 1) [Cleaved into: Processed cyclic AMP-dependent transcription factor ATF-6 beta]	ATF6B CREBL1 G13
Q15041	AR6P1 _HUMAN	ADP-ribosylation factor-like protein 6-interacting protein 1 (ARL-6-interacting protein 1) (Aip-1) (Apoptotic regulator in the membrane of the endoplasmic reticulum)	ARL6IP1 ARL6IP ARMER KIAA0069
P18850	ATF6A _HUMAN	Cyclic AMP-dependent transcription factor ATF-6 alpha (cAMP-dependent transcription factor ATF-6 alpha) (Activating transcription factor 6 alpha) (ATF6-alpha) [Cleaved into: Processed cyclic AMP-dependent transcription factor ATF-6 alpha]	ATF6
Q9UHQ4	BAP29 _HUMAN	B-cell receptor-associated protein 29 (BCR-associated protein 29) (Bap29)	BCAP29 BAP29
P82251	BAT1 _HUMAN	b(0,+)-type amino acid transporter 1 (b(0,+)AT1) (Glycoprotein-associated amino acid transporter b0,+AT1) (Solute carrier family 7 member 9)	SLC7A9 BAT1
P51572	BAP31 _HUMAN	B-cell receptor-associated protein 31 (BCR-associated protein 31) (Bap31) (6C6-AG tumor-associated antigen) (Protein CDM) (p28)	BCAP31 BAP31 DXS1357E
O60238	BNI3L _HUMAN	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like (Adenovirus E1B19K-binding protein B5) (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3A) (NIP3-like protein X) (NIP3L)	BNIP3L BNIP3A BNIP3H NIX
Q9UMX3	BOK _HUMAN	Bcl-2-related ovarian killer protein (hBOK) (Bcl-2-like protein 9) (Bcl2-L-9)	BOK BCL2L9
P15291	B4GT1 _HUMAN	Beta-1,4-galactosyltransferase 1 (Beta-1,4-GalTase 1) (Beta4Gal-T1) (b4Gal-T1) (EC 2.4.1.-) (UDP-Gal:beta-GlcNAc beta-1,4-galactosyltransferase 1) (UDP-galactose:beta-N-acetylglucosamine beta-1,4-galactosyltransferase 1) [Cleaved into: Processed beta-1,4-galactosyltransferase 1] [Includes: Lactose synthase A protein (EC 2.4.1.22); N-acetyllactosamine synthase (EC 2.4.1.90) (Nal synthase); Beta-N-acetylglucosaminylglycopeptide beta-1,4-galactosyltransferase (EC 2.4.1.38); Beta-N-acetylglucosaminyl-glycolipid beta-1,4-galactosyltransferase (EC 2.4.1.-)]	B4GALT1 GGTB2
Q12983	BNIP3 _HUMAN	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3	BNIP3 NIP3
Q16602	CALRL _HUMAN	Calcitonin gene-related peptide type 1 receptor (CGRP type 1 receptor) (Calcitonin receptor-like receptor)	CALCRL CGRPR
P10966	CD8B _HUMAN	T-cell surface glycoprotein CD8 beta chain (CD antigen CD8b)	CD8B CD8B1
P09693	CD3G _HUMAN	T-cell surface glycoprotein CD3 gamma chain (T-cell receptor T3 gamma chain) (CD antigen CD3g)	CD3G T3G
P60033	CD81 _HUMAN	CD81 antigen (26 kDa cell surface protein TAPA-1) (Target of the antiproliferative antibody 1) (Tetraspanin-28) (Tspan-28) (CD antigen CD81)	CD81 TAPA1 TSPAN28
P01732	CD8A _HUMAN	T-cell surface glycoprotein CD8 alpha chain (T-lymphocyte differentiation antigen T8/Leu-2) (CD antigen CD8a)	CD8A MAL
P06126	CD1A _HUMAN	T-cell surface glycoprotein CD1a (T-cell surface antigen T6/Leu-6) (hTa1 thymocyte antigen) (CD antigen CD1a)	CD1A
P40259	CD79B _HUMAN	B-cell antigen receptor complex-associated protein beta chain (B-cell-specific glycoprotein B29) (Ig-beta) (Immunoglobulin-associated B29 protein) (CD antigen CD79b)	CD79B B29 IGB
P11912	CD79A _HUMAN	B-cell antigen receptor complex-associated protein alpha chain (Ig-alpha) (MB-1 membrane glycoprotein) (Membrane-bound immunoglobulin-associated protein) (Surface IgM-associated protein) (CD antigen CD79a)	CD79A IGA MB1
P15812	CD1E _HUMAN	T-cell surface glycoprotein CD1e, membrane-associated (hCD1e) (R2G1) (CD antigen CD1e) [Cleaved into: T-cell surface glycoprotein CD1e, soluble (sCD1e)]	CD1E

P20963	CD3Z _HUMAN	T-cell surface glycoprotein CD3 zeta chain (T-cell receptor T3 zeta chain) (CD antigen CD247)	CD247 CD3Z T3Z TCRZ
A6NJW9	CD8B2 _HUMAN	Putative T-cell surface glycoprotein CD8 beta-2 chain (CD8b pseudogene)	CD8B2 CD8BP
P29017	CD1C _HUMAN	T-cell surface glycoprotein CD1c (CD antigen CD1c)	CD1C
P13688	CEAM1 _HUMAN	Carcinoembryonic antigen-related cell adhesion molecule 1 (Biliary glycoprotein 1) (BGP-1) (CD antigen CD66a)	CEACAM1 BGP BGP1
P29016	CD1B _HUMAN	T-cell surface glycoprotein CD1b (CD antigen CD1b)	CD1B
P15813	CD1D _HUMAN	Antigen-presenting glycoprotein CD1d (R3G1) (CD antigen CD1d)	CD1D
P20273	CD22 _HUMAN	B-cell receptor CD22 (B-lymphocyte cell adhesion molecule) (BL-CAM) (Sialic acid-binding Ig-like lectin 2) (Siglec-2) (T-cell surface antigen Leu-14) (CD antigen CD22)	CD22 SIGLEC2
P04234	CD3D _HUMAN	T-cell surface glycoprotein CD3 delta chain (T-cell receptor T3 delta chain) (CD antigen CD3d)	CD3D T3D
P07766	CD3E _HUMAN	T-cell surface glycoprotein CD3 epsilon chain (T-cell surface antigen T3/Leu-4 epsilon chain) (CD antigen CD3e)	CD3E T3E
P48960	CD97 _HUMAN	CD97 antigen (Leukocyte antigen CD97) (CD antigen CD97) [Cleaved into: CD97 antigen subunit alpha; CD97 antigen subunit beta]	CD97
Q9UHP7	CLC2D _HUMAN	C-type lectin domain family 2 member D (Lectin-like NK cell receptor) (Lectin-like transcript 1) (LLT-1) (Osteoclast inhibitory lectin)	CLEC2D CLAX LLT1 OCIL
P51790	CLCN3 _HUMAN	H(+)/Cl(-) exchange transporter 3 (Chloride channel protein 3) (ClC-3) (Chloride transporter ClC-3)	CLCN3
Q68CJ9	CR3L3 _HUMAN	Cyclic AMP-responsive element-binding protein 3-like protein 3 (cAMP-responsive element-binding protein 3-like protein 3) (Transcription factor CREB-H) [Cleaved into: Processed cyclic AMP-responsive element-binding protein 3-like protein 3]	CREB3L3 CREBH HYST1481
P34998	CRFR1 _HUMAN	Corticotropin-releasing factor receptor 1 (CRF-R-1) (CRF-R1) (CRFR-1) (Corticotropin-releasing hormone receptor 1) (CRH-R-1) (CRH-R1)	CRHR1 CRFR CRFR1 CRHR
Q9HC73	CRLF2 _HUMAN	Cytokine receptor-like factor 2 (Cytokine receptor-like 2) (IL-XR) (Thymic stromal lymphopoietin protein receptor) (TSLP receptor)	CRLF2 CRL2 ILXR TSLPR
P09603	CSF1 _HUMAN	Macrophage colony-stimulating factor 1 (CSF-1) (M-CSF) (MCSF) (Lanimostim) [Cleaved into: Processed macrophage colony-stimulating factor 1]	CSF1
P15509	CSF2R _HUMAN	Granulocyte-macrophage colony-stimulating factor receptor subunit alpha (GM-CSF-R-alpha) (GMCSFR-alpha) (GMR-alpha) (CDw116) (CD antigen CD116)	CSF2RA CSF2R CSF2RY
P06340	DOA _HUMAN	HLA class II histocompatibility antigen, DO alpha chain (MHC DN-alpha) (MHC DZ alpha) (MHC class II antigen DOA)	HLA-DOA HLA-DNA HLA-DZA
P01906	DQA2 _HUMAN	HLA class II histocompatibility antigen, DQ alpha 2 chain (DX alpha chain) (HLA class II histocompatibility antigen, DQ(6) alpha chain) (HLA-DQA1) (MHC class II DQA2)	HLA-DQA2 HLA-DXA
P13762	DRB4 _HUMAN	HLA class II histocompatibility antigen, DR beta 4 chain (MHC class II antigen DRB4)	HLA-DRB4
P13765	DOB _HUMAN	HLA class II histocompatibility antigen, DO beta chain (MHC class II antigen DOB)	HLA-DOB
P04440	DPB1 _HUMAN	HLA class II histocompatibility antigen, DP beta 1 chain (HLA class II histocompatibility antigen, DP(W4) beta chain) (MHC class II antigen DPB1)	HLA-DPB1 HLA-DP1B

P01909	DQA1 _HUMAN	HLA class II histocompatibility antigen, DQ alpha 1 chain (DC-1 alpha chain) (DC-alpha) (HLA-DCA) (MHC class II DQA1)	HLA-DQA1
P28067	DMA _HUMAN	HLA class II histocompatibility antigen, DM alpha chain (MHC class II antigen DMA) (Really interesting new gene 6 protein)	HLA-DMA DMA RING6
P79483	DRB3 _HUMAN	HLA class II histocompatibility antigen, DR beta 3 chain (MHC class II antigen DRB3)	HLA-DRB3
P28068	DMB _HUMAN	HLA class II histocompatibility antigen, DM beta chain (MHC class II antigen DMB) (Really interesting new gene 7 protein)	HLA-DMB DMB RING7
P01903	DRA _HUMAN	HLA class II histocompatibility antigen, DR alpha chain (MHC class II antigen DRA)	HLA-DRA HLA-DRA1
P20036	DPA1 _HUMAN	HLA class II histocompatibility antigen, DP alpha 1 chain (DP(W3)) (DP(W4)) (HLA-SB alpha chain) (MHC class II DP3-alpha) (MHC class II DPA1)	HLA-DPA1 HLA-DP1A HLASB
P05538	DQB2 _HUMAN	HLA class II histocompatibility antigen, DQ beta 2 chain (HLA class II histocompatibility antigen, DX beta chain) (MHC class II antigen DQB2)	HLA-DQB2 HLA-DXB
P27487	DPP4 _HUMAN	Dipeptidyl peptidase 4 (EC 3.4.14.5) (ADABP) (Adenosine deaminase complexing protein 2) (ADCP-2) (Dipeptidyl peptidase IV) (DPP IV) (T-cell activation antigen CD26) (TP103) (CD antigen CD26) [Cleaved into: Dipeptidyl peptidase 4 membrane form (Dipeptidyl peptidase IV membrane form); Dipeptidyl peptidase 4 soluble form (Dipeptidyl peptidase IV soluble form)]	DPP4 ADCP2 CD26
Q30154	DRB5 _HUMAN	HLA class II histocompatibility antigen, DR beta 5 chain (DR beta-5) (DR2-beta-2) (Dw2) (MHC class II antigen DRB5)	HLA-DRB5
P61565	ENK21 _HUMAN	Endogenous retrovirus group K member 21 Env polyprotein (EnvK1 protein) (Envelope polyprotein) (HERV-K_12q14.1 provirus ancestral Env polyprotein) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	ERVK-21
Q902F9	EN113 _HUMAN	Endogenous retrovirus group K member 113 Env polyprotein (EnvK5 protein) (Envelope polyprotein) (HERV-K113 envelope protein) (HERV-K_19p13.11 provirus ancestral Env polyprotein) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	HERVK_113
P60507	EFC1 _HUMAN	Endogenous retrovirus group FC1 Env polyprotein (Envelope polyprotein) (Fc1env) (HERV-F(c)1_Xq21.33 provirus ancestral Env polyprotein) (HERV-Fc1env) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	ERVFC1
P13224	GP1BB _HUMAN	Platelet glycoprotein Ib beta chain (GP-Ib beta) (GPIb-beta) (GPIbB) (Antigen CD42b-beta) (CD antigen CD42c)	GP1BB
Q9HB15	KCNKC _HUMAN	Potassium channel subfamily K member 12 (Tandem pore domain halothane-inhibited potassium channel 2) (THIK-2)	KCNK12
P23276	KELL _HUMAN	Kell blood group glycoprotein (EC 3.4.24.-) (CD antigen CD238)	KEL
Q13241	KLRD1 _HUMAN	Natural killer cells antigen CD94 (KP43) (Killer cell lectin-like receptor subfamily D member 1) (NK cell receptor) (CD antigen CD94)	KLRD1 CD94
O14649	KCNK3 _HUMAN	Potassium channel subfamily K member 3 (Acid-sensitive potassium channel protein TASK-1) (TWIK-related acid-sensitive K(+) channel 1) (Two pore potassium channel KT3.1) (Two pore K(+) channel KT3.1)	KCNK3 TASK TASK1
Q09470	KCNA1 _HUMAN	Potassium voltage-gated channel subfamily A member 1 (Voltage-gated K(+) channel HuKI) (Voltage-gated potassium channel HBK1) (Voltage-gated potassium channel subunit Kv1.1)	KCNA1
O00180	KCNK1 _HUMAN	Potassium channel subfamily K member 1 (Inward rectifying potassium channel protein TWIK-1) (Potassium channel K2P1) (Potassium channel KCNO1)	KCNK1 HOHO1 KCNO1 TWIK1
Q9NPC2	KCNK9 _HUMAN	Potassium channel subfamily K member 9 (Acid-sensitive potassium channel protein TASK-3) (TWIK-related acid-sensitive K(+) channel 3) (Two pore potassium channel KT3.2) (Two pore K(+) channel KT3.2)	KCNK9 TASK3
P54851	EMP2 _HUMAN	Epithelial membrane protein 2 (EMP-2) (Protein XMP)	EMP2 XMP
O71037	ENK19 _HUMAN	Endogenous retrovirus group K member 19 Env polyprotein (EnvK3 protein) (Envelope polyprotein) (HERV-K(C19) envelope protein) (HERV-K_19q11	ERVK-19

		provirus ancestral Env polyprotein) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	
P61567	ENK7 _HUMAN	Endogenous retrovirus group K member 7 Env polyprotein (Envelope polyprotein) (HERV-K(III) envelope protein) (HERV-K102 envelope protein) (HERV-K_1q22 provirus ancestral Env polyprotein) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	ERVK-7
Q9UKH3	ENK9 _HUMAN	Endogenous retrovirus group K member 9 Env polyprotein (EnvK4 protein) (Envelope polyprotein) (HERV-K(C6) envelope protein) (HERV-K109 envelope protein) (HERV-K_6q14.1 provirus ancestral Env polyprotein) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	ERVK-9
Q9NZ08	ERAP1 _HUMAN	Endoplasmic reticulum aminopeptidase 1 (EC 3.4.11.-) (ARTS-1) (Adipocyte-derived leucine aminopeptidase) (A-LAP) (Aminopeptidase PILS) (Puromycin-insensitive leucyl-specific aminopeptidase) (PILS-AP) (Type 1 tumor necrosis factor receptor shedding aminopeptidase regulator)	ERAP1 APPILS ARTS1 KIAA0525 UNQ584/P RO1154
Q9N2K0	ENH1 _HUMAN	HERV-H_2q24.3 provirus ancestral Env polyprotein (Env protein HERV-H/p62) (Env protein HERV-H19) (Env protein HERV-Hcl.3) (Envelope polyprotein) (HERV-H/env62) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	
Q9NX77	ENK13 _HUMAN	Endogenous retrovirus group K member 13-1 Env polyprotein (Envelope polyprotein) (HERV-K_16p13.3 provirus ancestral Env polyprotein) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	ERVK13-1
P61566	ENK24 _HUMAN	Endogenous retrovirus group K member 24 Env polyprotein (Envelope polyprotein) (HERV-K101 envelope protein) (HERV-K_22q11.21 provirus ancestral Env polyprotein) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	ERVK-24
Q69384	ENK6 _HUMAN	Endogenous retrovirus group K member 6 Env polyprotein (EnvK2 protein) (Envelope polyprotein) (HERV-K(C7) envelope protein) (HERV-K(HML-2.HOM) envelope protein) (HERV-K108 envelope protein) (HERV-K_7p22.1 provirus ancestral Env polyprotein) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	ERVK-6 ERVK6
Q9N2J8	ENH3 _HUMAN	HERV-H_2q24.1 provirus ancestral Env polyprotein (Env protein HERV-H/p59) (Envelope polyprotein) (HERV-H/env59) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	
O42043	ENK18 _HUMAN	Endogenous retrovirus group K member 18 Env polyprotein (Envelope polyprotein) (HERV-K(C1a) envelope protein) (HERV-K110 envelope protein) (HERV-K18 envelope protein) (HERV-K18 superantigen) (HERV-K_1q23.3 provirus ancestral Env polyprotein) (IDDMK1,2 22 envelope protein) (IDDMK1,2 22 superantigen) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	ERVK-18
Q902F8	ENK8 _HUMAN	Endogenous retrovirus group K member 8 Env polyprotein (EnvK6 protein) (Envelope polyprotein) (HERV-K115 envelope protein) (HERV-K_8p23.1 provirus ancestral Env polyprotein) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	ERVK-8
P29317	EPHA2 _HUMAN	Ephrin type-A receptor 2 (EC 2.7.10.1) (Epithelial cell kinase) (Tyrosine-protein kinase receptor ECK)	EPHA2 ECK
P61570	ENK25 _HUMAN	Endogenous retrovirus group K member 25 Env polyprotein (Envelope polyprotein) (HERV-K_11q22.1 provirus ancestral Env polyprotein) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	ERVK-25
P98073	ENTK _HUMAN	Enteropeptidase (EC 3.4.21.9) (Enterokinase) (Serine protease 7) (Transmembrane protease serine 15) [Cleaved into: Enteropeptidase non-catalytic heavy chain; Enteropeptidase catalytic light chain]	TMPRSS1 5 ENTK PRSS7
Q6P179	ERAP2 _HUMAN	Endoplasmic reticulum aminopeptidase 2 (EC 3.4.11.-) (Leukocyte-derived arginine aminopeptidase) (L-RAP)	ERAP2 LRAP
P55899	FCGRN _HUMAN	IgG receptor FcRn large subunit p51 (FcRn) (IgG Fc fragment receptor transporter alpha chain) (Neonatal Fc receptor)	FCGRT FCRN

O75899	GABR2_HUMAN	Gamma-aminobutyric acid type B receptor subunit 2 (GABA-B receptor 2) (GABA-B-R2) (GABA-BR2) (GABABR2) (Gb2) (G-protein coupled receptor 51) (HG20)	GABBR2 GPR51 GPRC3B
Q14318	FKBP8_HUMAN	Peptidyl-prolyl cis-trans isomerase FKBP8 (PPIase FKBP8) (EC 5.2.1.8) (38 kDa FK506-binding protein) (38 kDa FKBP) (FKBP-38) (hFKBP38) (FK506-binding protein 8) (FKBP-8) (FKBPR38) (Rotamase)	FKBP8 FKBP38
Q9UBS5	GABR1_HUMAN	Gamma-aminobutyric acid type B receptor subunit 1 (GABA-B receptor 1) (GABA-B-R1) (GABA-BR1) (GABABR1) (Gb1)	GABBR1 GPRC3A
Q9UG22	GIMA2_HUMAN	GTPase IMAP family member 2 (Immunity-associated protein 2) (hIMAP2)	GIMAP2 IMAP2
Q6P531	GGT6_HUMAN	Gamma-glutamyltransferase 6 (GGT 6) (EC 2.3.2.2) (Gamma-glutamyltranspeptidase 6) (Glutathione hydrolase 6) (EC 3.4.19.13) [Cleaved into: Gamma-glutamyltransferase 6 heavy chain; Gamma-glutamyltransferase 6 light chain]	GGT6
Q9UJ14	GGT7_HUMAN	Gamma-glutamyltransferase 7 (GGT 7) (EC 2.3.2.2) (Gamma-glutamyltransferase-like 3) (Gamma-glutamyltransferase-like 5) (Gamma-glutamyltranspeptidase 7) (Glutathione hydrolase 7) (EC 3.4.19.13) [Cleaved into: Gamma-glutamyltransferase 7 heavy chain; Gamma-glutamyltransferase 7 light chain]	GGT7 GGTL3 GGTL5
P14770	GPIX_HUMAN	Platelet glycoprotein IX (GP-IX) (GPIX) (Glycoprotein 9) (CD antigen CD42a)	GP9
P19440	GGT1_HUMAN	Gamma-glutamyltranspeptidase 1 (GGT 1) (EC 2.3.2.2) (Gamma-glutamyltransferase 1) (Glutathione hydrolase 1) (EC 3.4.19.13) (Leukotriene-C4 hydrolase) (EC 3.4.19.14) (CD antigen CD224) [Cleaved into: Gamma-glutamyltranspeptidase 1 heavy chain; Gamma-glutamyltranspeptidase 1 light chain]	GGT1 GGT
P36269	GGT5_HUMAN	Gamma-glutamyltransferase 5 (GGT 5) (EC 2.3.2.2) (Gamma-glutamyl transpeptidase-related enzyme) (GGT-rel) (Gamma-glutamyltransferase-like activity 1) (Gamma-glutamyltranspeptidase 5) (Glutathione hydrolase 5) (EC 3.4.19.13) (Leukotriene-C4 hydrolase) (EC 3.4.19.14) [Cleaved into: Gamma-glutamyltransferase 5 heavy chain; Gamma-glutamyltransferase 5 light chain]	GGT5 GGTLA1
P07359	GP1BA_HUMAN	Platelet glycoprotein Ib alpha chain (GP-Ib alpha) (GPIb-alpha) (GPIbA) (Glycoprotein Ibalpha) (Antigen CD42b-alpha) (CD antigen CD42b) [Cleaved into: Glycocalicin]	GP1BA
P32249	GP183_HUMAN	G-protein coupled receptor 183 (Epstein-Barr virus-induced G-protein coupled receptor 2) (EBI2) (EBV-induced G-protein coupled receptor 2) (hEBI2)	GPR183 EBI2
P17693	HLAG_HUMAN	HLA class I histocompatibility antigen, alpha chain G (HLA G antigen) (MHC class I antigen G)	HLA-G HLA-6.0 HLAG
P01893	HLAH_HUMAN	Putative HLA class I histocompatibility antigen, alpha chain H (HLA-12.4) (HLA-AR) (MHC class I antigen H)	HLA-H HLAH
P13747	HLAE_HUMAN	HLA class I histocompatibility antigen, alpha chain E (MHC class I antigen E)	HLA-E HLA-6.2 HLAE
Q99665	I12R2_HUMAN	Interleukin-12 receptor subunit beta-2 (IL-12 receptor subunit beta-2) (IL-12R subunit beta-2) (IL-12R-beta-2) (IL-12RB2)	IL12RB2
P30511	HLAF_HUMAN	HLA class I histocompatibility antigen, alpha chain F (CDA12) (HLA F antigen) (Leukocyte antigen F) (MHC class I antigen F)	HLA-F HLA-5.4 HLAF
P42701	I12R1_HUMAN	Interleukin-12 receptor subunit beta-1 (IL-12 receptor subunit beta-1) (IL-12R subunit beta-1) (IL-12R-beta-1) (IL-12RB1) (IL-12 receptor beta component) (CD antigen CD212)	IL12RB1 IL12R IL12RB
Q96F46	I17RA_HUMAN	Interleukin-17 receptor A (IL-17 receptor A) (IL-17RA) (CDw217) (CD antigen CD217)	IL17RA IL17R
Q9NPH3	IL1AP_HUMAN	Interleukin-1 receptor accessory protein (IL-1 receptor accessory protein) (IL-1RAcP) (Interleukin-1 receptor 3) (IL-1R-3) (IL-1R3)	IL1RAP C3orf13 IL1R3
P32927	IL3RB_HUMAN	Cytokine receptor common subunit beta (CDw131) (GM-CSF/IL-3/IL-5 receptor common beta subunit) (CD antigen CD131)	CSF2RB IL3RB

			IL5RB
Q08334	I10R2 _HUMAN	Interleukin-10 receptor subunit beta (IL-10 receptor subunit beta) (IL-10R subunit beta) (IL-10RB) (Cytokine receptor class-II member 4) (Cytokine receptor family 2 member 4) (CRF2-4) (Interleukin-10 receptor subunit 2) (IL-10R subunit 2) (IL-10R2) (CD antigen CDw210b)	IL10RB CRFB4 D21S58 D21S66
Q8NAC3	I17RC _HUMAN	Interleukin-17 receptor C (IL-17 receptor C) (IL-17RC) (Interleukin-17 receptor homolog) (IL17Rhom) (Interleukin-17 receptor-like protein) (IL-17RL) (ZcytoR14)	IL17RC UNQ6118/ PRO20040/ PRO38901
Q8NFR9	I17RE _HUMAN	Interleukin-17 receptor E (IL-17 receptor E) (IL-17RE)	IL17RE UNQ3056/ PRO9877
O95256	I18RA _HUMAN	Interleukin-18 receptor accessory protein (IL-18 receptor accessory protein) (IL-18RAcP) (Accessory protein-like) (AcPL) (CD218 antigen-like family member B) (CDw218b) (IL-1R accessory protein-like) (IL-1RAcPL) (Interleukin-1 receptor 7) (IL-1R-7) (IL-1R7) (Interleukin-18 receptor accessory protein-like) (Interleukin-18 receptor beta) (IL-18R-beta) (IL-18Rbeta) (CD antigen CD218b)	IL18RAP IL1R7
Q6UXL0	I20RB _HUMAN	Interleukin-20 receptor subunit beta (IL-20 receptor subunit beta) (IL-20R-beta) (IL-20RB) (Fibronectin type III domain containing 6) (FNDC6) (IL-20R2)	IL20RB DIRS1 UNQ557/P RO1114
Q9HBE5	IL21R _HUMAN	Interleukin-21 receptor (IL-21 receptor) (IL-21R) (Novel interleukin receptor) (CD antigen CD360)	IL21R NILR UNQ3121/ PRO10273
Q9UHF4	I20RA _HUMAN	Interleukin-20 receptor subunit alpha (IL-20 receptor subunit alpha) (IL-20R-alpha) (IL-20RA) (Cytokine receptor class-II member 8) (Cytokine receptor family 2 member 8) (CRF2-8) (IL-20R1) (ZcytoR7)	IL20RA UNQ681/P RO1315
P14778	IL1R1 _HUMAN	Interleukin-1 receptor type 1 (IL-1R-1) (IL-1RT-1) (IL-1RT1) (CD121 antigen-like family member A) (Interleukin-1 receptor alpha) (IL-1R-alpha) (Interleukin-1 receptor type I) (p80) (CD antigen CD121a) [Cleaved into: Interleukin-1 receptor type 1, membrane form (mIL-1R1) (mIL-1RI); Interleukin-1 receptor type 1, soluble form (sIL-1R1) (sIL-1RI)]	IL1R1 IL1R IL1RA IL1RT1
Q8N6P7	I22R1 _HUMAN	Interleukin-22 receptor subunit alpha-1 (IL-22 receptor subunit alpha-1) (IL-22R-alpha-1) (IL-22RA1) (Cytokine receptor class-II member 9) (Cytokine receptor family 2 member 9) (CRF2-9) (ZcytoR11)	IL22RA1 IL22R
Q13478	IL18R _HUMAN	Interleukin-18 receptor 1 (IL-18R-1) (IL-18R1) (CD218 antigen-like family member A) (CDw218a) (IL1 receptor-related protein) (IL-1Rrp) (IL1R-rp) (CD antigen CD218a)	IL18R1 IL1RRP
P26951	IL3RA _HUMAN	Interleukin-3 receptor subunit alpha (IL-3 receptor subunit alpha) (IL-3R subunit alpha) (IL-3R-alpha) (IL-3RA) (CD antigen CD123)	IL3RA IL3R
Q01344	IL5RA _HUMAN	Interleukin-5 receptor subunit alpha (IL-5 receptor subunit alpha) (IL-5R subunit alpha) (IL-5R-alpha) (IL-5RA) (CDw125) (CD antigen CD125)	IL5RA IL5R
Q8NI17	IL31R _HUMAN	Interleukin-31 receptor subunit alpha (IL-31 receptor subunit alpha) (IL-31R subunit alpha) (IL-31R-alpha) (IL-31RA) (Cytokine receptor-like 3) (GLM-R) (hGLM-R) (Gp130-like monocyte receptor) (Gp130-like receptor) (ZcytoR17)	IL31RA CRL3 GPL UNQ6368/ PRO21073/ PRO21384
Q9UKX5	ITA11 _HUMAN	Integrin alpha-11	ITGA11 MSTP018
P17301	ITA2 _HUMAN	Integrin alpha-2 (CD49 antigen-like family member B) (Collagen receptor) (Platelet membrane glycoprotein Ia) (GPIa) (VLA-2 subunit alpha) (CD antigen CD49b)	ITGA2 CD49B
Q13683	ITA7 _HUMAN	Integrin alpha-7 [Cleaved into: Integrin alpha-7 heavy chain; Integrin alpha-7 light chain; Integrin alpha-7 70 kDa form]	ITGA7 UNQ406/P RO768
P53708	ITA8 _HUMAN	Integrin alpha-8 [Cleaved into: Integrin alpha-8 heavy chain; Integrin alpha-8 light chain]	ITGA8

P38570	ITAE_HUMAN	Integrin alpha-E (HML-1 antigen) (Integrin alpha-IEL) (Mucosal lymphocyte 1 antigen) (CD antigen CD103) [Cleaved into: Integrin alpha-E light chain; Integrin alpha-E heavy chain]	ITGAE
P05107	ITB2_HUMAN	Integrin beta-2 (Cell surface adhesion glycoproteins LFA-1/CR3/p150,95 subunit beta) (Complement receptor C3 subunit beta) (CD antigen CD18)	ITGB2 CD18 MFI7
P18564	ITB6_HUMAN	Integrin beta-6	ITGB6
Q8IU57	INLR1_HUMAN	Interferon lambda receptor 1 (IFN-lambda receptor 1) (IFN-lambda-R1) (Cytokine receptor class-II member 12) (Cytokine receptor family 2 member 12) (CRF2-12) (Interleukin-28 receptor subunit alpha) (IL-28 receptor subunit alpha) (IL-28R-alpha) (IL-28RA) (Likely interleukin or cytokine receptor 2) (LICR2)	IFNLR1 IL28RA LICR2
Q5VWK5	IL23R_HUMAN	Interleukin-23 receptor (IL-23 receptor) (IL-23R)	IL23R
P17181	INAR1_HUMAN	Interferon alpha/beta receptor 1 (IFN-R-1) (IFN-alpha/beta receptor 1) (Cytokine receptor class-II member 1) (Cytokine receptor family 2 member 1) (CRF2-1) (Type I interferon receptor 1)	IFNAR1 IFNAR
P16871	IL7RA_HUMAN	Interleukin-7 receptor subunit alpha (IL-7 receptor subunit alpha) (IL-7R subunit alpha) (IL-7R-alpha) (IL-7RA) (CDw127) (CD antigen CD127)	IL7R
Q96T52	IMP2L_HUMAN	Mitochondrial inner membrane protease subunit 2 (EC 3.4.21.-) (IMP2-like protein)	IMMP2L
P13612	ITA4_HUMAN	Integrin alpha-4 (CD49 antigen-like family member D) (Integrin alpha-IV) (VLA-4 subunit alpha) (CD antigen CD49d)	ITGA4 CD49D
Q13349	ITAD_HUMAN	Integrin alpha-D (ADB2) (CD11 antigen-like family member D) (Leukointegrin alpha D) (CD antigen CD11d)	ITGAD
P78508	KCJ10_HUMAN	ATP-sensitive inward rectifier potassium channel 10 (ATP-dependent inwardly rectifying potassium channel Kir4.1) (Inward rectifier K(+) channel Kir1.2) (Potassium channel, inwardly rectifying subfamily J member 10)	KCNJ10
P11215	ITAM_HUMAN	Integrin alpha-M (CD11 antigen-like family member B) (CR-3 alpha chain) (Cell surface glycoprotein MAC-1 subunit alpha) (Leukocyte adhesion receptor MO1) (Neutrophil adherence receptor) (CD antigen CD11b)	ITGAM CD11B CR3A
P16144	ITB4_HUMAN	Integrin beta-4 (GP150) (CD antigen CD104)	ITGB4
P26012	ITB8_HUMAN	Integrin beta-8	ITGB8
Q9NPI9	KCJ16_HUMAN	Inward rectifier potassium channel 16 (Inward rectifier K(+) channel Kir5.1) (Potassium channel, inwardly rectifying subfamily J member 16)	KCNJ16
P38484	INGR2_HUMAN	Interferon gamma receptor 2 (IFN-gamma receptor 2) (IFN-gamma-R2) (Interferon gamma receptor accessory factor 1) (AF-1) (Interferon gamma receptor beta-chain) (IFN-gamma-R-beta) (Interferon gamma transducer 1)	IFNGR2 IFNGT1
P56199	ITA1_HUMAN	Integrin alpha-1 (CD49 antigen-like family member A) (Laminin and collagen receptor) (VLA-1) (CD antigen CD49a)	ITGA1
P26006	ITA3_HUMAN	Integrin alpha-3 (CD49 antigen-like family member C) (FRP-2) (Galactoprotein B3) (GAPB3) (VLA-3 subunit alpha) (CD antigen CD49c) [Cleaved into: Integrin alpha-3 heavy chain; Integrin alpha-3 light chain]	ITGA3 MSK18
P20702	ITAX_HUMAN	Integrin alpha-X (CD11 antigen-like family member C) (Leu M5) (Leukocyte adhesion glycoprotein p150,95 alpha chain) (Leukocyte adhesion receptor p150,95) (CD antigen CD11c)	ITGAX CD11C
P18084	ITB5_HUMAN	Integrin beta-5	ITGB5
Q9UJ96	KCNG2_HUMAN	Potassium voltage-gated channel subfamily G member 2 (Cardiac potassium channel subunit) (Voltage-gated potassium channel subunit Kv6.2)	KCNG2 KCNF2
O95069	KCNK2_HUMAN	Potassium channel subfamily K member 2 (Outward rectifying potassium channel protein TREK-1) (TREK-1 K(+) channel subunit) (Two pore domain potassium channel TREK-1) (Two pore potassium channel TPKC1)	KCNK2 TREK TREK1
Q9H427	KCNKF_HUMAN	Potassium channel subfamily K member 15 (Acid-sensitive potassium channel protein TASK-5) (TWIK-related acid-sensitive K(+) channel 5) (Two pore potassium channel KT3.3) (Two pore K(+) channel KT3.3)	KCNK15 TASK5
O75578	ITA10	Integrin alpha-10	ITGA10

	_HUMAN		UNQ468/PRO827
P08514	ITA2B_HUMAN	Integrin alpha-IIb (GPalpha IIb) (GPIIb) (Platelet membrane glycoprotein IIb) (CD antigen CD41) [Cleaved into: Integrin alpha-IIb heavy chain; Integrin alpha-IIb light chain, form 1; Integrin alpha-IIb light chain, form 2]	ITGA2B GP2B ITGAB
P23229	ITA6_HUMAN	Integrin alpha-6 (CD49 antigen-like family member F) (VLA-6) (CD antigen CD49f) [Cleaved into: Integrin alpha-6 heavy chain; Integrin alpha-6 light chain; Processed integrin alpha-6 (Alpha6p)]	ITGA6
P08648	ITA5_HUMAN	Integrin alpha-5 (CD49 antigen-like family member E) (Fibronectin receptor subunit alpha) (Integrin alpha-F) (VLA-5) (CD antigen CD49e) [Cleaved into: Integrin alpha-5 heavy chain; Integrin alpha-5 light chain]	ITGA5 FNRA
Q13797	ITA9_HUMAN	Integrin alpha-9 (Integrin alpha-RLC)	ITGA9
P20701	ITAL_HUMAN	Integrin alpha-L (CD11 antigen-like family member A) (Leukocyte adhesion glycoprotein LFA-1 alpha chain) (LFA-1A) (Leukocyte function-associated molecule 1 alpha chain) (CD antigen CD11a)	ITGAL CD11A
P26010	ITB7_HUMAN	Integrin beta-7 (Gut homing receptor beta subunit)	ITGB7
P48551	INAR2_HUMAN	Interferon alpha/beta receptor 2 (IFN-R-2) (IFN-alpha binding protein) (IFN-alpha/beta receptor 2) (Interferon alpha binding protein) (Type I interferon receptor 2)	IFNAR2 IFNABR IFNARB
P15260	INGR1_HUMAN	Interferon gamma receptor 1 (IFN-gamma receptor 1) (IFN-gamma-R1) (CDw119) (Interferon gamma receptor alpha-chain) (IFN-gamma-R-alpha) (CD antigen CD119)	IFNGR1
Q01650	LAT1_HUMAN	Large neutral amino acids transporter small subunit 1 (4F2 light chain) (4F2 LC) (4F2LC) (CD98 light chain) (Integral membrane protein E16) (L-type amino acid transporter 1) (hLAT1) (Solute carrier family 7 member 5) (y+ system cationic amino acid transporter)	SLC7A5 CD98LC LAT1 MPE16
Q9UHI5	LAT2_HUMAN	Large neutral amino acids transporter small subunit 2 (L-type amino acid transporter 2) (hLAT2) (Solute carrier family 7 member 8)	SLC7A8 LAT2
P42702	LIFR_HUMAN	Leukemia inhibitory factor receptor (LIF receptor) (LIF-R) (CD antigen CD118)	LIFR
O75096	LRP4_HUMAN	Low-density lipoprotein receptor-related protein 4 (LRP-4) (Multiple epidermal growth factor-like domains 7)	LRP4 KIAA0816 LRP10 MEGF7
Q07820	MCL1_HUMAN	Induced myeloid leukemia cell differentiation protein Mcl-1 (Bcl-2-like protein 3) (Bcl2-L-3) (Bcl-2-related protein EAT/mcl1) (mcl1/EAT)	MCL1 BCL2L3
Q29980	MICB_HUMAN	MHC class I polypeptide-related sequence B (MIC-B)	MICB PERB11.2
Q99650	OSMR_HUMAN	Oncostatin-M-specific receptor subunit beta (Interleukin-31 receptor subunit beta) (IL-31 receptor subunit beta) (IL-31R subunit beta) (IL-31R-beta) (IL-31RB)	OSMR OSMRB
Q86UW1	OSTA_HUMAN	Organic solute transporter subunit alpha (OST-alpha) (Solute carrier family 51 subunit alpha)	SLC51A OSTA
P41143	OPRD_HUMAN	Delta-type opioid receptor (D-OR-1) (DOR-1)	OPRD1 OPRD
Q96QU1	PCD15_HUMAN	Protocadherin-15	PCDH15 USH1F
P07202	PERT_HUMAN	Thyroid peroxidase (TPO) (EC 1.11.1.8)	TPO
Q29983	MICA_HUMAN	MHC class I polypeptide-related sequence A (MIC-A)	MICA PERB11.1
Q7L4E1	MIGA2_HUMAN	Mitoguardin 2 (Protein FAM73B)	MIGA2 C9orf54 FAM73B PSEC0112
Q9BPX6	MICU1_HUMAN	Calcium uptake protein 1, mitochondrial (Atopy-related autoantigen CALC) (ara CALC) (Calcium-binding atopy-related autoantigen 1) (allergen Hom s 4)	MICU1 CALC CBARA1

Q8TCY5	MRAP _HUMAN	Melanocortin-2 receptor accessory protein (B27) (Fat cell-specific low molecular weight protein) (Fat tissue-specific low MW protein)	MRAP C21orf61 FALP
Q8NAN2	MIGA1 _HUMAN	Mitoguardin 1 (Protein FAM73A)	MIGA1 FAM73A
Q13585	MTR1L _HUMAN	Melatonin-related receptor (G protein-coupled receptor 50) (H9)	GPR50
O15146	MUSK _HUMAN	Muscle, skeletal receptor tyrosine-protein kinase (EC 2.7.10.1) (Muscle-specific tyrosine-protein kinase receptor) (MuSK) (Muscle-specific kinase receptor)	MUSK
Q96G30	MRAP2 _HUMAN	Melanocortin-2 receptor accessory protein 2 (MC2R accessory protein 2)	MRAP2 C6orf117
O43908	NKG2F _HUMAN	NKG2-F type II integral membrane protein (NK cell receptor F) (NKG2-F-activating NK receptor)	KLRC4 NKG2F
Q9Y2A7	NCKP1 _HUMAN	Nck-associated protein 1 (NAP 1) (Membrane-associated protein HEM-2) (p125Nap1)	NCKAP1 HEM2 KIAA0587 NAP1
O00533	NCHL1 _HUMAN	Neural cell adhesion molecule L1-like protein (Close homolog of L1) [Cleaved into: Processed neural cell adhesion molecule L1-like protein]	CHL1 CALL
P26715	NKG2A _HUMAN	NKG2-A/NKG2-B type II integral membrane protein (CD159 antigen-like family member A) (NK cell receptor A) (NKG2-A/B-activating NK receptor) (CD antigen CD159a)	KLRC1 NKG2A
Q9NZ94	NLGN3 _HUMAN	Neuroligin-3 (Gliotactin homolog)	NLGN3 KIAA1480 NL3
P26717	NKG2C _HUMAN	NKG2-C type II integral membrane protein (CD159 antigen-like family member C) (NK cell receptor C) (NKG2-C-activating NK receptor) (CD antigen CD159c)	KLRC2 NKG2C
Q07444	NKG2E _HUMAN	NKG2-E type II integral membrane protein (NK cell receptor E) (NKG2-E-activating NK receptor)	KLRC3 NKG2E
Q99466	NOTC4 _HUMAN	Neurogenic locus notch homolog protein 4 (Notch 4) (hNotch4) [Cleaved into: Notch 4 extracellular truncation; Notch 4 intracellular domain]	NOTCH4 INT3
O14786	NRP1 _HUMAN	Neuropilin-1 (Vascular endothelial cell growth factor 165 receptor) (CD antigen CD304)	NRP1 NRP VEGF165R
Q04721	NOTC2 _HUMAN	Neurogenic locus notch homolog protein 2 (Notch 2) (hN2) [Cleaved into: Notch 2 extracellular truncation (N2ECD); Notch 2 intracellular domain (N2ICD)]	NOTCH2
O60462	NRP2 _HUMAN	Neuropilin-2 (Vascular endothelial cell growth factor 165 receptor 2)	NRP2 VEGF165R 2
Q86UW2	OSTB _HUMAN	Organic solute transporter subunit beta (OST-beta) (Solute carrier family 51 subunit beta)	SLC51B OSTB
Q9P0L9	PK2L1 _HUMAN	Polycystic kidney disease 2-like 1 protein (Polycystin-2 homolog) (Polycystin-2L1) (Polycystin-L) (Polycystin-L1)	PKD2L1 PKD2L PKDL TRPP3
Q8TDX9	PK1L1 _HUMAN	Polycystic kidney disease protein 1-like 1 (PC1-like 1 protein) (Polycystin-1L1)	PKD1L1 UNQ5785/ PRO19563
Q16651	PRSS8 _HUMAN	Prostasin (EC 3.4.21.-) (Channel-activating protease 1) (CAP1) (Serine protease 8) [Cleaved into: Prostasin light chain; Prostasin heavy chain]	PRSS8
Q96HA9	PX11C _HUMAN	Peroxisomal membrane protein 11C (Peroxin-11C) (Peroxisomal biogenesis factor 11C) (Protein PEX11 homolog gamma) (PEX11-gamma)	PEX11G PEX11C
O60896	RAMP3 _HUMAN	Receptor activity-modifying protein 3 (Calcitonin-receptor-like receptor activity-modifying protein 3) (CRLR activity-modifying protein 3)	RAMP3
O60895	RAMP2 _HUMAN	Receptor activity-modifying protein 2 (Calcitonin-receptor-like receptor activity-modifying protein 2) (CRLR activity-modifying protein 2)	RAMP2
O60894	RAMP1 _HUMAN	Receptor activity-modifying protein 1 (Calcitonin-receptor-like receptor activity-modifying protein 1) (CRLR activity-modifying protein 1)	RAMP1
O43157	PLXB1	Plexin-B1 (Semaphorin receptor SEP)	PLXNB1

	_HUMAN		KIAA0407 PLXN5 SEP
O15031	PLXB2 _HUMAN	Plexin-B2 (MM1)	PLXNB2 KIAA0315
Q9UG56	PISD _HUMAN	Phosphatidylserine decarboxylase proenzyme, mitochondrial (EC 4.1.1.65) [Cleaved into: Phosphatidylserine decarboxylase beta chain; Phosphatidylserine decarboxylase alpha chain]	PISD
O75915	PRAF3 _HUMAN	PRA1 family protein 3 (ADP-ribosylation factor-like protein 6-interacting protein 5) (ARL-6-interacting protein 5) (Aip-5) (Cytoskeleton-related vitamin A-responsive protein) (Dermal papilla-derived protein 11) (GTRAP3-18) (Glutamate transporter EAAC1-interacting protein) (JM5) (Prenylated Rab acceptor protein 2) (Protein JWa) (Putative MAPK-activating protein PM27)	ARL6IP5 DERP11 JWA PRA2 PRAF3 HSPC127
O96011	PX11B _HUMAN	Peroxisomal membrane protein 11B (Peroxin-11B) (Peroxisomal biogenesis factor 11B) (Protein PEX11 homolog beta) (PEX11-beta)	PEX11B
O75192	PX11A _HUMAN	Peroxisomal membrane protein 11A (HsPEX11p) (28 kDa peroxisomal integral membrane protein) (PMP28) (Peroxin-11A) (Peroxisomal biogenesis factor 11A) (Protein PEX11 homolog alpha) (PEX11-alpha)	PEX11A PEX11
Q6ISU1	PTCRA _HUMAN	Pre T-cell antigen receptor alpha (pT-alpha) (pTa) (pT-alpha-TCR)	PTCRA
Q68DV7	RNF43 _HUMAN	E3 ubiquitin-protein ligase RNF43 (EC 2.3.2.27) (RING finger protein 43) (RING-type E3 ubiquitin transferase RNF43)	RNF43
P51811	XK _HUMAN	Membrane transport protein XK (Kell complex 37 kDa component) (Kx antigen) (XK-related protein 1)	XK XKR1 XRG1
Q04912	RON _HUMAN	Macrophage-stimulating protein receptor (MSP receptor) (EC 2.7.10.1) (CDw136) (Protein-tyrosine kinase 8) (p185-Ron) (CD antigen CD136) [Cleaved into: Macrophage-stimulating protein receptor alpha chain; Macrophage-stimulating protein receptor beta chain]	MST1R PTK8 RON
Q96DX8	RTP4 _HUMAN	Receptor-transporting protein 4 (28 kDa interferon-responsive protein) (3CxxC-type zinc finger protein 4)	RTP4 IFRG28 Z3CXXC4
Q8WTV0	SCRB1 _HUMAN	Scavenger receptor class B member 1 (SRB1) (CD36 and LIMPII analogous 1) (CLA-1) (CD36 antigen-like 1) (Collagen type I receptor, thrombospondin receptor-like 1) (SR-BI) (CD antigen CD36)	SCARB1 CD36L1 CLA1
Q12884	SEPR _HUMAN	Prolyl endopeptidase FAP (EC 3.4.21.26) (170 kDa melanoma membrane-bound gelatinase) (Dipeptidyl peptidase FAP) (EC 3.4.14.5) (Fibroblast activation protein alpha) (FAPalpha) (Gelatine degradation protease FAP) (EC 3.4.21.-) (Integral membrane serine protease) (Post-proline cleaving enzyme) (Serine integral membrane protease) (SIMP) (Surface-expressed protease) (Seprase) [Cleaved into: Antiplasmin-cleaving enzyme FAP, soluble form (APCE) (EC 3.4.14.5) (EC 3.4.21.-) (EC 3.4.21.26)]	FAP
Q9Y5M8	SRPRB _HUMAN	Signal recognition particle receptor subunit beta (SR-beta) (Protein APMCF1)	SRPRB PSEC0230
O15270	SPTC2 _HUMAN	Serine palmitoyltransferase 2 (EC 2.3.1.50) (Long chain base biosynthesis protein 2) (LCB 2) (Long chain base biosynthesis protein 2a) (LCB2a) (Serine-palmitoyl-CoA transferase 2) (SPT 2)	SPTLC2 KIAA0526 LCB2
Q07837	SLC31 _HUMAN	Neutral and basic amino acid transport protein rBAT (NBAT) (D2h) (Solute carrier family 3 member 1) (b(0,+)-type amino acid transport protein)	SLC3A1 RBAT
O15269	SPTC1 _HUMAN	Serine palmitoyltransferase 1 (EC 2.3.1.50) (Long chain base biosynthesis protein 1) (LCB 1) (Serine-palmitoyl-CoA transferase 1) (SPT 1) (SPT1)	SPTLC1 LCB1
P30874	SSR2 _HUMAN	Somatostatin receptor type 2 (SS-2-R) (SS2-R) (SS2R) (SRIF-1)	SSTR2
Q9NUV7	SPTC3 _HUMAN	Serine palmitoyltransferase 3 (EC 2.3.1.50) (Long chain base biosynthesis protein 2b) (LCB2b) (Long chain base biosynthesis protein 3) (LCB 3) (Serine-palmitoyl-CoA transferase 3) (SPT 3)	SPTLC3 C20orf38 SPTLC2L
P32745	SSR3 _HUMAN	Somatostatin receptor type 3 (SS-3-R) (SS3-R) (SS3R) (SSR-28)	SSTR3

P35346	SSR5_HUMAN	Somatostatin receptor type 5 (SS-5-R) (SS5-R) (SS5R)	SSTR5
P60508	SYCY2_HUMAN	Syncytin-2 (Endogenous retrovirus group FRD member 1) (Envelope polyprotein) (HERV-FRD) (HERV-FRD_6p24.1 provirus ancestral Env polyprotein) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	ERVFRD-1 ERVFRDE1 UNQ6191/PRO20218
Q86SS6	SYT9_HUMAN	Synaptotagmin-9 (Synaptotagmin IX) (SytIX)	SYT9
Q9BQG1	SYT3_HUMAN	Synaptotagmin-3 (Synaptotagmin III) (SytIII)	SYT3
Q5T7P8	SYT6_HUMAN	Synaptotagmin-6 (Synaptotagmin VI) (SytVI)	SYT6
Q6XYQ8	SYT10_HUMAN	Synaptotagmin-10 (Synaptotagmin X) (SytX)	SYT10
Q03518	TAP1_HUMAN	Antigen peptide transporter 1 (APT1) (ATP-binding cassette sub-family B member 2) (Peptide supply factor 1) (Peptide transporter PSF1) (PSF-1) (Peptide transporter TAP1) (Peptide transporter involved in antigen processing 1) (Really interesting new gene 4 protein)	TAP1 ABCB2 PSF1 RING4 Y3
Q03519	TAP2_HUMAN	Antigen peptide transporter 2 (APT2) (ATP-binding cassette sub-family B member 3) (Peptide supply factor 2) (Peptide transporter PSF2) (PSF-2) (Peptide transporter TAP2) (Peptide transporter involved in antigen processing 2) (Really interesting new gene 11 protein)	TAP2 ABCB3 PSF2 RING11 Y1
P01848	TCA_HUMAN	T-cell receptor alpha chain C region	TRAC TCRA
Q9UKZ4	TEN1_HUMAN	Teneurin-1 (Ten-1) (Protein Odd Oz/ten-m homolog 1) (Tenascin-M1) (Ten-m1) (Teneurin transmembrane protein 1) [Cleaved into: Ten-1 intracellular domain (IDten-1) (Ten-1 ICD); Teneurin C-terminal-associated peptide (TCPA-1) (Ten-1 extracellular domain) (Ten-1 ECD)]	TENM1 ODZ1 TNM1
Q9UL52	TM11E_HUMAN	Transmembrane protease serine 11E (EC 3.4.21.-) (Serine protease DESC1) (Transmembrane protease serine 11E2) [Cleaved into: Transmembrane protease serine 11E non-catalytic chain; Transmembrane protease serine 11E catalytic chain]	TMPRSS11E DESC1 TMPRSS11E2 UNQ742/PRO1461
P35590	TIE1_HUMAN	Tyrosine-protein kinase receptor Tie-1 (EC 2.7.10.1)	TIE1 TIE
Q9Y2C9	TLR6_HUMAN	Toll-like receptor 6 (CD antigen CD286)	TLR6
Q9NT68	TEN2_HUMAN	Teneurin-2 (Ten-2) (Protein Odd Oz/ten-m homolog 2) (Tenascin-M2) (Ten-m2) (Teneurin transmembrane protein 2) [Cleaved into: Ten-2, soluble form; Ten-2 intracellular domain (Ten-2 ICD)]	TENM2 KIAA1127 ODZ2 TNM2
Q6N022	TEN4_HUMAN	Teneurin-4 (Ten-4) (Protein Odd Oz/ten-m homolog 4) (Tenascin-M4) (Ten-m4) (Teneurin transmembrane protein 4)	TENM4 KIAA1302 ODZ4 TNM4
Q15399	TLR1_HUMAN	Toll-like receptor 1 (Toll/interleukin-1 receptor-like protein) (TIL) (CD antigen CD281)	TLR1 KIAA0012
Q7RTY8	TMPS7_HUMAN	Transmembrane protease serine 7 (EC 3.4.21.-) (Matriptase-3)	TMPRSS7
P01850	TRBC1_HUMAN	T-cell receptor beta-1 chain C region	TRBC1
O15533	TPSN_HUMAN	Tapasin (TPN) (TPSN) (NGS-17) (TAP-associated protein) (TAP-binding protein)	TAPBP NGS17 TAPA

Q9BX84	TRPM6 _HUMAN	Transient receptor potential cation channel subfamily M member 6 (EC 2.7.11.1) (Channel kinase 2) (Melastatin-related TRP cation channel 6)	TRPM6 CHAK2
P30530	UFO _HUMAN	Tyrosine-protein kinase receptor UFO (EC 2.7.10.1) (AXL oncogene)	AXL UFO
O00526	UPK2 _HUMAN	Uroplakin-2 (UP2) (Uroplakin II) (UPII)	UPK2
Q92536	YLAT2 _HUMAN	Y+L amino acid transporter 2 (Cationic amino acid transporter, y+ system) (Solute carrier family 7 member 6) (y(+)L-type amino acid transporter 2) (Y+LAT2) (y+LAT-2)	SLC7A6 KIAA0245
Q9UM01	YLAT1 _HUMAN	Y+L amino acid transporter 1 (Monocyte amino acid permease 2) (MOP-2) (Solute carrier family 7 member 7) (y(+)L-type amino acid transporter 1) (Y+LAT1) (y+LAT-1)	SLC7A7
O75631	UPK3A _HUMAN	Uroplakin-3a (UP3a) (Uroplakin III) (UPIII)	UPK3A UPK3
Q9BT76	UPK3B _HUMAN	Uroplakin-3b (UP3b) (Uroplakin IIIb) (UPIIIb) (p35)	UPK3B
P35916	VGFR3 _HUMAN	Vascular endothelial growth factor receptor 3 (VEGFR-3) (EC 2.7.10.1) (Fms-like tyrosine kinase 4) (FLT-4) (Tyrosine-protein kinase receptor FLT4)	FLT4 VEGFR3
O75841	UPK1B _HUMAN	Uroplakin-1b (UP1b) (Tetraspanin-20) (Tspan-20) (Uroplakin Ib) (UPIb)	UPK1B TSPAN20
Q9P0L0	VAPA _HUMAN	Vesicle-associated membrane protein-associated protein A (VAMP-A) (VAMP-associated protein A) (VAP-A) (33 kDa VAMP-associated protein) (VAP-33)	VAPA VAP33
Q9ULK5	VANG2 _HUMAN	Vang-like protein 2 (Loop-tail protein 1 homolog) (Strabismus 1) (Van Gogh-like protein 2)	VANGL2 KIAA1215 STB1
Q8TAA9	VANG1 _HUMAN	Vang-like protein 1 (Loop-tail protein 2 homolog) (LPP2) (Strabismus 2) (Van Gogh-like protein 1)	VANGL1 STB2
O95292	VAPB _HUMAN	Vesicle-associated membrane protein-associated protein B/C (VAMP-B/VAMP-C) (VAMP-associated protein B/C) (VAP-B/VAP-C)	VAPB UNQ484/PRO983
Q9UPY5	XCT _HUMAN	Cystine/glutamate transporter (Amino acid transport system xc-) (Calcium channel blocker resistance protein CCBR1) (Solute carrier family 7 member 11) (xCT)	SLC7A11
Q6XR72	ZNT10 _HUMAN	Zinc transporter 10 (ZnT-10) (Manganese transporter SLC30A10) (Solute carrier family 30 member 10)	SLC30A10 ZNT10 ZNT8
A0A024R0A1	A0A024R0A1 _HUMAN	Macrophage colony-stimulating factor 1 (CSF-1) (MCSF)	CSF1 hCG_40247
P31785	IL2RG _HUMAN	Cytokine receptor common subunit gamma (Interleukin-2 receptor subunit gamma) (IL-2 receptor subunit gamma) (IL-2R subunit gamma) (IL-2RG) (gammaC) (p64) (CD antigen CD132)	IL2RG
P32927	IL3RB _HUMAN	Cytokine receptor common subunit beta (CDw131) (GM-CSF/IL-3/IL-5 receptor common beta subunit) (CD antigen CD131)	CSF2RB IL3RB IL5RB
P26951	IL3RA _HUMAN	Interleukin-3 receptor subunit alpha (IL-3 receptor subunit alpha) (IL-3R subunit alpha) (IL-3R-alpha) (IL-3RA) (CD antigen CD123)	IL3RA IL3R
P15509	CSF2R _HUMAN	Granulocyte-macrophage colony-stimulating factor receptor subunit alpha (GM-CSF-R-alpha) (GMCSFR-alpha) (GMR-alpha) (CDw116) (CD antigen CD116)	CSF2RA CSF2R CSF2RY
Q01344	IL5RA _HUMAN	Interleukin-5 receptor subunit alpha (IL-5 receptor subunit alpha) (IL-5R subunit alpha) (IL-5R-alpha) (IL-5RA) (CDw125) (CD antigen CD125)	IL5RA IL5R
P08887	IL6RA _HUMAN	Interleukin-6 receptor subunit alpha (IL-6 receptor subunit alpha) (IL-6R subunit alpha) (IL-6R-alpha) (IL-6RA) (IL-6R 1) (Membrane glycoprotein 80) (gp80) (CD antigen CD126)	IL6R
P40189	IL6RB _HUMAN	Interleukin-6 receptor subunit beta (IL-6 receptor subunit beta) (IL-6R subunit beta) (IL-6R-beta) (IL-6RB) (CDw130) (Interleukin-6 signal transducer) (Membrane glycoprotein 130) (gp130) (Oncostatin-M receptor subunit alpha) (CD antigen CD130)	IL6ST

Q14626	I11RA _HUMAN	Interleukin-11 receptor subunit alpha (IL-11 receptor subunit alpha) (IL-11R subunit alpha) (IL-11R-alpha) (IL-11RA)	IL11RA
P42702	LIFR _HUMAN	Leukemia inhibitory factor receptor (LIF receptor) (LIF-R) (CD antigen CD118)	LIFR
Q99650	OSMR _HUMAN	Oncostatin-M-specific receptor subunit beta (Interleukin-31 receptor subunit beta) (IL-31 receptor subunit beta) (IL-31R subunit beta) (IL-31R-beta) (IL-31RB)	OSMR OSMRB
P31785	IL2RG _HUMAN	Cytokine receptor common subunit gamma (Interleukin-2 receptor subunit gamma) (IL-2 receptor subunit gamma) (IL-2R subunit gamma) (IL-2RG) (gammaC) (p64) (CD antigen CD132)	IL2RG
P01589	IL2RA _HUMAN	Interleukin-2 receptor subunit alpha (IL-2 receptor subunit alpha) (IL-2-RA) (IL-2R subunit alpha) (IL2-RA) (TAC antigen) (p55) (CD antigen CD25)	IL2RA
P14784	IL2RB _HUMAN	Interleukin-2 receptor subunit beta (IL-2 receptor subunit beta) (IL-2R subunit beta) (IL-2RB) (High affinity IL-2 receptor subunit beta) (Interleukin-15 receptor subunit beta) (p70-75) (p75) (CD antigen CD122)	IL2RB IL15RB
P24394	IL4RA _HUMAN	Interleukin-4 receptor subunit alpha (IL-4 receptor subunit alpha) (IL-4R subunit alpha) (IL-4R-alpha) (IL-4RA) (CD antigen CD124) [Cleaved into: Soluble interleukin-4 receptor subunit alpha (Soluble IL-4 receptor subunit alpha) (Soluble IL-4R-alpha) (sIL4Ralpha/prot) (IL-4-binding protein) (IL4-BP)]	IL4R IL4RA 582J2.1
P16871	IL7RA _HUMAN	Interleukin-7 receptor subunit alpha (IL-7 receptor subunit alpha) (IL-7R subunit alpha) (IL-7R-alpha) (IL-7RA) (CDw127) (CD antigen CD127)	IL7R
Q01113	IL9R _HUMAN	Interleukin-9 receptor (IL-9 receptor) (IL-9R) (CD antigen CD129)	IL9R
P78552	I13R1 _HUMAN	Interleukin-13 receptor subunit alpha-1 (IL-13 receptor subunit alpha-1) (IL-13R subunit alpha-1) (IL-13R-alpha-1) (IL-13RA1) (Cancer/testis antigen 19) (CT19) (CD antigen CD213a1)	IL13RA1 IL13R IL13RA
Q14627	I13R2 _HUMAN	Interleukin-13 receptor subunit alpha-2 (IL-13 receptor subunit alpha-2) (IL-13R subunit alpha-2) (IL-13R-alpha-2) (IL-13RA2) (Interleukin-13-binding protein) (CD antigen CD213a2)	IL13RA2 IL13R
Q13651	I10R1 _HUMAN	Interleukin-10 receptor subunit alpha (IL-10 receptor subunit alpha) (IL-10R subunit alpha) (IL-10RA) (CDw210a) (Interleukin-10 receptor subunit 1) (IL-10R subunit 1) (IL-10R1) (CD antigen CD210)	IL10RA IL10R
P26992	CNTFR _HUMAN	Ciliary neurotrophic factor receptor subunit alpha (CNTF receptor subunit alpha) (CNTFR-alpha)	CNTFR
Q9NPF7	IL23A _HUMAN	Interleukin-23 subunit alpha (IL-23 subunit alpha) (IL-23-A) (Interleukin-23 subunit p19) (IL-23p19)	IL23A SGRF UNQ2498/ PRO5798
P29459	IL12A _HUMAN	Interleukin-12 subunit alpha (IL-12A) (Cytotoxic lymphocyte maturation factor 35 kDa subunit) (CLMF p35) (IL-12 subunit p35) (NK cell stimulatory factor chain 1) (NKSF1)	IL12A NKSF1
Q8NEV9	IL27A _HUMAN	Interleukin-27 subunit alpha (IL-27 subunit alpha) (IL-27-A) (IL27-A) (Interleukin-30) (p28)	IL27 IL27A IL30
Q14213	IL27B _HUMAN	Interleukin-27 subunit beta (IL-27 subunit beta) (IL-27B) (Epstein-Barr virus-induced gene 3 protein) (EBV-induced gene 3 protein)	EBI3 IL27B

In various embodiments, the present heterodimeric proteins may be engineered to target one or more molecules that reside on human leukocytes including, without limitation, the extracellular domains (where applicable) of SLAMF4, IL-2Rα, IL-2 R β, ALCAM, B7-1, IL-4 R, B7-H3, BLAME/SLAMFS, CEACAM1, IL-6 R, IL-7 Rα, IL-10R α, IL-I 0 R β, IL-12 R β 1, 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, KIR2DL1, CD2S, KIR2DL3, KIR2DL4/CD15Sd, CD31/PECAM-1, KIR2DS4, 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, CX3CR1, CX3CL1, L-Selectin, SIRP β 1, SLAM, TCCR/WSX-1, DNAM-1, Thymopoietin, EMMPRIN/CD147, TIM-1, EphB6, TIM-2, TIM-3, TIM-4, Fcγ RIII/CD16, TIM-6, Granulysin, ICAM-1/CD54, ICAM-2/CD102, IFN-γR1, IFN-γ R2, TSLP, IL-1 R1 and TSLP R.

In some embodiments, the present heterodimeric proteins may be engineered to target one or more molecules involved in immune inhibition, including for example: CTLA-4, PD-L1, PD-L2, PD-1, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA/VSIG8, KIR, 2B4, TIGIT, CD160 (also referred to as BY55), CHK 1 and CHK2 kinases, A2aR, CEACAM (*e.g.*, CEACAM-1, CEACAM-3 and/or CEACAM-5), and various B-7 family ligands (including, but are not limited to, B7-1, B7-2, B7-DC, B7-H1, B7-H2, B7-H3, B7-H4, B7-H5, B7-H6 and B7-H7).

In some embodiments, the present heterodimeric proteins comprise an extracellular domain of an immune inhibitory agent.

In some embodiments, the present heterodimeric proteins comprise an extracellular domain of a soluble or membrane protein which has immune inhibitory properties.

In some embodiments, the present heterodimeric proteins simulate binding of an inhibitory signal ligand to its cognate receptor but inhibit the inhibitory signal transmission to an immune cell (*e.g.,* a T cell, macrophage or other leukocyte).

In various embodiments, the heterodimeric protein comprises an immune inhibitory receptor extracellular domain and an immune stimulatory ligand extracellular domain which can, without limitation, deliver an immune stimulation to a T cell while masking a tumor cell's immune inhibitory signals. In various embodiments, the heterodimeric protein delivers a signal that has the net result of T cell activation.

In some embodiments, the present heterodimeric proteins comprise an extracellular domain of a soluble or membrane protein which has immune stimulatory properties.

In embodiments, a heterodimeric protein useful in the present invention comprises the extracellular domain of Gp130. Gp130 (also known as Interleukin-6 receptor subunit beta, IL-6R-beta, IL-6RB, and IL-6ST) is a signal-transducing molecule. The receptor systems for IL6, LIF, OSM, CNTF, IL11, CTF1 and BSF3 can utilize Gp130 for initiating signal transmission. Binding of IL6 to IL6R induces IL6ST homodimerization and formation of a high-affinity receptor complex, which activates Janus kinases. That causes phosphorylation of Gp130 tyrosine residues which in turn activates STAT3. Gp130 mediates signals which regulate immune response, hematopoiesis, pain control and bone metabolism (By similarity).

In embodiments, a heterodimeric protein useful in the present invention comprises a variant of the extracellular domain of Gp130. As examples, the variant may have 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 amino acid sequence of Gp130, *e.g.,* human Gp130.

In embodiments, the extracellular domain of Gp130 has the following amino acid sequence:

ELLDPCGYISPESPVVQLHSNFTAVCVLKEKCMDYFHVNANYIVWKTNHFTIPKEQYTIINRTASSVTFTD
IASLNIQLTCNILTFGQLEQNVYGITIISGLPPEKPKNLSCIVNEGKKMRCEWDGGRETHLETNFTLKSEW
ATHKFADCKAKRDTPTSCTVDYSTVYFVNIEVWVEAENALGKVTSDHINFDPVYKVKPNPPHNLSVINS
EELSSILKLTWTNPSIKSVIILKYNIQYRTKDASTWSQIPPEDTASTRSSFTVQDLKPFTEYVFRIRCMKED
GKGYWSDWSEEASGITYEDRPSKAPSFWYKIDPSHTQGYRTVQLVWKTLPPFEANGKILDYEVTLTRW
KSHLQNYTVNATKLTVNLTNDRYLATLTVRNLVGKSDAAVLTIPACDFQATHPVMDLKAFPKDNMLWVE
WTTPRESVKKYILEWCVLSDKAPCITDWQQEDGTVHRTYLRGNLAESKCYLITVTPVYADGPGSPESIK
AYLKQAPPSKGPTVRTKKVGKNEAVLEWDQLPVDVQNGFIRNYTIFYRTIIGNETAVNVDSSHTEYTLSS
LTSDTLYMVRMAAYTDEGGKDGPEFTFTTPKFAQGEIE (SEQ ID NO: 18)

In embodiments, a heterodimeric protein comprises a variant of the extracellular domain of Gp130. As examples, the variant may have 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 SEQ ID NO: 18.

In embodiments, one chain of the heterodimeric protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 18.

One of ordinary skill may select variants of the known amino acid sequence of Gp130 by consulting the literature, *e.g.*, Hibi et al, "Molecular cloning and expression of an IL-6 signal transducer, gp130" Cell 63 (6), 1149-1157 (1990); Waetzig et al., "N-linked glycosylation is essential for the stability but not the signaling function of the interleukin-6 signal transducer glycoprotein 130", J. Biol. Chem. 285 (3), 1781-1789 (2010); Schutt et al., "gp130 activation is regulated by D2-D3 interdomain connectivity", Biochem. J. 450 (3), 487-496 (2013); Bravo et al., "Crystal structure of a cytokine-binding region of gp130", EMBO J. 17 (6), 1665-1674 (1998); Chow et al., "Structure of an extracellular gp130 cytokine receptor signaling complex", Science 291 (5511), 2150-2155 (2001); Boulanger et al., "Hexameric structure and assembly of the interleukin-6/IL-6 alpha-receptor/gp130 complex", Science 300 (5628), 2101-2104 (2003); Xu et al., "Crystal structure of the entire ectodomain of gp130: insights into the molecular assembly of the tall cytokine receptor complexes", J. Biol. Chem. 285 (28), 21214-21218 (2010), each of which is incorporated by reference in its entirety.

In embodiments, a heterodimeric protein useful in the present invention comprises the extracellular domain of IL-6RA. IL-6RA (also known as Interleukin-6 receptor subunit alpha, IL-6 receptor subunit alpha, IL-6R subunit alpha, and IL-6R-alpha) is part of the receptor for interleukin 6. Binds to IL6 with low affinity, but does not transduce a signal. Signal activation necessitate an association with gp130. Activation may lead to the regulation of the immune response, acute-phase reactions and hematopoiesis. Low concentration of a soluble form of IL6 receptor acts as an agonist of IL6 activity. Dysregulated production of IL6 and this receptor are implicated in the pathogenesis of many diseases, such as multiple myeloma, autoimmune diseases and prostate cancer.

In embodiments, a heterodimeric protein useful in the present invention comprises a variant of the extracellular domain of IL-6RA. As examples, the variant may have 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 amino acid sequence of IL-6RA, *e.g.,* human IL-6RA.

In embodiments, the extracellular domain of IL-6RA has the following amino acid sequence:

LAPRRCPAQEVARGVLTSLPGDSVTLTCPGVEPEDNATVHWVLRKPAAGSHPSRWAGMGRRLLLRSV
QLHDSGNYSCYRAGRPAGTVHLLVDVPPEEPQLSCFRKSPLSNVVCEWGPRSTPSLTTKAVLLVRKFQ
NSPAEDFQEPCQYSQESQKFSCQLAVPEGDSSFYIVSMCVASSVGSKFSKTQTFQGCGILQPDPPANI
TVTAVARNPRWLSVTWQDPHSWNSSFYRLRFELRYRAERSKTFTTWMVKDLQHHCVIHDAWSGLRH
VVQLRAQEEFGQGEWSEWSPEAMGTPWTESRSPPAENEVSTPMQALTTNKDDDNILFRDSANATSLP
VQDSSSVPLP (SEQ ID NO: 19)

In embodiments, a heterodimeric protein comprises a variant of the extracellular domain of IL-6RA. As examples, the variant may have 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 SEQ ID NO: 19.

In embodiments, one chain of the heterodimeric protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 19.

One of ordinary skill may select variants of the known amino acid sequence of IL-6RA by consulting the literature, *e.g.*, Yamasaki et al., "Cloning and expression of the human interleukin-6 (BSF-2/IFN beta 2) receptor" Science 241 (4867), 825-828 (1988); Buk et al., "Increased association with detergent-resistant membranes/lipid rafts of apically targeted mutants of the interleukin-6 receptor gp80" Eur. J. Cell Biol. 84 (10), 819-831 (2005); Yawata et al., Structure-function analysis of human IL-6 receptor: dissociation of amino acid residues required for IL-6-binding and for IL-6 signal transduction through gp130" EMBO J. 12 (4), 1705-1712 (1993); Horiuchi et al., "Soluble interleukin-6 receptors released from T cell or granulocyte/macrophage cell lines and human peripheral blood mononuclear cells are generated through an alternative splicing mechanism" Eur. J. Immunol. 24 (8), 1945-1948 (1994); Boulanger et al., "Hexameric structure and assembly of the interleukin-6/IL-6 alpha-receptor/gp130 complex", Science 300 (5628), 2101-2104 (2003), each of which is incorporated by reference in its entirety.

In embodiments, a heterodimeric protein useful in the present invention comprises the extracellular domain of IL-12A. IL-12A (also known as Interleukin-12 subunit alpha and IL-12 subunit p35) is a cytokine that can act as a growth factor for activated T and NK cells, enhance the lytic activity of NK/lymphokine-activated killer cells, and stimulate the production of IFN-gamma by resting PBMC. The cytokine is a disulfide-linked heterodimer composed of the 35-kD subunit encoded by this gene, and a 40-kD subunit that is a member of the cytokine receptor family. This cytokine is required for the T-cell-dependent induction of interferon gamma (INF-γ), and is important for the differentiation of both Th1 and Th2 cells. The responses of lymphocytes to this cytokine are mediated by the activator of transcription protein STAT4.

In embodiments, a heterodimeric protein useful in the present invention comprises a variant of the extracellular domain of IL-12A. As examples, the variant may have 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 amino acid sequence of IL-12A, *e.g.,* human IL-12A.

In embodiments, the extracellular domain of IL-12A has the following amino acid sequence:

RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTK
NESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLA
VIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 20)

In embodiments, a heterodimeric protein comprises a variant of the extracellular domain of IL-12A. As examples, the variant may have 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 SEQ ID NO: 20.

In embodiments, one chain of the heterodimeric protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 20.

One of ordinary skill may select variants of the known amino acid sequence of IL-12A by consulting the literature, *e.g.*, Wolf et al., "Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells", J. Immunol. 146 (9), 3074-3081 (1991); Devergne et al., "Epstein-Barr virus-induced gene 3 and the p35 subunit of interleukin 12 form a novel heterodimeric hematopoietin", Proc. Natl. Acad. Sci. U.S.A. 94 (22), 12041-12046 (1997); Yoon et al., "Charged residues dominate a unique interlocking topography in the heterodimeric cytokine interleukin-12", EMBO J. 19 (14), 3530-3541 (2000), each of which is incorporated by reference in its entirety.

In embodiments, a heterodimeric protein useful in the present invention comprises the extracellular domain of IL-27B. IL-27b (also known as Interleukin-27 subunit beta, IL-27 subunit beta, and IL-27B), together with IL-12a forms Interleukin 35 (IL-35). IL-35 is a dimeric protein composed of IL-12α and IL-27β chains, which are encoded by two separate genes called IL12A and EBI3, respectively. IL-27 has pro- and anti-inflammatory properties, that can regulate T-helper cell development, suppress T-cell proliferation, stimulate cytotoxic T-cell activity, induce isotype switching in B-cells, and that has diverse effects on innate immune cells. Its gene was identified by its induced expression in B lymphocytes in response Epstein-Barr virus infection. IL-27 regulates T cell and inflammatory responses, in part by activating the Jak/STAT pathway of CD4+ T cells.

In embodiments, a heterodimeric protein useful in the present invention comprises a variant of the extracellular domain of IL-27B. As examples, the variant may have 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 amino acid sequence of IL-27B, *e.g.,* human IL-27B.

In embodiments, the extracellular domain of IL-27B has the following amino acid sequence:

RKGPPAALTLPRVQCRASRYPIAVDCSWTLPPAPNSTSPVSFIATYRLGMAARGHSWPCLQQTPTSTSC
TITDVQLFSMAPYVLNVTAVHPWGSSSSFVPFITEHIIKPDPPEGVRLSPLAERQLQVQWEPPGSWPFP
EIFSLKYWIRYKRQGAARFHRVGPIEATSFILRAVRPRARYYVQVAAQDLTDYGELSDWSLPATATMSLG
K (SEQ ID NO: 21)

In embodiments, a heterodimeric protein comprises a variant of the extracellular domain of IL-27B. As examples, the variant may have 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 SEQ ID NO: 21.

In embodiments, one chain of the heterodimeric protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 21.

One of ordinary skill may select variants of the known amino acid sequence of IL-27B by consulting the literature, *e.g.*, Devergne "Epstein-Barr virus-induced gene 3 and the p35 subunit of interleukin 12 form a novel heterodimeric hematopoietin", Proc. Natl. Acad. Sci. U.S.A. 94 (22), 12041-12046 (1997); Pflanz et al., "IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4+ T cells", Immunity 16 (6), 779-790 (2002); Batten and Ghilardi "The biology and therapeutic potential of interleukin 27", J. Mol. Med. 85 (7), 661-672 (2007), each of which is incorporated by reference in its entirety.

In embodiments, an alpha chain useful in a heterodimeric chimeric protein of the present invention comprises: (1) a first domain comprising the amino acid sequence of SEQ ID NO: 18, or a variant thereof, (b) a second domain comprises the amino acid sequence of SEQ ID NO: 20, or a variant thereof, and (c) an alpha core domain, or variant thereof, which comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 16 or SEQ ID NO: 24. Such an alpha chain may be referred to as "Gp130-Alpha-IL12A".

In embodiments, a Gp130-Alpha-IL12A chain used in the present invention and has the following amino acid sequence:

ELLDPCGYISPESPVVQLHSNFTAVCVLKEKCMDYFHVNANYIVWKTNHFTIPKEQYTIINRTASSVTFTDI
ASLNIQLTCNILTFGQLEQNVYGITIISGLPPEKPKNLSCIVNEGKKMRCEWDGGRETHLETNFTLKSEWA
THKFADCKAKRDTPTSCTVDYSTVYFVNIEVWVEAENALGKVTSDHINFDPVYKVKPNPPHNLSVINSE
ELSSILKLTWTNPSIKSVIILKYNIQYRTKDASTWSQIPPEDTASTRSSFTVQDLKPFTEYVFRIRCMKEDG

KGYWSDWSEEASGITYEDRPSKAPSFWYKIDPSHTQGYRTVQLVWKTLPPFEANGKILDYEVTLTRWK
SHLQNYTVNATKLTVNLTNDRYLATLTVRNLVGKSDAAVLTIPACDFQATHPVMDLKAFPKDNMLWVEWT
TPRESVKKYILEWCVLSDKAPCITDWQQEDGTVHRTYLRGNLAESKCYLITVTPVYADGPGSPESIKAYL
KQAPPSKGPTVRTKKVGKNEAVLEWDQLPVDVQNGFIRNYTIFYRTIIGNETAVNVDSSHTEYTLSSLTS
DTLYMVRMAAYTDEGGKDGPEFTFTTPKFAQGEIEGSGSRKGGKRGSKYGPPCPPCPAPEFLGGPSV
FLFPPKPKDQLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTV
LHQDWLSGKEYKCKVSSKGLPSSIEKTISNATGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLSL
GKDEGGEDGSGSRNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDK
TSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMD
PKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS*
(SEQ ID NO: 22)

In embodiments, an beta chain useful in a heterodimeric chimeric protein of the present invention comprises: (1) a first domain comprising the amino acid sequence of SEQ ID NO: 19, or a variant thereof, (b) a second domain comprises the amino acid sequence of SEQ ID NO: 21, or a variant thereof, and (c) an alpha core domain, or variant thereof, which comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 17 or SEQ ID NO: 25. Such an alpha chain may be referred to as "IL6RA-Beta-IL27B".In embodiments, an IL6RA-Beta-IL27B chain used in the present invention and has the following amino acid sequence:

LAPRRCPAQEVARGVLTSLPGDSVTLTCPGVEPEDNATVHWVLRKPAAGSHPSRWAGMGRRLLLRSV
QLHDSGNYSCYRAGRPAGTVHLLVDVPPEEPQLSCFRKSPLSNVVCEWGPRSTPSLTTKAVLLVRKFQ
NSPAEDFQEPCQYSQESQKFSCQLAVPEGDSSFYIVSMCVASSVGSKFSKTQTFQGCGILQPDPPANI
TVTAVARNPRWLSVTWQDPHSWNSSFYRLRFELRYRAERSKTFTTWMVKDLQHHCVIHDAWSGLRH
VVQLRAQEEFGQGEWSEWSPEAMGTPWTESRSPPAENEVSTPMQALTTNKDDDNILFRDSANATSLP
VQDSSSVPLPGSGSDEGGEDGSKYGPPCPPCPAPEFLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDV
SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIE
KTISNATGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSRLTVDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLSLGKRKGGKRGSGSRKGPPAALTLP
RVQCRASRYPIAVDCSWTLPPAPNSTSPVSFIATYRLGMAARGHSWPCLQQTPTSTSCTITDVQLFSM
APYVLNVTAVHPWGSSSSFVPFITEHIIKPDPPEGVRLSPLAERQLQVQWEPPGSWPFPEIFSLKYWIRY
KRQGAARFHRVGPIEATSFILRAVRPRARYYVQVAAQDLTDYGELSDWSLPATATMSLGK* (SEQ ID
NO: 23)

When a Gp130-Alpha-IL12A chain and an IL6RA-Beta-IL27B chain are combined (within a cell or *in vitro*), they form a heterodimeric protein referred to herein as IL-6R-Fc-IL-35.

In embodiments, a heterodimeric protein useful in the present invention comprises the extracellular domain of IL-21r. Interleukin-21 receptor (also known as IL-21 receptor and IL-21R) is a receptor for interleukin-21 belongs to the type I cytokine receptors, and has been shown to form a heterodimeric receptor complex with the common gamma-chain, a receptor subunit also shared by the receptors for interleukin 2, 4, 7, 9, and 15. This receptor transduces the growth-promoting signal of IL21, and is important for the proliferation and differentiation of T cells, B cells, and natural killer (NK) cells. The ligand binding of this receptor leads to the activation of multiple downstream signaling molecules, including JAK1, JAK3, STAT1, and STAT3. Knockout studies of a similar gene in mouse suggest a role for this gene in regulating immunoglobulin production.

In embodiments, a heterodimeric protein useful in the present invention comprises a variant of the extracellular domain of IL-21r. As examples, the variant may have 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 amino acid sequence of IL-21r, *e.g.,* human IL-21r.

In embodiments, the extracellular domain of IL-21r has the following amino acid sequence:

> CPDLVCYTDYLQTVICILEMWNLHPSTLTLTWQDQYEELKDEATSCSLHRSAHNATHATYTCHMDVFHF MADDIFSVNITDQSGNYSQECGSFLLAESIKPAPPFNVTVTFSGQYNISWRSDYEDPAFYMLKGKLQYE LQYRNRGDPWAVSPRRKLISVDSRSVSLLPLEFRKDSSYELQVRAGPMPGSSYQGTWSEWSDPVIFQ TQSEELKE (SEQ ID NO: 26)

In embodiments, a heterodimeric protein comprises a variant of the extracellular domain of IL-21r. As examples, the variant may have 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 SEQ ID NO: 26.

In embodiments, one chain of the heterodimeric protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 26.

One of ordinary skill may select variants of the known amino acid sequence of IL-21r by consulting the literature, *e.g.*, Ozaki et al., "Cloning of a type I cytokine receptor most related to the IL-2 receptor beta chain", Proc. Natl. Acad. Sci. U.S.A. 97 (21), 11439-11444 (2000); Kotlarz et al., "Loss-of-function mutations in the IL-21 receptor gene cause a primary immunodeficiency syndrome" J. Exp. Med. 210 (3), 433-443 (2013); Hamming et al., "Crystal structure of interleukin-21 receptor (IL-21R) bound to IL-21 reveals that sugar chain interacting with WSXWS motif is integral part of IL-21R" J. Biol. Chem. 287 (12), 9454-9460 (2012), each of which is incorporated by reference in its entirety.

In embodiments, a heterodimeric protein useful in the present invention comprises the extracellular domain of IL2RG. Interleukin-2 receptor subunit gamma (also known as Cytokine receptor common subunit gamma, IL-2 receptor subunit gamma, IL-2R subunit gamma, and IL-2RG) is a common subunit for the receptors for a variety

of interleukins, including those of interleukin -2, -4, -7 and -21, and is thus referred to as the common gamma chain. Mutations in this gene cause X-linked severe combined immunodeficiency (XSCID), as well as X-linked combined immunodeficiency (XCID), a less severe immunodeficiency disorder.

In embodiments, a heterodimeric protein useful in the present invention comprises a variant of the extracellular domain of IL2RG. As examples, the variant may have 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 amino acid sequence of IL2RG, *e.g.,* human IL2RG.

In embodiments, the extracellular domain of IL2RG has the following amino acid sequence:

LNTTILTPNGNEDTTADFFLTTMPTDSLSVSTLPLPEVQCFVFNVEYMNCTWNSSSEPQPTNLTLHYWY
KNSDNDKVQKCSHYLFSEEITSGCQLQKKEIHLYQTFVVQLQDPREPRRQATQMLKLQNLVIPWAPENL
TLHKLSESQLELNWNNRFLNHCLEHLVQYRTDWDHSWTEQSVDYRHKFSLPSVDGQKRYTFRVRSRF
NPLCGSAQHWSEWSHPIHWGSNTSKENPFLFALEA (SEQ ID NO: 27)

In embodiments, a heterodimeric protein comprises a variant of the extracellular domain of IL2RG. As examples, the variant may have 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 SEQ ID NO: 27.

In embodiments, one chain of the heterodimeric protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 27.

One of ordinary skill may select variants of the known amino acid sequence of IL2RG by consulting the literature, *e.g.*, Takeshita et al., "Cloning of the gamma chain of the human IL-2 receptor", Science 257 (5068), 379-382 (1992); Ratthe et al., "Interleukin-15 enhances human neutrophil phagocytosis by a Syk-dependent mechanism: importance of the IL-15Ralpha chain", J. Leukoc. Biol. 76 (1), 162-168 (2004); Bamborough et al., "The interleukin-2 and interleukin-4 receptors studied by molecular modelling", Structure 2 (9), 839-851 (1994);

Wang et al., "Structure of the quaternary complex of interleukin-2 with its alpha, beta, and gammac receptors" Science 310 (5751), 1159-1163 (2005); Stauber et al., "Crystal structure of the IL-2 signaling complex: paradigm for a heterotrimeric cytokine receptor", Proc. Natl. Acad. Sci. U.S.A. 103 (8), 2788-2793 (2006), each of which is incorporated by reference in its entirety.

In embodiments, an alpha chain useful in a heterodimeric chimeric protein of the present invention comprises: (1) a first domain comprising the amino acid sequence of SEQ ID NO: 26, or a variant thereof, (b) a second domain comprises the amino acid sequence of SEQ ID NO: 20, or a variant thereof, and (c) an alpha core domain, or variant thereof, which comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 16 or SEQ ID NO: 24. Such an alpha chain may be referred to as "IL21r-Alpha-IL12a".

In embodiments, an IL21r-Alpha-IL12a chain used in the present invention and has the following amino acid sequence:

CPDLVCYTDYLQTVICILEMWNLHPSTLTLTWQDQYEELKDEATSCSLHRSAHNATHATYTCHMDVFHF
MADDIFSVNITDQSGNYSQECGSFLLAESIKPAPPFNVTVTFSGQYNISWRSDYEDPAFYMLKGKLQYE
LQYRNRGDPWAVSPRRKLISVDSRSVSLLPLEFRKDSSYELQVRAGPMPGSSYQGTWSEWSDPVIFQ
TQSEELKEGSGSRKGGKRGSKYGPPCPPCPAPEFLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIEKTI
SNATGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSRLTVDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLSLGKDEGGEDGSGSRNLPVATPDPGMFP
CLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITN
GSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSET
VPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 28)

In embodiments, an beta chain useful in a heterodimeric chimeric protein of the present invention comprises: (1) a first domain comprising the amino acid sequence of SEQ ID NO: 27, or a variant thereof, (b) a second domain comprises the amino acid sequence of SEQ ID NO: 21, or a variant thereof, and (c) an alpha core domain, or variant thereof, which comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 17 or SEQ ID NO: 25. Such a beta chain may be referred to as "IL2rg-Beta-IL27B".

In embodiments, an IL2rg-Beta-IL27B chain used in the present invention and has the following amino acid sequence:

LNTTILTPNGNEDTTADFFLTTMPTDSLSVSTLPLPEVQCFVFNVEYMNCTWNSSSEPQPTNLTLHYWY
KNSDNDKVQKCSHYLFSEEITSGCQLQKKEIHLYQTFVVQLQDPREPRRQATQMLKLQNLVIPWAPENL
TLHKLSESQLELNWNNRFLNHCLEHLVQYRTDWDHSWTEQSVDYRHKFSLPSVDGQKRYTFRVRSRF
NPLCGSAQHWSEWSHPIHWGSNTSKENPFLFALEAGSGSDEGGEDGSKYGPPCPPCPAPEFLGGPS
VFLFPPKPKDQLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLT
VLHQDWLSGKEYKCKVSSKGLPSSIEKTISNATGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVLHEALHNHYTQKSLSL
SLGKRKGGKRGSGSRKGPPAALTLPRVQCRASRYPIAVDCSWTLPPAPNSTSPVSFIATYRLGMAARG
HSWPCLQQTPTSTSCTITDVQLFSMAPYVLNVTAVHPWGSSSSFVPFITEHIIKPDPPEGVRLSPLAERQ
LQVQWEPPGSWPFPEIFSLKYWIRYKRQGAARFHRVGPIEATSFILRAVRPRARYYVQVAAQDLTDYG
ELSDWSLPATATMSLGK (SEQ ID NO: 29)

When an IL21r-Alpha-IL12a chain and an IL2rg-Beta-IL27B chain are combined (within a cell or *in vitro*), they form a heterodimeric protein referred to herein as IL-21R-Fc-IL-35.

In embodiments, an alpha chain useful in a heterodimeric chimeric protein of the present invention comprises: (1) a first domain comprising the amino acid sequence of SEQ ID NO: 26, or a variant thereof, (b) a second domain comprises the amino acid sequence of SEQ ID NO: 20, or a variant thereof, and (c) an alpha core domain, or variant thereof, which comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 17 or SEQ ID NO: 25. Such an alpha chain may be referred to as "IL21r-Beta-IL12a".

In embodiments, an IL21r-Beta-IL12a chain used in the present invention and has the following amino acid sequence:

CPDLVCYTDYLQTVICILEMWNLHPSTLTLTWQDQYEELKDEATSCSLHRSAHNATHATYTCHMDVFHF
MADDIFSVNITDQSGNYSQECGSFLLAESIKPAPPFNVTVTFSGQYNISWRSDYEDPAFYMLKGKLQYE
LQYRNRGDPWAVSPRRKLISVDSRSVSLLPLEFRKDSSYELQVRAGPMPGSSYQGTWSEWSDPVIFQ
TQSEELKEGSGSDEGGEDGSKYGPPCPPCPAPEFLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIEKTI
SNATGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSRLTVDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLSLGKRKGGKRGSGSRNLPVATPDPGMF
PCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFIT
NGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSE
TVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 37)

In embodiments, an beta chain useful in a heterodimeric chimeric protein of the present invention comprises: (1) a first domain comprising the amino acid sequence of SEQ ID NO: 27, or a variant thereof, (b) a second domain comprises the amino acid sequence of SEQ ID NO: 21, or a variant thereof, and (c) an alpha core domain, or variant thereof, which comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 16 or SEQ ID NO: 24. Such a beta chain may be referred to as "IL2rg-Alpha-IL27B".

In embodiments, an IL2rg-Alpha-IL27B chain used in the present invention and has the following amino acid sequence:

LNTTILTPNGNEDTTADFFLTTMPTDSLSVSTLPLPEVQCFVFNVEYMNCTWNSSSEPQPTNLTLHYWY
KNSDNDKVQKCSHYLFSEEITSGCQLQKKEIHLYQTFVVQLQDPREPRRQATQMLKLQNLVIPWAPENL
TLHKLSESQLELNWNNRFLNHCLEHLVQYRTDWDHSWTEQSVDYRHKFSLPSVDGQKRYTFRVRSRF
NPLCGSAQHWSEWSHPIHWGSNTSKENPFLFALEAGSGSRKGGKRGSKYGPPCPPCPAPEFLGGPS
VFLFPPKPKDQLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLT
VLHQDWLSGKEYKCKVSSKGLPSSIEKTISNATGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLS
LGKDEGGEDGSGSRKGPPAALTLPRVQCRASRYPIAVDCSWTLPPAPNSTSPVSFIATYRLGMAARGH
SWPCLQQTPTSTSCTITDVQLFSMAPYVLNVTAVHPWGSSSSFVPFITEHIIKPDPPEGVRLSPLAERQL
QVQWEPPGSWPFPEIFSLKYWIRYKRQGAARFHRVGPIEATSFILRAVRPRARYYVQVAAQDLTDYGE
LSDWSLPATATMSLGK (SEQ ID NO: 36)

When an IL21r-Beta-IL12a chain and an IL2rg-Alpha-IL27B chain are combined (within a cell or *in vitro*), they form a heterodimeric protein may also be referred to herein as IL-21R-Fc-IL-35.

In embodiments, a heterodimeric protein useful in the present invention comprises the extracellular domain of IFNgR. IFNgR (also known as Interferon gamma receptor 1, IFN-gamma receptor 1, IFN-gamma-R1, IFN-gamma-R-alpha, IFNγR, and IFNGR1) associates with IFNGR2 to form a receptor for the cytokine interferon gamma (IFNG). Ligand binding stimulates activation of the JAK/STAT signaling pathway. It plays an essential role in the

IFN-gamma pathway that is required for the cellular response to infectious agents. A genetic variation in IFNGR1 is associated with susceptibility to Helicobacter pylori infection. In addition, defects in IFNGR1 are a cause of Mendelian susceptibility to mycobacterial disease, also known as familial disseminated atypical mycobacterial infection.

In embodiments, a heterodimeric protein useful in the present invention comprises a variant of the extracellular domain of IFNgR. As examples, the variant may have 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 amino acid sequence of IFNgR, *e.g.,* human IFNgR.

In embodiments, the extracellular domain of IFNgR has the following amino acid sequence:

EMGTADLGPSSVPTPTNVTIESYNMNPIVYWEYQIMPQVPVFTVEVKNYGVKNSEWIDACINISHHYCNI
SDHVGDPSNSLWVRVKARVGQKESAYAKSEEFAVCRDGKIGPPKLDIRKEEKQIMIDIFHPSVFVNGDE
QEVDYDPETTCYIRVYNVYVRMNGSEIQYKILTQKEDDCDEIQCQLAIPVSSLNSQYCVSAEGVLHVWG
VTTEKSKEVCITIFNSSIKG (SEQ ID NO: 30)

In embodiments, a heterodimeric protein comprises a variant of the extracellular domain of IFNgR. As examples, the variant may have 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 SEQ ID NO: 30.

In embodiments, one chain of the heterodimeric protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 30.

One of ordinary skill may select variants of the known amino acid sequence of IFNgR by consulting the literature, *e.g.*, Aguet et al., "Molecular cloning and expression of the human interferon-gamma receptor" Cell 55 (2), 273-280 (1988); Stuber et al., "Alignment of disulfide bonds of the extracellular domain of the interferon gamma receptor and investigation of their role in biological activity", Biochemistry 32 (9), 2423-2430 (1993);

Sakatsume et al., "The Jak kinases differentially associate with the alpha and beta (accessory factor) chains of the interferon gamma receptor to form a functional receptor unit capable of activating STAT transcription factors", J. Biol. Chem. 270 (29), 17528-17534 (1995); Walter et al., "Crystal structure of a complex between interferon-gamma and its soluble high-affinity receptor", Nature 376 (6537), 230-235 (1995); Sogabe et al., "Neutralizing epitopes on the extracellular interferon gamma receptor (IFNgammaR) alpha-chain characterized by homolog scanning mutagenesis and X-ray crystal structure of the A6 fab-IFNgammaR1-108 complex", J. Mol. Biol. 273 (4), 882-897 (1997); Thiel et al., "Observation of an unexpected third receptor molecule in the crystal structure of human interferon-gamma receptor complex", Structure 8 (9), 927-936 (2000); van de Wetering et al., "Functional analysis of naturally occurring amino acid substitutions in human IFN-gammaR1." Mol. Immunol. 47:1023-1030(2010), each of which is incorporated by reference in its entirety.

In embodiments, a heterodimeric protein useful in the present invention comprises the extracellular domain of IFNGR2. IFNGR2 (also known as Interferon gamma receptor 2, also known as IFN-gamma receptor 2, and IFN-gamma-R2) is the non-ligand-binding beta chain of the gamma interferon receptor. Human interferon-gamma receptor is a heterodimer of IFNGR1 and IFNGR2. Ligand binding stimulates activation of the JAK/STAT signaling pathway. IFNGR2 is required for signal transduction in contrast to other receptor subunit responsible for ligand binding. Defects in IFNGR2 are a cause of Mendelian susceptibility to mycobacterial disease (MSMD), also known as familial disseminated atypical mycobacterial infection. MSMD is a genetically heterogeneous disease with autosomal recessive, autosomal dominant or X-linked inheritance.

In embodiments, a heterodimeric protein useful in the present invention comprises a variant of the extracellular domain of IFNGR2. As examples, the variant may have 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 amino acid sequence of IFNGR2, *e.g.,* human IFNGR2.

In embodiments, the extracellular domain of IFNGR2 has the following amino acid sequence:

SQLPAPQHPKIRLYNAEQVLSWEPVALSNSTRPVVYQVQFKYTDSKWFTADIMSIGVNCTQITATECDF
TAASPSAGFPMDFNVTLRLRAELGALHSAWVTMPWFQHYRNVTVGPPENIEVTPGEGSLIIRFSSPFDI
ADTSTAFFCYYVHYWEKGGIQQVKGPFRSNSISLDNLKPSRVYCLQVQAQLLWNKSNIFRVGHLSNISC
YETMADASTELQQ (SEQ ID NO: 31)

In embodiments, a heterodimeric protein comprises a variant of the extracellular domain of IFNGR2. As examples, the variant may have 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 SEQ ID NO: 31.

In embodiments, one chain of the heterodimeric protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 31.

One of ordinary skill may select variants of the known amino acid sequence of IFNGR2 by consulting the literature, *e.g.*, Soh et al., "Identification and sequence of an accessory factor required for activation of the human interferon gamma receptor", Cell 76 (5), 793-802 (1994); Sakatsume et al., "The Jak kinases differentially associate with the alpha and beta (accessory factor) chains of the interferon gamma receptor to form a functional receptor unit capable of activating STAT transcription factors", J. Biol. Chem. 270 (29), 17528-17534 (1995); Rosenzweig et al., "Characterization of a dipeptide motif regulating IFN-gamma receptor 2 plasma membrane accumulation and IFN-gamma responsiveness", J. Immunol. 173 (6), 3991-3999 (2004); Mikulecky et al., "Crystal structure of human interferon-gamma receptor 2 reveals the structural basis for receptor specificity", Acta Crystallogr. D 75, 1017-1024 (2016); Kotenko et al., "Interaction between the components of the interferon gamma receptor complex." J. Biol. Chem. 270:20915-20921(1995), each of which is incorporated by reference in its entirety.

In embodiments, an alpha chain useful in a heterodimeric chimeric protein of the present invention comprises: (1) a first domain comprising the amino acid sequence of SEQ ID NO: 30, or a variant thereof, (b) a second domain comprises the amino acid sequence of SEQ ID NO: 20, or a variant thereof, and (c) an alpha core domain, or variant thereof, which comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 16 or SEQ ID NO: 24. Such an alpha chain may be referred to as "IFNgR-Alpha-IL12a".

In embodiments, an IFNgR-Alpha-IL12a chain used in the present invention and has the following amino acid sequence:

EMGTADLGPSSVPTPTNVTIESYNMNPIVYWEYQIMPQVPVFTVEVKNYGVKNSEWIDACINISHHYCNI
SDHVGDPSNSLWVRVKARVGQKESAYAKSEEFAVCRDGKIGPPKLDIRKEEKQIMIDIFHPSVFVNGDE
QEVDYDPETTCYIRVYNVYVRMNGSEIQYKILTQKEDDCDEIQCQLAIPVSSLNSQYCVSAEGVLHVWG
VTTEKSKEVCITIFNSSIKGGSGSRKGGKRGSKYGPPCPPCPAPEFLGGPSVFLFPPKPKDQLMISRTPE
VTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSS
KGLPSSIEKTISNATGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLSLGKDEGGEDGSGSRNLPV
ATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCL
NSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELM
QALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 32)

In embodiments, an beta chain useful in a heterodimeric chimeric protein of the present invention comprises: (1) a first domain comprising the amino acid sequence of SEQ ID NO: 31, or a variant thereof, (b) a second domain comprises the amino acid sequence of SEQ ID NO: 21, or a variant thereof, and (c) an alpha core domain, or variant thereof, which comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 17 or SEQ ID NO: 25. Such a beta chain may be referred to as "IFNGR2-Beta-IL27B".

In embodiments, an IFNGR2-Beta-IL27B chain used in the present invention and has the following amino acid sequence:

SQLPAPQHPKIRLYNAEQVLSWEPVALSNSTRPVVYQVQFKYTDSKWFTADIMSIGVNCTQITATECDF
TAASPSAGFPMDFNVTLRLRAELGALHSAWVTMPWFQHYRNVTVGPPENIEVTPGEGSLIIRFSSPFDI
ADTSTAFFCYYVHYWEKGGIQQVKGPFRSNSISLDNLKPSRVYCLQVQAQLLWNKSNIFRVGHLSNISC
YETMADASTELQQGSGSDEGGEDGSKYGPPCPPCPAPEFLGGPSVFLFPPKPKDQLMISRTPEVTCV
VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLP
SSIEKTISNATGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSRLTVDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLSLGKRKGGKRGSGSRKGPPAAL
TLPRVQCRASRYPIAVDCSWTLPPAPNSTSPVSFIATYRLGMAARGHSWPCLQQTPTSTSCTITDVQLF
SMAPYVLNVTAVHPWGSSSSFVPFITEHIIKPDPPEGVRLSPLAERQLQVQWEPPGSWPFPEIFSLKYW
IRYKRQGAARFHRVGPIEATSFILRAVRPRARYYVQVAAQDLTDYGELSDWSLPATATMSLGK (SEQ ID NO: 33)

When an IFNgR-Alpha-IL12a chain and an IFNGR2-Beta-IL27B chain are combined (within a cell or *in vitro*), they form a heterodimeric protein referred to herein as IFNγR-Fc-IL-35.

In embodiments, an alpha chain useful in a heterodimeric chimeric protein of the present invention comprises: (1) a first domain comprising the amino acid sequence of SEQ ID NO: 30, or a variant thereof, (b) a second domain comprises the amino acid sequence of SEQ ID NO: 20, or a variant thereof, and (c) an alpha core domain, or variant thereof, which comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 17 or SEQ ID NO: 25. Such an alpha chain may be referred to as "IFNgR-Beta-IL12a".

In embodiments, an IFNgR-Beta-IL12a chain used in the present invention and has the following amino acid sequence:

EMGTADLGPSSVPTPTNVTIESYNMNPIVYWEYQIMPQVPVFTVEVKNYGVKNSEWIDACINISHHYCNI
SDHVGDPSNSLWVRVKARVGQKESAYAKSEEFAVCRDGKIGPPKLDIRKEEKQIMIDIFHPSVFVNGDE
QEVDYDPETTCYIRVYNVYVRMNGSEIQYKILTQKEDDCDEIQCQLAIPVSSLNSQYCVSAEGVLHVWG
VTTEKSKEVCITIFNSSIKGGSGSDEGGEDGSKYGPPCPPCPAPEFLGGPSVFLFPPKPKDQLMISRTP
EVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVS
SKGLPSSIEKTISNATGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLSLGKRKGGKRGSGSRNL
PVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNES
CLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDE
LMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 38)

In embodiments, an beta chain useful in a heterodimeric chimeric protein of the present invention comprises: (1) a first domain comprising the amino acid sequence of SEQ ID NO: 31, or a variant thereof, (b) a second domain comprises the amino acid sequence of SEQ ID NO: 21, or a variant thereof, and (c) an alpha core domain, or

variant thereof, which comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 16 or SEQ ID NO: 24. Such a beta chain may be referred to as "IFNGR2-Alpha-IL27B".

In embodiments, an IFNGR2-Alpha-IL27B chain used in the present invention and has the following amino acid sequence:

SQLPAPQHPKIRLYNAEQVLSWEPVALSNSTRPVVYQVQFKYTDSKWFTADIMSIGVNCTQITATECDF
TAASPSAGFPMDFNVTLRLRAELGALHSAWVTMPWFQHYRNVTVGPPENIEVTPGEGSLIIRFSSPFDI
ADTSTAFFCYYVHYWEKGGIQQVKGPFRSNSISLDNLKPSRVYCLQVQAQLLWNKSNIFRVGHLSNISC
YETMADASTELQQGSGSRKGGKRGSKYGPPCPPCPAPEFLGGPSVFLFPPKPKDQLMISRTPEVTCVV
VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPS
SIEKTISNATGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSRLTVDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLSLGKDEGGEDGSGSRKGPPAALTL
PRVQCRASRYPIAVDCSWTLPPAPNSTSPVSFIATYRLGMAARGHSWPCLQQTPTSTSCTITDVQLFS
MAPYVLNVTAVHPWGSSSSFVPFITEHIIKPDPPEGVRLSPLAERQLQVQWEPPGSWPFPEIFSLKYWI
RYKRQGAARFHRVGPIEATSFILRAVRPRARYYVQVAAQDLTDYGELSDWSLPATATMSLGK (SEQ ID NO: 39)

When an IFNgR-Beta-IL12a chain and an IFNGR2-Alpha-IL27B chain are combined (within a cell or *in vitro*), they form a heterodimeric protein may also be referred to herein as IFNγR-Fc-IL-35.

One embodiment of the IL-6R-Fc-IL-35 heterodimeric protein is disclosed above, *i.e.,* comprising a Gp130-Alpha-IL12A chain and an IL6RA-Beta-IL27B chain. In alternate embodiment, an IL-6R-Fc-IL-35 heterodimeric protein can comprise an IL6RA-Alpha-IL12a chain and a Gp130-Beta-IL27b.

In embodiments, an alpha chain useful in a heterodimeric chimeric protein of the present invention comprises: (1) a first domain comprising the amino acid sequence of SEQ ID NO: 19, or a variant thereof, (b) a second domain comprises the amino acid sequence of SEQ ID NO: 20, or a variant thereof, and (c) an alpha core domain, or variant thereof, which comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 16 or SEQ ID NO: 24. Such an alpha chain may be referred to as "IL6RA-Alpha-IL12a".

In embodiments, an IL6RA-Alpha-IL12a chain used in the present invention and has the following amino acid sequence:

LAPRRCPAQEVARGVLTSLPGDSVTLTCPGVEPEDNATVHWVLRKPAAGSHPSRWAGMGRRLLLRSV
QLHDSGNYSCYRAGRPAGTVHLLVDVPPEEPQLSCFRKSPLSNVVCEWGPRSTPSLTTKAVLLVRKFQ
NSPAEDFQEPCQYSQESQKFSCQLAVPEGDSSFYIVSMCVASSVGSKFSKTQTFQGCGILQPDPPANIT
VTAVARNPRWLSVTWQDPHSWNSSFYRLRFELRYRAERSKTFTTWMVKDLQHHCVIHDAWSGLRHVV
QLRAQEEFGQGEWSEWSPEAMGTPWTESRSPPAENEVSTPMQALTTNKDDDNILFRDSANATSLPVQ
DSSSVPLPGSGSRKGGKRGSKYGPPCPPCPAPEFLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIEKTI
SNATGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSRLTVDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLSLGKDEGGEDGSGSRNLPVATPDPGMFP
CLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITN
GSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSET
VPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 34)

In embodiments, an beta chain useful in a heterodimeric chimeric protein of the present invention comprises: (1) a first domain comprising the amino acid sequence of SEQ ID NO: 18, or a variant thereof, (b) a second domain

comprises the amino acid sequence of SEQ ID NO: 21, or a variant thereof, and (c) an alpha core domain, or variant thereof, which comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 17 or SEQ ID NO: 25. Such a beta chain may be referred to as "Gp130-Beta-IL27b".

In embodiments, a Gp130-Beta-IL27b chain used in the present invention and has the following amino acid sequence:

ELLDPCGYISPESPVVQLHSNFTAVCVLKEKCMDYFHVNANYIVWKTNHFTIPKEQYTIINRTASSVTFTD
IASLNIQLTCNILTFGQLEQNVYGITIISGLPPEKPKNLSCIVNEGKKMRCEWDGGRETHLETNFTLKSEW
ATHKFADCKAKRDTPTSCTVDYSTVYFVNIEVWVEAENALGKVTSDHINFDPVYKVKPNPPHNLSVINS
EELSSILKLTWTNPSIKSVIILKYNIQYRTKDASTWSQIPPEDTASTRSSFTVQDLKPFTEYVFRIRCMKED
GKGYWSDWSEEASGITYEDRPSKAPSFWYKIDPSHTQGYRTVQLVWKTLPPFEANGKILDYEVTLTRW
KSHLQNYTVNATKLTVNLTNDRYLATLTVRNLVGKSDAAVLTIPACDFQATHPVMDLKAFPKDNMLWVE
WTTPRESVKKYILEWCVLSDKAPCITDWQQEDGTVHRTYLRGNLAESKCYLITVTPVYADGPGSPESIK
AYLKQAPPSKGPTVRTKKVGKNEAVLEWDQLPVDVQNGFIRNYTIFYRTIIGNETAVNVDSSHTEYTLSS
LTSDTLYMVRMAAYTDEGGKDGPEFTFTTPKFAQGEIE (SEQ ID NO: 35)

When an IL6RA-Alpha-IL12a chain and a Gp130-Beta-IL27b chain are combined (within a cell or *in vitro*), they form a heterodimeric protein may also be referred to herein as IL-6R-Fc-IL-35.

In various embodiments, the present heterodimeric protein may comprise variants of any of the known cytokines, growth factors, and/or hormones. In various embodiments, the present heterodimeric proteins may comprise variants of any of the known receptors for cytokines, growth factors, and/or hormones. In various embodiments, the present heterodimeric proteins may comprises variants of any of the known extracellular domains, for instance, a sequence having 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 amino acid or nucleic acid sequences.

In various embodiments, the present heterodimeric protein may comprise an amino acid sequence having one or more amino acid mutations relative to any of the known protein sequences. 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).

Mutations may also be made to the nucleotide sequences of the heterodimeric proteins by reference to the genetic code, including taking into account codon degeneracy.

In various embodiments, the present heterodimeric proteins are capable of, and can be used in methods comprising, promoting immune activation (*e.g.,* against tumors). In various embodiments, the present heterodimeric proteins are capable of, and can be used in methods comprising, suppressing immune inhibition (*e.g.,* that allows tumors to survive). In various embodiments, the present heterodimeric protein provides improved immune activation and/or improved suppression of immune inhibition.

In various embodiments, the present heterodimeric proteins are capable of, or can be used in methods comprising, modulating the amplitude of an immune response, *e.g.,* modulating the level of effector output. In some embodiments, *e.g.,* when used for the treatment of cancer, the present heterodimeric protein alters the extent of immune stimulation as compared to immune inhibition to increase the amplitude of a T cell response, including, without limitation, stimulating increased levels of cytokine production, proliferation or target killing potential.

In various embodiments, the present heterodimeric proteins, in some embodiments are capable of, or find use in methods involving, masking an inhibitory ligand on the surface of a tumor cell and replacing that immune inhibitory ligand with an immune stimulatory ligand. Accordingly, the present heterodimeric proteins, in some embodiments are capable of, or find use in methods involving, reducing or eliminating an inhibitory immune signal and/or increasing or activating an immune stimulatory signal. For example, a tumor cell bearing an inhibitory signal (and thus evading an immune response) may be substituted for a positive signal binding on a T cell that can then attack

a tumor cell. Accordingly, in some embodiments, an inhibitory immune signal is masked by the present heterodimeric proteins and a stimulatory immune signal is activated. Such beneficial properties are enhanced by the single construct approach of the present heterodimeric proteins. For instance, the signal replacement can be effected nearly simultaneously and the signal replacement is tailored to be local at a site of clinical importance (*e.g.,* the tumor microenvironment).

In various embodiments, the present heterodimeric proteins are capable of, or find use in methods comprising, stimulating or enhancing the binding of immune stimulatory receptor/ligand pairs.

In other embodiments, the present heterodimeric proteins are capable of, or find use in methods involving, enhancing, restoring, promoting and/or stimulating immune modulation. In some embodiments, the present heterodimeric proteins described herein, restore, promote and/or stimulate the activity or activation of one or more immune cells against tumor cells including, but not limited to: 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, and dendritic cells. In some embodiments, the present heterodimeric proteins enhance, restore, promote and/or stimulate the activity and/or activation of T cells, including, by way of a non-limiting example, activating and/or stimulating one or more T- cell intrinsic signals, including a pro-survival signal; an autocrine or paracrine growth signal; a p38 MAPK-, ERK-, STAT-, JAK-, AKT- or PI3K-mediated signal; an anti-apoptotic signal; and/or a signal promoting and/or necessary for one or more of: proinflammatory cytokine production or T cell migration or T cell tumor infiltration.

In some embodiments, the present heterodimeric proteins are capable of, or find use in methods involving, causing an increase of one or more of T cells (including without limitation cytotoxic T lymphocytes, T helper cells, natural killer T (NKT) cells), B cells, natural killer (NK) cells, natural killer T (NKT) cells, dendritic cells, monocytes, and macrophages (*e.g.,* one or more of M1 and M2) into a tumor or the tumor microenvironment. In some embodiments, the present heterodimeric proteins are capable of, or find use in methods involving, inhibiting and/or causing a decrease in recruitment of immunosuppressive cells (*e.g.,* myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), tumor associated neutrophils (TANs), M2 macrophages, and tumor associated macrophages (TAMs)) to the tumor and/or tumor microenvironment (TME). In some embodiments, the present therapies may alter the ratio of M1 versus M2 macrophages in the tumor site and/or TME to favor M1 macrophages.

In various embodiments, the present heterodimeric proteins are capable of, and can be used in methods comprising, inhibiting and/or reducing T cell inactivation and/or immune tolerance to a tumor, comprising administering an effective amount of a heterodimeric protein described herein to a subject. In some embodiments, the present heterodimeric proteins are able to increase the serum levels of various cytokines including, but not limited to, one or more of IFNγ, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, and IL-22. In some embodiments, the present heterodimeric proteins are capable of enhancing IL-2, IL-4, IL-5, IL-10, IL-13, IL-17A, IL-22, or IFNγ in the serum of a treated subject.

In various embodiments, the present heterodimeric proteins inhibit, block and/or reduce cell death of an anti-tumor CD8+ and/or CD4+ T cell; or stimulate, induce, and/or increase cell death of a pro-tumor T cell. T cell exhaustion is a state of T cell dysfunction characterized by progressive loss of proliferative and effector functions, culminating in clonal deletion. Accordingly, a pro-tumor T cell refers to a state of T cell dysfunction that arises during many chronic infections and cancer. This dysfunction is defined by poor proliferative and/or effector functions, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T cells. Exhaustion prevents optimal control of infection and tumors. In addition, an anti-tumor CD8+ and/or CD4+ T cell refers to T cells that can mount an immune response to a tumor. Illustrative pro-tumor T cells include, but are not limited to, Tregs, CD4+ and/or CD8+ T cells expressing one or more checkpoint inhibitory receptors, Th2 cells and Th17 cells. Checkpoint inhibitory receptors refers to receptors (*e.g.,* CTLA-4, B7-H3, B7-H4, TIM-3) expressed on immune cells that prevent or inhibit uncontrolled immune responses.

In various embodiments, the present heterodimeric proteins are capable of, and can be used in methods comprising, increasing a ratio of effector T cells to regulatory T cells. Illustrative effector T cells include $ICOS^+$ effector T cells; 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, $CD4^+CD45RO^+CCR7(^-)$ effector T cells; and effector T cells secreting IL-2, IL-4 and/or IFN-γ. Illustrative regulatory T cells include $ICOS^+$ regulatory T cells, $CD4^+CD25^+FOXP3^+$ regulatory T cells, $CD4^+CD25^+$ regulatory T cells, $CD4^+CD25^-$ regulatory T cells, $CD4^+$CD25high regulatory T cells, $TIM\text{-}3^+PD\text{-}1^+$ regulatory T cells, lymphocyte activation gene-3 (LAG-3)$^+$ regulatory T cells, CTLA-4/$CD152^+$ regulatory T cells, neuropilin-1 (Nrp-1)$^+$ regulatory T cells, $CCR4^+CCR8^+$ regulatory T cells, CD62L (L-selectin)$^+$ regulatory T cells, CD45RBlow regulatory T cells, CD127low regulatory T cells, LRRC32/$GARP^+$ regulatory T cells, $CD39^+$ regulatory T cells, $GITR^+$ regulatory T cells, LAP^+ regulatory T cells, $1B11^+$ regulatory T cells, $BTLA^+$ regulatory T cells, type 1 regulatory T cells (Tr1 cells),T helper type 3 (Th3) cells, regulatory cell of natural killer T cell phenotype (NKTregs), $CD8^+$ regulatory T cells, $CD8^+CD28^-$ regulatory T cells and/or regulatory T-cells secreting IL-10, IL-35, TGF-β, TNF-α, Galectin-1, IFN-γ and/or MCP1.

In various embodiments, the present heterodimeric proteins are capable of, and can be used in methods comprising, transiently stimulating effector T cells for no longer than about 12 hours, about 24 hours, about 48

hours, about 72 hours or about 96 hours or about 1 week or about 2 weeks. In various embodiments, the present heterodimeric proteins are capable of, and can be used in methods comprising, transiently depleting or inhibiting regulatory T cells for no longer than about 12 hours, about 24 hours, about 48 hours, about 72 hours or about 96 hours or about 1 week or about 2 weeks. In various embodiments, the transient stimulation of effector T cells and/or transient depletion or inhibition of regulatory T cells occurs substantially in a patient's bloodstream or in a particular tissue/location including lymphoid tissues such as for example, the bone marrow, lymph-node, spleen, thymus, mucosa-associated lymphoid tissue (MALT), non-lymphoid tissues, or in the tumor microenvironment.

In various embodiments, the present heterodimeric proteins provide advantages including, without limitation, ease of use and ease of production. This is because two distinct immunotherapy agents are combined into a single product which allows for a single manufacturing process instead of two independent manufacturing processes. In addition, administration of a single agent instead of two separate agents allows for easier administration and greater patient compliance. Further, in contrast to, for example, monoclonal antibodies, which are large multimeric proteins containing numerous disulfide bonds and post-translational modifications such as glycosylation, the present heterodimeric proteins are easier and more cost effective to manufacture.

In various embodiments, the present heterodimeric proteins provide synergistic therapeutic effects as it allows for improved site-specific interplay of two immunotherapy agents. In some embodiments, the present heterodimeric proteins provide the potential for reducing off-site and/or systemic toxicity.

Diseases; Methods of Treatment, and Patient Selections

In various embodiments, the present invention pertains to the use of the heterodimeric proteins for the treatment of one or more autoimmune diseases or disorders. In various embodiments, the treatment of an autoimmune disease or disorder may involve modulating the immune system with the present heterodimeric proteins to favor immune inhibition over immune stimulation. Illustrative autoimmune diseases or disorders treatable with the present heterodimeric proteins include those in which the body's own antigens become targets for an immune response, such as, for example, rheumatoid arthritis, systemic lupus erythematosus, diabetes mellitus, ankylosing spondylitis, Sjögren's syndrome, inflammatory bowel diseases (*e.g.,* colitis ulcerosa, Crohn's disease), multiple sclerosis, sarcoidosis, psoriasis, Grave's disease, Hashimoto's thyroiditis, , psoriasis, hypersensitivity reactions (*e.g.*, allergies, hay fever, asthma, and acute edema cause type I hypersensitivity reactions), and vasculitis.

Exemplary autoimmune diseases or conditions that may be treated or prevented using the heterodimeric protein of the invention include, but are not limited to, 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, Sjögren's syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, Reiter's syndrome, Grave's disease, and other autoimmune diseases.

In various embodiments, the present invention pertains to cancers and/or tumors; for example, the treatment or prevention of cancers and/or tumors. As described elsewhere herein, the treatment of cancer may involve in various embodiments, modulating the immune system with the present heterodimeric proteins to favor immune stimulation over immune inhibition.

Cancers or tumors refer to an uncontrolled growth of cells and/or abnormal increased cell survival and/or inhibition of apoptosis which interferes with the normal functioning of the bodily organs and systems. Included are benign and malignant cancers, polyps, hyperplasia, as well as dormant tumors or micrometastases. Also, included are cells having abnormal proliferation that is not impeded by the immune system (*e.g.,* virus infected cells). The cancer may be a primary cancer or a metastatic cancer. The primary cancer may be an area of cancer cells at an originating site that becomes clinically detectable, and may be a primary tumor. In contrast, the metastatic cancer may be the spread of a disease from one organ or part to another non-adjacent organ or part. The metastatic cancer may be caused by a cancer cell that acquires the ability to penetrate and infiltrate surrounding normal tissues in a local area, forming a new tumor, which may be a local metastasis. The cancer may also be caused by a cancer cell that acquires the ability to penetrate the walls of lymphatic and/or blood vessels, after which the cancer cell is able to circulate through the bloodstream (thereby being a circulating tumor cell) to other sites and tissues in the body. The cancer may be due to a process such as lymphatic or hematogeneous spread. The cancer may also be caused by a tumor cell that comes to rest at another site, re-penetrates through the vessel or walls, continues to multiply, and eventually forms another clinically detectable tumor. The cancer may be this new tumor, which may be a metastatic (or secondary) tumor.

The cancer may be caused by tumor cells that have metastasized, which may be a secondary or metastatic tumor. The cells of the tumor may be like those in the original tumor. As an example, if a breast cancer or colon cancer metastasizes to the liver, the secondary tumor, while present in the liver, is made up of abnormal breast or colon cells, not of abnormal liver cells. The tumor in the liver may thus be a metastatic breast cancer or a metastatic colon cancer, not liver cancer.

The cancer may have an origin from any tissue. The cancer may originate from melanoma, colon, breast, or prostate, and thus may be made up of cells that were originally skin, colon, breast, or prostate, respectively. The cancer may also be a hematological malignancy, which may be leukemia or lymphoma. The cancer may invade a tissue such as liver, lung, bladder, or intestinal.

Representative cancers and/or tumors of the present invention include, but are not limited to, a 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 (such as that associated with brain tumors), and Meigs' syndrome.

In some embodiments, the heterodimeric protein is used to treat a subject that has a treatment-refractory cancer. In some embodiments, the heterodimeric protein is used to treat a subject that is refractory to one or more immune-modulating agents. For example, in some embodiments, the heterodimeric protein is used to treat a subject that presents no response to treatment, or even progress, after 12 weeks or so of treatment. For instance, in some embodiments, the subject is refractory to a PD-1 and/or PD-L1 and/or PD-L2 agent, including, for example, 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), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and/or MPDL328OA (ROCHE)-refractory patients. For instance, in some embodiments, the subject is refractory to an anti-CTLA-4 agent, *e.g.,* ipilimumab (YERVOY)-refractory patients (*e.g.,* melanoma patients). Accordingly, in various embodiments, the present invention provides methods of cancer treatment that rescue patients that are non-responsive to various therapies, including monotherapy of one or more immune-modulating agents.

In various embodiments, the present invention provides heterodimeric proteins which target a cell or tissue within the tumor microenviroment. In some embodiments, the cell or tissue within the tumor microenvironment expresses one or more targets or binding partners of the heterodimeric protein. The tumor microenvironment refers to the cellular milieu, including cells, secreted proteins, physiological small molecules, and blood vessels in which the tumor exists. In some embodiments, the cells or tissue within the tumor microenvironment are one or more of: tumor vasculature; tumor-infiltrating lymphocytes; fibroblast reticular cells; endothelial progenitor cells (EPC); cancer-associated fibroblasts; pericytes; other stromal cells; components of the extracellular matrix (ECM); dendritic cells; antigen presenting cells; T-cells; regulatory T cells; macrophages; neutrophils; and other immune cells located proximal to a tumor. In various embodiments, the present heterodimeric protein targets a cancer cell. In some embodiments, the cancer cell expresses one or more of targets or binding partners of the heterodimeric protein.

In various embodiments, the heterodimeric protein of the invention may target a cell (*e.g.*, cancer cell or immune cell) that expresses any of the receptors as described herein. For example, the heterodimeric protein of the invention may target a cell that expresses any of the receptors for a cytokine, growth factor, and/or hormone as described herein.

In some embodiments, the present methods provide treatment with the heterodimeric protein in a patient who is refractory to an additional agent, such "additional agents" being described elsewhere herein, inclusive, without limitation, of the various chemotherapeutic agents described herein.

In some aspects, the present chimeric agents are used to eliminate intracellular pathogens. In some aspects, the present chimeric agents are used to treat one or more infections. In some embodiments, the present heterodimeric proteins are used in methods of treating viral infections (including, for example, HIV and HCV), parasitic infections (including, for example, malaria), and bacterial infections. In various embodiments, the infections induce immunosuppression. For example, HIV infections often result in immunosuppression in the infected subjects. Accordingly, as described elsewhere herein, the treatment of such infections may involve, in various embodiments, modulating the immune system with the present heterodimeric proteins to favor immune stimulation over immune inhibition. Alternatively, the present invention provides methods for treating infections that induce immunoactivation. For example, intestinal helminth infections have been associated with chronic immune activation. In these embodiments, the treatment of such infections may involve modulating the immune system with the present heterodimeric proteins to favor immune inhibition over immune stimulation.

In various embodiments, the present invention provides methods of treating viral infections including, without limitation, acute or chronic viral infections, for example, of the respiratory tract, of papilloma virus infections, of herpes simplex virus (HSV) infection, of human immunodeficiency virus (HIV) infection, and of viral infection of internal organs such as infection with hepatitis viruses. In some embodiments, the viral infection is caused by a virus of family Flaviviridae. In some embodiments, the virus of family Flaviviridae is selected from Yellow Fever Virus, West Nile virus, Dengue virus, Japanese Encephalitis Virus, St. Louis Encephalitis Virus, and Hepatitis C Virus. In other embodiments, the viral infection is caused by a virus of family Picornaviridae, *e.g.*, poliovirus, rhinovirus, coxsackievirus. In other embodiments, the viral infection is caused by a member of Orthomyxoviridae, *e.g.*, an influenza virus. In other embodiments, the viral infection is caused by a member of Retroviridae, *e.g.*, a lentivirus. In other embodiments, the viral infection is caused by a member of Paramyxoviridae, *e.g.*, respiratory syncytial virus, a human parainfluenza virus, rubulavirus (*e.g.*, mumps virus), measles virus, and human metapneumovirus. In other embodiments, the viral infection is caused by a member of Bunyaviridae, *e.g.*, hantavirus. In other embodiments, the viral infection is caused by a member of Reoviridae, *e.g.*, a rotavirus.

In various embodiments, the present invention provides methods of treating parasitic infections such as protozoan or helminths infections. In some embodiments, the parasitic infection is by a protozoan parasite. In some embodiments, the oritiziab parasite is selected from intestinal protozoa, tissue protozoa, or blood protozoa. Illustrative protozoan parasites include, but are not limited to, *Entamoeba hystolytica, Giardia lamblia,*

Cryptosporidium muris, Trypanosomatida gambiense, Trypanosomatida rhodesiense, Trypanosomatida crusi, Leishmania mexicana, Leishmania braziliensis, Leishmania tropica, Leishmania donovani, Toxoplasma gondii, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, Plasmodium falciparum, Trichomonas vaginalis, and *Histomonas meleagridis.* In some embodiments, the parasitic infection is by a helminthic parasite such as nematodes (*e.g.*, Adenophorea). In some embodiments, the parasite is selected from Secementea (*e.g.*, *Trichuris trichiura, Ascaris lumbricoides, Enterobius vermicularis, Ancylostoma duodenale, Necator americanus, Strongyloides stercoralis, Wuchereria bancrofti, Dracunculus medinensis*). In some embodiments, the parasite is selected from trematodes (*e.g.,* blood flukes, liver flukes, intestinal flukes, and lung flukes). In some embodiments, the parasite is selected from: *Schistosoma mansoni, Schistosoma haematobium, Schistosoma japonicum, Fasciola hepatica, Fasciola gigantica, Heterophyes heterophyes, Paragonimus westermani.* In some embodiments, the parasite is selected from cestodes (*e.g.*, *Taenia solium, Taenia saginata, Hymenolepis nana, Echinococcus granulosus*).

In various embodiments, the present invention provides methods of treating bacterial infections. In various embodiments, the bacterial infection is by gram-positive bacteria, gram-negative bacteria, aerobic and/or anaerobic bacteria. In various embodiments, the bacteria are selected from, but not limited to, *Staphylococcus, Lactobacillus, Streptococcus, Sarcina, Escherichia, Enterobacter, Klebsiella, Pseudomonas, Acinetobacter, Mycobacterium, Proteus, Campylobacter, Citrobacter, Nisseria, Baccillus, Bacteroides, Peptococcus, Clostridium, Salmonella, Shigella, Serratia, Haemophilus, Brucella* and other organisms. In some embodiments, the bacteria is selected from, but not limited to, *Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas acidovorans, Pseudomonas alcaligenes, Pseudomonas putida, Stenotrophomonas maltophilia, Burkholderia cepacia, Aeromonas hydrophilia, Escherichia coli, Citrobacter freundii, Salmonella typhimurium, Salmonella typhi, Salmonella paratyphi, Salmonella enteritidis, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Enterobacter cloacae, Enterobacter aerogenes, Klebsiella pneumoniae, Klebsiella oxytoca, Serratia marcescens, Francisella tularensis, Morganella morganii, Proteus mirabilis, Proteus vulgaris, Providencia alcalifaciens, Providencia rettgeri, Providencia stuartii, Acinetobacter baumannii, Acinetobacter calcoaceticus, Acinetobacter haemolyticus, Yersinia enterocolitica, Yersinia pestis, Yersinia pseudotuberculosis, Yersinia intermedia, Bordetella pertussis, Bordetella parapertussis, Bordetella bronchiseptica, Haemophilus influenzae, Haemophilus parainfluenzae, Haemophilus haemolyticus, Haemophilus parahaemolyticus, Haemophilus ducreyi, Pasteurella multocida, Pasteurella haemolytica, Branhamella catarrhalis, Helicobacter pylori, Campylobacter fetus, Campylobacter jejuni, Campylobacter coli, Borrelia burgdorferi, Vibrio cholerae, Vibrio parahaemolyticus, Legionella pneumophila, Listeria monocytogenes, Neisseria gonorrhoeae, Neisseria meningitidis, Kingella, Moraxella, Gardnerella vaginalis, Bacteroides fragilis, Bacteroides distasonis, Bacteroides* 3452A homology group, *Bacteroides vulgatus, Bacteroides ovalus, Bacteroides thetaiotaomicron, Bacteroides uniformis, Bacteroides eggerthii, Bacteroides splanchnicus, Clostridium difficile, Mycobacterium tuberculosis, Mycobacterium avium, Mycobacterium intracellulare, Mycobacterium leprae, Corynebacterium diphtheriae, Corynebacterium ulcerans, Streptococcus pneumoniae, Streptococcus agalactiae, Streptococcus pyogenes, Enterococcus faecalis, Enterococcus faecium,*

Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Staphylococcus intermedius, Staphylococcus hyicus subsp. *hyicus, Staphylococcus haemolyticus, Staphylococcus hominis*, or *Staphylococcus saccharolyticus*.

In still another other aspect, the present invention is directed toward methods of treating and preventing T cell-mediated diseases and disorders, such as, but not limited to diseases or disorders described elsewhere herein and inflammatory disease or disorder, graft-versus-host disease (GVHD), transplant rejection, and T cell proliferative disorder.

In some aspects, the present chimeric agents are used in methods of activating a T cell, *e.g.,* via the extracellular domain having an immune stimulatory signal.

In some aspects, the present chimeric agents are used in methods of preventing the cellular transmission of an immunosuppressive signal.

Combination Therapies and Conjugation

In some embodiments, the invention provides for heterodimeric proteins and methods that further comprise administering an additional agent to a subject. In some embodiments, the invention pertains to co-administration and/or co-formulation. Any of the compositions described herein may be co-formulated and/or co-administered.

In some embodiments, any heterodimeric protein described herein acts synergistically when co-administered with another agent and is administered at doses that are lower than the doses commonly employed when such agents are used as monotherapy. In various embodiments, any agent referenced herein may be used in combination with any of the heterodimeric proteins described herein.

In various embodiments, any of the heterodimeric proteins disclosed herein may be co-administered with another heterodimeric protein disclosed herein. Without wishing to be bound by theory, it is believed that a combined regimen involving the administration of one or more heterodimeric proteins which induce an innate immune response and one or more heterodimeric proteins which induce an adaptive immune response may provide synergistic effects (*e.g.*, synergistic anti-tumor effects).

In various embodiments, any heterodimeric protein which induces an innate immune response may be utilized in the present invention. In various embodiments, any heterodimeric protein which induces an adaptive immune response may be utilized in the present invention.

In some embodiments, inclusive of, without limitation, cancer applications, the present invention pertains to chemotherapeutic agents as additional agents. 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 various embodiments, inclusive of, without limitation, cancer applications, the present additional agent is one or more immune-modulating agents selected from an agent that blocks, reduces and/or inhibits PD-1 and PD-L1 or PD-L2 and/or the binding of PD-1 with PD-L1 or PD-L2 (by way of non-limiting example, one or more of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, Merck), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), atezolizumab (TECENTRIQ, GENENTECH), MPDL328OA (ROCHE)), an agent that increases and/or stimulates CD137 (4-1BB) and/or the binding of CD137 (4-1BB) with one or more of 4-1BB ligand (by way of non-limiting example, urelumab (BMS-663513 and anti-4-1BB antibody), and an agent that blocks, reduces and/or inhibits the activity of CTLA-4 and/or the binding of CTLA-4 with one or more of AP2M1, CD80, CD86, SHP-2, and PPP2R5A and/or the binding of OX40 with OX40L (by way of non-limiting example GBR 830 (GLENMARK), MEDI6469 (MEDIMMUNE).

In some embodiments, inclusive of, without limitation, infectious disease applications, the present invention pertains to anti-infectives as additional 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 some embodiments, inclusive, without limitation, of autoimmune applications, the additional agent is an immunosuppressive agent. In some embodiments, the immunosuppressive agent 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).

In some embodiments, the heterodimeric proteins (and/or additional agents) 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 turicamycin, *etc.* Additionally, the derivative can contain one or more non-classical amino acids. In still other embodiments, the heterodimeric proteins (and/or additional agents) 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 heterodimeric proteins (and/or additional agents) 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.

Formulations

The heterodimeric proteins (and/or additional agents) 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.

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

Further, any heterodimeric protein (and/or additional agents) 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. 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.

In some embodiments, the compositions described herein are resuspended in a saline buffer (including, without limitation TBS, PBS, and the like).

In various embodiments, the heterodimeric proteins may by conjugated and/or fused with another agent to extend half-life or otherwise improve pharmacodynamic and pharmacokinetic properties. In some embodiments, the heterodimeric proteins 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 heterodimeric proteins 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.

Administration, Dosing, and Treatment Regimens

The present invention includes the described heterodimeric protein (and/or additional agents) in various formulations. Any heterodimeric protein (and/or additional agents) described herein can take the form of solutions, suspensions, emulsion, drops, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. DNA or RNA constructs encoding the protein sequences may also be used. In one embodiment, the composition is in the form of a capsule (*see, e.g.*, U.S. Patent No. 5,698,155). 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.

Where necessary, the formulations comprising the heterodimeric protein (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. Compositions for administration can optionally include a local anesthetic such as, for example, lignocaine to lessen pain at the site of the injection.

The formulations comprising the heterodimeric protein (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 one embodiment, any heterodimeric protein (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: intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, oral, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by inhalation, or topically, particularly to the ears, nose, eyes, or skin. In some embodiments, the administering is effected orally or by parenteral injection. In most instances, administration results in the release of any agent described herein into the bloodstream.

Any heterodimeric protein (and/or additional agents) described herein can be administered orally. Such heterodimeric proteins (and/or additional agents) can also be administered by any other convenient route, for example, by intravenous infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, *etc.*) and can be administered together with another biologically active agent. Administration can be systemic or local. Various delivery systems are known, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, capsules, *etc.*, and can be used to administer.

In specific embodiments, it may be desirable to administer locally to the area in need of treatment. In one embodiment, for instance in the treatment of cancer, the heterodimeric protein (and/or additional agents) are administered in the tumor microenvironment (*e.g.,* cells, molecules, extracellular matrix and/or blood vessels that surround and/or feed a tumor cell, inclusive of, for example, tumor vasculature; tumor-infiltrating lymphocytes; fibroblast reticular cells; endothelial progenitor cells (EPC); cancer-associated fibroblasts; pericytes; other stromal cells; components of the extracellular matrix (ECM); dendritic cells; antigen presenting cells; T-cells; regulatory T cells; macrophages; neutrophils; and other immune cells located proximal to a tumor) or lymph node and/or targeted to the tumor microenvironment or lymph node. In various embodiments, for instance in the treatment of cancer, the heterodimeric protein (and/or additional agents) are administered intratumorally.

In the various embodiments, the present heterodimeric protein allows for a dual effect that provides less side effects than are seen in conventional immunotherapy (*e.g.,* treatments with one or more of OPDIVO, KEYTRUDA, YERVOY, and TECENTRIQ). For example, the present heterodimeric proteins reduce or prevent commonly

observed immune-related adverse events that affect various tissues and organs including the skin, the gastrointestinal tract, the kidneys, peripheral and central nervous system, liver, lymph nodes, eyes, pancreas, and the endocrine system; such as hypophysitis, colitis, hepatitis, pneumonitis, rash, and rheumatic disease. Further, the present local administration, *e.g.,* intratumorally, obviate adverse event seen with standard systemic administration, *e.g.,* IV infusions, as are used with conventional immunotherapy (*e.g.,* treatments with one or more of OPDIVO, KEYTRUDA, YERVOY, and TECENTRIQ).

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.

The dosage of any heterodimeric protein (and/or additional agents) described herein as well as the dosing schedule can depend on various parameters, including, but not limited to, the disease being treated, the subject's general health, and the administering physician's discretion. Any heterodimeric protein described herein, can be administered prior to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concurrently with, or subsequent to (*e.g.,* 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of an additional agent, to a subject in need thereof. In various embodiments any heterodimeric protein and additional agent described herein are administered 1 minute apart, 10 minutes apart, 30 minutes apart, less than 1 hour apart, 1 hour apart, 1 hour to 2 hours apart, 2 hours to 3 hours apart, 3 hours to 4 hours apart, 4 hours to 5 hours apart, 5 hours to 6 hours apart, 6 hours to 7 hours apart, 7 hours to 8 hours apart, 8 hours to 9 hours apart, 9 hours to 10 hours apart, 10 hours to 11 hours apart, 11 hours to 12 hours apart, 1 day apart, 2 days apart, 3 days apart, 4 days apart, 5 days apart, 6 days apart, 1 week apart, 2 weeks apart, 3 weeks apart, or 4 weeks apart.

In various embodiments, the present invention relates to the co-administration of a heterodimeric protein which induces an innate immune response and another heterodimeric protein which induces an adaptive immune response. In such embodiments, the heterodimeric protein which induces an innate immune response may be administered before, concurrently with, or subsequent to administration of the heterodimeric protein which induces an adaptive immune response. For example, the heterodimeric proteins may be administered 1 minute apart, 10 minutes apart, 30 minutes apart, less than 1 hour apart, 1 hour apart, 1 hour to 2 hours apart, 2 hours to 3 hours apart, 3 hours to 4 hours apart, 4 hours to 5 hours apart, 5 hours to 6 hours apart, 6 hours to 7 hours apart, 7 hours to 8 hours apart, 8 hours to 9 hours apart, 9 hours to 10 hours apart, 10 hours to 11 hours apart, 11 hours to 12 hours apart, 1 day apart, 2 days apart, 3 days apart, 4 days apart, 5 days apart, 6 days apart, 1 week apart, 2 weeks apart, 3 weeks apart, or 4 weeks apart. In an exemplary embodiment, the heterodimeric protein which induces an innate immune response and the heterodimeric protein which induces an adaptive response are

administered 1 week apart, or administered on alternate weeks (*i.e.*, administration of the heterodimeric protein inducing an innate immune response is followed 1 week later with administration of the heterodimeric protein which induces an adaptive immune response and so forth).

The dosage of any heterodimeric protein (and/or additional agents) described herein can depend on several factors including the severity of the condition, whether the condition is to be treated or prevented, and the age, weight, and health of the subject to be treated. Additionally, pharmacogenomic (the effect of genotype on the pharmacokinetic, pharmacodynamic or efficacy profile of a therapeutic) information about a particular subject may affect dosage used. Furthermore, the exact individual dosages can be adjusted somewhat depending on a variety of factors, including the specific combination of the agents being administered, the time of administration, the route of administration, the nature of the formulation, the rate of excretion, the particular disease being treated, the severity of the disorder, and the anatomical location of the disorder. Some variations in the dosage can be expected.

For administration of any heterodimeric protein (and/or additional agents) described herein by parenteral injection, the dosage may be about 0.1 mg to about 250 mg per day, about 1 mg to about 20 mg per day, or about 3 mg to about 5 mg per day. Generally, when orally or parenterally administered, the dosage of any agent described herein may be about 0.1 mg to about 1500 mg per day, or about 0.5 mg to about 10 mg per day, or about 0.5 mg to about 5 mg per day, or about 200 to about 1,200 mg per day (*e.g.*, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1,000 mg, about 1,100 mg, about 1,200 mg per day).

In some embodiments, administration of the heterodimeric protein (and/or additional agents) described herein is by parenteral injection at a dosage of about 0.1 mg to about 1500 mg per treatment, or about 0.5 mg to about 10 mg per treatment, or about 0.5 mg to about 5 mg per treatment, or about 200 to about 1,200 mg per treatment (*e.g.*, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1,000 mg, about 1,100 mg, about 1,200 mg per treatment).

In some embodiments, a suitable dosage of the heterodimeric protein (and/or additional agents) is in a range of about 0.01 mg/kg to about 100 mg/kg of body weight ,or about 0.01 mg/kg to about 10 mg/kg of body weight of the subject, for example, about 0.01 mg/kg, about 0.02 mg/kg, about 0.03 mg/kg, about 0.04 mg/kg, about 0.05 mg/kg, about 0.06 mg/kg, about 0.07 mg/kg, about 0.08 mg/kg, about 0.09 mg/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, inclusive of all values and ranges therebetween.

In another embodiment, delivery can be in a vesicle, in particular a liposome (*see* Langer, 1990, *Science* 249:1527-1533; Treat *et al.*, in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989).

Any heterodimeric protein (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, hydropropylmethyl cellulose, 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. 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, polymeric materials can be used (*see Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61; *see also* Levy *et al.*, 1985, *Science* 228:190; During *et al.*, 1989, *Ann. Neurol.* 25:351; Howard *et al.*, 1989, *J. Neurosurg.* 71:105).

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 (s*ee, 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.

Administration of any heterodimeric protein (and/or additional agents) described herein can, independently, be one to four times daily or one to four times per month or one to six times per year or once every two, three, four or five years. Administration can be for the duration of one day or one month, two months, three months, six months, one year, two years, three years, and may even be for the life of the subject.

The dosage regimen utilizing any heterodimeric protein (and/or additional agents) described herein can be selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the subject; the severity of the condition to be treated; the route of administration; the renal or hepatic function of the subject; the pharmacogenomic makeup of the individual; and the specific compound of the invention employed. Any heterodimeric protein (and/or additional agents) described herein can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three or four times daily. Furthermore, any heterodimeric protein (and/or additional agents) described herein can be administered continuously rather than intermittently throughout the dosage regimen.

Cells and Nucleic Acids

In various embodiments, the present invention provides an expression vector, comprising a nucleic acid encoding the heterodimeric protein (*e.g.*, a heterodimeric protein comprising a first and second polypeptide chains) described herein. In various embodiments, the expression vector comprises DNA or RNA. In various embodiments, the expression vector is a mammalian expression vector.

Both prokaryotic and eukaryotic vectors can be used for expression of the heterodimeric protein. Prokaryotic vectors include constructs based on *E. coli* sequences (*see, e.g.,* Makrides, *Microbiol Rev* 1996, 60:512-538). Non-limiting examples of regulatory regions that can be used for expression in *E. coli* include lac, trp, lpp, phoA, recA, tac, T3, T7 and λP_L. Non-limiting examples of prokaryotic expression vectors may include the λgt vector series such as λgt11 (Huynh *et al.*, in "DNA Cloning Techniques, Vol. I: A Practical Approach," 1984, (D. Glover, ed.), pp. 49-78, IRL Press, Oxford), and the pET vector series (Studier *et al.*, *Methods Enzymol* 1990, 185:60-89). Prokaryotic host-vector systems cannot perform much of the post-translational processing of mammalian cells, however. Thus, eukaryotic host- vector systems may be particularly useful. A variety of regulatory regions can be used for expression of the heterodimeric proteins in mammalian host cells. For example, the SV40 early and late promoters, the cytomegalovirus (CMV) immediate early promoter, and the Rous sarcoma virus long terminal repeat (RSV-LTR) promoter can be used. Inducible promoters that may be useful in mammalian cells include, without limitation, promoters associated with the metallothionein II gene, mouse mammary tumor virus glucocorticoid responsive long terminal repeats (MMTV-LTR), the β-interferon gene, and the hsp70 gene (*see*, Williams *et al.*, *Cancer Res* 1989, 49:2735-42; and Taylor *et al.*, *Mol Cell Biol* 1990, 10:165-75). Heat shock promoters or stress promoters also may be advantageous for driving expression of the fusion proteins in recombinant host cells.

In some embodiments, expression vectors of the invention comprise a nucleic acid encoding at least the first and/or second polypeptide chains of the heterodimeric proteins (and/or additional agents), or a complement thereof, operably linked to an expression control region, or complement thereof, that is functional in a mammalian cell. The expression control region is capable of driving expression of the operably linked blocking and/or stimulating agent encoding nucleic acid such that the blocking and/or stimulating agent is produced in a human cell transformed with the expression vector.

Expression control regions are regulatory polynucleotides (sometimes referred to herein as elements), such as promoters and enhancers, that influence expression of an operably linked nucleic acid. An expression control region of an expression vector of the invention is capable of expressing operably linked encoding nucleic acid in a human cell. In an embodiment, the cell is a tumor cell. In another embodiment, the cell is a non-tumor cell. In an embodiment, the expression control region confers regulatable expression to an operably linked nucleic acid. A signal (sometimes referred to as a stimulus) can increase or decrease expression of a nucleic acid operably linked to such an expression control region. Such expression control regions that increase expression in response to a signal are often referred to as inducible. Such expression control regions that decrease expression in response to a signal are often referred to as repressible. Typically, the amount of increase or decrease conferred by such

elements is proportional to the amount of signal present; the greater the amount of signal, the greater the increase or decrease in expression.

In an embodiment, the present invention contemplates the use of inducible promoters capable of effecting high level of expression transiently in response to a cue. For example, when in the proximity of a tumor cell, a cell transformed with an expression vector for the heterodimeric protein (and/or additional agents) comprising such an expression control sequence is induced to transiently produce a high level of the agent by exposing the transformed cell to an appropriate cue. Illustrative inducible expression control regions include those comprising an inducible promoter that is stimulated with a cue such as a small molecule chemical compound. Particular examples can be found, for example, in U.S. Pat. Nos. 5,989,910, 5,935,934, 6,015,709, and 6,004,941, each of which is incorporated herein by reference in its entirety.

Expression control regions and locus control regions include full-length promoter sequences, such as native promoter and enhancer elements, as well as subsequences or polynucleotide variants which retain all or part of full-length or non-variant function. As used herein, the term "functional" and grammatical variants thereof, when used in reference to a nucleic acid sequence, subsequence or fragment, means that the sequence has one or more functions of native nucleic acid sequence (*e.g.*, non-variant or unmodified sequence).

As used herein, “operable linkage” refers to a physical juxtaposition of the components so described as to permit them to function in their intended manner. In the example of an expression control element in operable linkage with a nucleic acid, the relationship is such that the control element modulates expression of the nucleic acid. Typically, an expression control region that modulates transcription is juxtaposed near the 5' end of the transcribed nucleic acid (*i.e.*, “upstream”). Expression control regions can also be located at the 3’ end of the transcribed sequence (*i.e.*, “downstream”) or within the transcript (*e.g.*, in an intron). Expression control elements can be located at a distance away from the transcribed sequence (*e.g.*, 100 to 500, 500 to 1000, 2000 to 5000, or more nucleotides from the nucleic acid). A specific example of an expression control element is a promoter, which is usually located 5' of the transcribed sequence. Another example of an expression control element is an enhancer, which can be located 5' or 3' of the transcribed sequence, or within the transcribed sequence.

Expression systems functional in human cells are well known in the art, and include viral systems. Generally, a promoter functional in a human cell is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and typically a TATA box located 25-30 base pairs upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A promoter will also typically contain an upstream promoter element (enhancer element), typically located within 100 to 200 base pairs upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation. Of particular use as promoters are the promoters from mammalian viral genes, since the viral genes are often highly expressed and have a broad host range. Examples include the SV40 early promoter, mouse

mammary tumor virus LTR promoter, adenovirus major late promoter, herpes simplex virus promoter, and the CMV promoter.

Typically, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the mature mRNA is formed by site-specific post-translational cleavage and polyadenylation. Examples of transcription terminator and polyadenylation signals include those derived from SV40. Introns may also be included in expression constructs.

There are a variety of techniques available for introducing nucleic acids into viable cells. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, polymer-based systems, DEAE-dextran, viral transduction, the calcium phosphate precipitation method, *etc.* For *in vivo* gene transfer, a number of techniques and reagents may also be used, including liposomes; natural polymer-based delivery vehicles, such as chitosan and gelatin; viral vectors are also suitable for *in vivo* transduction. In some situations, it is desirable to provide a targeting agent, such as an antibody or ligand specific for a tumor cell surface membrane protein. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.*, capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, J. Biol. Chem. 262, 4429-4432 (1987); and Wagner *et al.*, Proc. Natl. Acad. Sci. USA 87, 3410-3414 (1990).

Where appropriate, gene delivery agents such as, *e.g.*, integration sequences can also be employed. Numerous integration sequences are known in the art (*see*, *e.g.*, Nunes-Duby *et al.*, Nucleic Acids Res. 26:391-406, 1998; Sadwoski, J. Bacteriol., 165:341-357, 1986; Bestor, Cell, 122(3):322-325, 2005; Plasterk *et al.*, TIG 15:326-332, 1999; Kootstra *et al.*, Ann. Rev. Pharm. Toxicol., 43:413-439, 2003). These include recombinases and transposases. Examples include Cre (Sternberg and Hamilton, J. Mol. Biol., 150:467-486, 1981), lambda (Nash, Nature, 247, 543-545, 1974), Flp (Broach, *et al.*, Cell, 29:227-234, 1982), R (Matsuzaki, *et al.*, J. Bacteriology, 172:610-618, 1990), cpC31 (*see*, *e.g.*, Groth *et al.*, J. Mol. Biol. 335:667-678, 2004), sleeping beauty, transposases of the mariner family (Plasterk *et al.*, supra), and components for integrating viruses such as AAV, retroviruses, and antiviruses having components that provide for virus integration such as the LTR sequences of retroviruses or lentivirus and the ITR sequences of AAV (Kootstra *et al.*, Ann. Rev. Pharm. Toxicol., 43:413-439, 2003). In addition, direct and targeted genetic integration strategies may be used to insert nucleic acid sequences encoding the chimeric fusion proteins including CRISPR/CAS9, zinc finger, TALEN, and meganuclease gene-editing technologies.

In one aspect, the invention provides expression vectors for the expression of the heterodimeric proteins (and/or additional agents) that are viral vectors. Many viral vectors useful for gene therapy are known (*see*, *e.g.*, Lundstrom, Trends Biotechnol., 21: 1 17, 122, 2003. Illustrative viral vectors include those selected from Antiviruses (LV),

retroviruses (RV), adenoviruses (AV), adeno-associated viruses (AAV), and α viruses, though other viral vectors may also be used. For *in vivo* uses, viral vectors that do not integrate into the host genome are suitable for use, such as α viruses and adenoviruses. Illustrative types of α viruses include Sindbis virus, Venezuelan equine encephalitis (VEE) virus, and Semliki Forest virus (SFV). For *in vitro* uses, viral vectors that integrate into the host genome are suitable, such as retroviruses, AAV, and Antiviruses. In one embodiment, the invention provides methods of transducing a human cell *in vivo*, comprising contacting a solid tumor *in vivo* with a viral vector of the invention.

In various embodiments, the present invention provides a host cell, comprising the expression vector comprising the heterodimeric protein described herein.

Expression vectors can be introduced into host cells for producing the present heterodimeric proteins. Cells may be cultured *in vitro* or genetically engineered, for example. Useful mammalian host cells include, without limitation, cells derived from humans, monkeys, and rodents (*see*, for example, Kriegler in "Gene Transfer and Expression: A Laboratory Manual," 1990, New York, Freeman & Co.). These include monkey kidney cell lines transformed by SV40 (*e.g.*, COS-7, ATCC CRL 1651); human embryonic kidney lines (*e.g.*, 293, 293-EBNA, or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J Gen Virol* 1977, 36:59); baby hamster kidney cells (*e.g.*, BHK, ATCC CCL 10); Chinese hamster ovary-cells-DHFR (*e.g.*, CHO, Urlaub and Chasin, *Proc Natl Acad Sci USA* 1980, 77:4216); DG44 CHO cells, CHO-K1 cells, mouse sertoli cells (Mather, *Biol Reprod* 1980, 23:243-251); mouse fibroblast cells (*e.g.*, NIH-3T3), monkey kidney cells (*e.g.*, CV1 ATCC CCL 70); African green monkey kidney cells. (*e.g.*, VERO-76, ATCC CRL-1587); human cervical carcinoma cells (*e.g.*, HELA, ATCC CCL 2); canine kidney cells (*e.g.*, MDCK, ATCC CCL 34); buffalo rat liver cells (*e.g.*, BRL 3A, ATCC CRL 1442); human lung cells (*e.g.*, W138, ATCC CCL 75); human liver cells (*e.g.*, Hep G2, HB 8065); and mouse mammary tumor cells (*e.g.*, MMT 060562, ATCC CCL51). Illustrative cancer cell types for expressing the fusion proteins described herein include mouse fibroblast cell line, NIH3T3, mouse Lewis lung carcinoma cell line, LLC, mouse mastocytoma cell line, P815, mouse lymphoma cell line, EL4 and its ovalbumin transfectant, *E.G*7, mouse melanoma cell line, B16F10, mouse fibrosarcoma cell line, MC57, and human small cell lung carcinoma cell lines, SCLC#2 and SCLC#7.

Host cells can be obtained from normal or affected subjects, including healthy humans, cancer patients, and patients with an infectious disease, private laboratory deposits, public culture collections such as the American Type Culture Collection, or from commercial suppliers.

Cells that can be used for production of the present heterodimeric proteins *in vitro*, *ex vivo*, and/or *in vivo* include, without limitation, epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells (*e.g.*, as obtained from bone marrow), umbilical cord blood, peripheral blood, fetal liver, *etc.* The choice of cell type depends on the type of tumor or infectious disease being treated or prevented, and can be determined by one of skill in the art.

Production and purification of Fc-containing macromolecules (such as Fc fusion proteins) has become a standardized process, with minor modifications between products. For example, many Fc containing macromolecules are produced by human embryonic kidney (HEK) cells (or variants thereof) or Chinese Hamster Ovary (CHO) cells (or variants thereof) or in some cases by bacterial or synthetic methods. Following production, the Fc containing macromolecules that are secreted by HEK or CHO cells are purified through binding to Protein A columns and subsequently 'polished' using various methods. Generally speaking, purified Fc containing macromolecules are stored in liquid form for some period of time, frozen for extended periods of time or in some cases lyophilized. In various embodiments, production of the heterodimeric proteins contemplated herein may have unique characteristics as compared to traditional Fc containing macromolecules. In certain examples, the heterodimeric proteins may be purified using specific chromatography resins, or using chromatography methods that do not depend upon Protein A capture. In other embodiments, the heterodimeric proteins may be purified in an oligomeric state, or in multiple oligomeric states, and enriched for a specific oligomeric state using specific methods. Without being bound by theory, these methods could include treatment with specific buffers including specified salt concentrations, pH and additive compositions. In other examples, such methods could include treatments that favor one oligomeric state over another. The heterodimeric proteins obtained herein may be additionally 'polished' using methods that are specified in the art. In some embodiments, the heterodimeric proteins are highly stable and able to tolerate a wide range of pH exposure (between pH 3-12), are able to tolerate a large number of freeze/thaw stresses (greater than 3 freeze/thaw cycles) and are able to tolerate extended incubation at high temperatures (longer than 2 weeks at 40 degrees C). In other embodiments, the heterodimeric proteins are shown to remain intact, without evidence of degradation, deamidation, etc. under such stress conditions.

Subjects and/or Animals

In some embodiments, the subject and/or animal is a mammal, *e.g.*, a human, mouse, rat, guinea pig, dog, cat, horse, cow, pig, rabbit, sheep, or non-human primate, such as a monkey, chimpanzee, or baboon. In other embodiments, the subject and/or animal is a non-mammal, such, for example, a zebrafish. In some embodiments, the subject and/or animal may comprise fluorescently-tagged cells (with *e.g.,* GFP). In some embodiments, the subject and/or animal is a transgenic animal comprising a fluorescent cell.

In some embodiments, the subject and/or animal is a human. In some embodiments, the human is a pediatric human. In other embodiments, the human is an adult human. In other embodiments, the human is a geriatric human. In other embodiments, the human may be referred to as a patient.

In certain embodiments, the human has an age in a range of from about 0 months to about 6 months old, from about 6 to about 12 months old, from about 6 to about 18 months old, from about 18 to about 36 months old, from about 1 to about 5 years old, from about 5 to about 10 years old, from about 10 to about 15 years old, from about 15 to about 20 years old, from about 20 to about 25 years old, from about 25 to about 30 years old, from about 30 to about 35 years old, from about 35 to about 40 years old, from about 40 to about 45 years old, from about 45 to about 50 years old, from about 50 to about 55 years old, from about 55 to about 60 years old, from about 60 to about 65 years old, from about 65 to about 70 years old, from about 70 to about 75 years old, from about 75 to

about 80 years old, from about 80 to about 85 years old, from about 85 to about 90 years old, from about 90 to about 95 years old or from about 95 to about 100 years old.

In other embodiments, the subject is a non-human animal, and therefore the invention pertains to veterinary use. In a specific embodiment, the non-human animal is a household pet. In another specific embodiment, the non-human animal is a livestock animal.

Kits

The invention provides kits that can simplify the administration of any agent described herein. An illustrative kit of the invention comprises any composition described herein in unit dosage form. In one embodiment, the unit dosage form is a container, such as a pre-filled syringe, which can be sterile, containing any agent described herein and a pharmaceutically acceptable carrier, diluent, excipient, or vehicle. The kit can further comprise a label or printed instructions instructing the use of any agent described herein. The kit may also include a lid speculum, topical anesthetic, and a cleaning agent for the administration location. The kit can also further comprise one or more additional agent described herein. In one embodiment, the kit comprises a container containing an effective amount of a composition of the invention and an effective amount of another composition, such those described herein.

Definitions

As used in this Specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

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

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About is understood to be within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from the context, all numerical values provided herein are modified by the term "about."

A stated range is understood to be any value between and at the limits of the stated range. As examples, a range between 1 and 5 includes 1, 2, 3, 4, and 5; a range between 1 and 10 includes 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; and a range between 1 and 100 includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although other probes, compositions, methods, and kits similar, or equivalent, to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein. It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

Any aspect or embodiment described herein can be combined with any other aspect or embodiment as disclosed herein.

The invention will be further described in the following example, which does not limit the scope of the invention described in the claims.

EXAMPLES

Example 1: Construction and Characterization of the IL-6R-Fc-IL-35 Heterodimeric Protein

A heterodimeric protein comprising the IL6 receptor (IL6R) linked by a charge polarized core domain to IL-35 was constructed (*see, e.g.,* FIG. 1). Specifically, the heterodimeric protein comprises two polypeptide chains. The first polypeptide chain comprises the IL6R subunit Gp130 linked by a charge polarized core domain to the IL-35 subunit IL12α. The second polypeptide chain comprises the IL6R subunit IL6Rα linked by a charge polarized core domain to the IL-35 subunit IL27β. The IL-6R-Fc-IL-35 heterodimeric protein was expressed in mammalian cells by a dual transient transfection with both the IL6RA-Beta-IL27β and gp130-Alpha-IL12α constructs. Coomassie staining indicated the presence of expressed proteins, which were confirmed using an anti-human IgG Western blot to include proteins corresponding to the approximate molecular weights of the Alpha and Beta constructs (see, *e.g.*, FIG. 2).

Additional analysis of the purified protein under non-reduced, reduced and reduced and deglycosylated conditions provided further evidence for assembly of the heterodimeric construct. Specifically, anti-human Fc and anti-human IL-6R staining by Western blot demonstrated the presence of a single high-molecular weight band corresponding to the approximate molecular weight of the alpha/beta heterodimer comprising IL-6R-Fc-IL-35. This heterodimer could be disassembled into the constituent alpha and beta strands under reducing conditions, which showed an apparent molecular weight higher than the predicted molecular weight based on amino acid content alone. This was expected due to the known presence of glycosylation sites, and deglycosylation of the alpha and beta strands led to those individual proteins appearing at their predicted molecular weights by Western analysis (see, *e.g.*, FIG. 3).

Additionally, because the presence of SDS may disrupt any charge interactions which contribute to protein multimerization, the IL-6R-Fc-IL-35 construct was further analyzed by Blue Native PAGE. These data indicate that a majority of the secreted protein (estimated at 60%) represents the alpha/beta heterodimer (FIG. 3). The concentration of the purified IL-6R-Fc-IL-35 protein was confirmed by spectrophotometric analysis (see, *e.g.*, FIG. 4).

Size-exclusion chromatography (SEC) was performed of the IL-6R-Fc-IL-35 heterodimeric protein following dual transfection of the gp130-Fc(alpha)-IL12A and IL6RA-Fc(beta)-IL27B constructs in CHO cells followed by purification of the secreted protein using protein A. The appearance of a single peak by SEC indicated that there is likely only a single species of heterodimeric protein present, which was intended from using charge polarized linker domains (Fc-alpha and Fc-beta) in the two constructs (FIG. 5).

To confirm that the assembled IL-6R-Fc-IL-35 heterodimer retained the ability to engage with the cognate ligand (*e.g.*, IL-6) and be recognized by specific antibodies against each constituent protein of the assembled heterodimer (*i.e.*, IL-6RA, gp130, IL27β/EBI3 and IL12α), a series of ELISA assays were performed to demonstrate the specific presence of the IL-6R-Fc-IL-35 heterodimer. In FIG. 6 to FIG. 15, the schematic of the ELISA assays is illustrated in the top portion of each figure. In the schematic, the capture and detection strategy is illustrated. In each case, the presence of the IL-6R-Fc-IL-35 heterodimer was observed through capture with recombinant IL-6 and detection with anti-IL-27B/EBI3 (FIG. 6), capture with recombinant IL-6 and detection with anti-human IL-6RA (FIG. 7), capture with anti-human gp130 and detection with anti-IL27B/EBI3 (FIG. 8), capture with anti-human gp130 and detection with anti-human IL-6RA (FIG. 9), capture with anti-IL-6RA and detection with anti-IL27B/EBI3 (FIG. 10), capture with anti-IL-6RA detection and with anti-IL-6RA (FIG. 11), capture with anti-human p35 and detection with anti-IL-27B/EBI3 (FIG. 12), capture with anti-human p35 and detection with anti-human IL-6RA (FIG. 13), capture with anti-human p35 and detection with anti-IL27B/EBI3 (FIG. 14), and capture with anti-IL27B/EBI3 and detection with anti-human IL-6RA (FIG. 15).

The sequence of an illustrative charge polarized core domain (negative – positive, *i.e.,* "alpha core domain") is provided by SEQ ID NO: 16 and an illustrative alpha core domain comprising knob in hole mutations is provided in SEQ ID NO: 24.

The sequence of an illustrative charge polarized core domain (positive – negative, *i.e.,* "beta core domain") is provided by SEQ ID NO: 17 and an illustrative beta core domain comprising knob in hole mutations is provided in SEQ ID NO: 25.

The sequences of the components of illustrative polypeptide chains are set forth in SEQ ID NO: 18 for the Gp130 ECD (Type 1), in SEQ ID NO: 19 for the IL-6RA ECD (Type 1), in SEQ ID NO: 20 for the IL-12a (Type 2, first part of IL-35), and in SEQ ID NO: 21 for the IL-27b (Type 2, second part of IL-35).

An illustrative Gp130-Alpha-IL12A chain had the sequence set forth in SEQ ID NO: 22, and an illustrative IL6RA-Beta-IL27B chain had the sequence set forth in SEQ ID NO: 23.

In alternate embodiment, an IL-6R-Fc-IL-35 heterodimeric protein can comprise an IL6RA-Alpha-IL12a chain (SEQ ID NO: 34) and a Gp130-Beta-IL27b chain (SEQ ID NO: 35).

Example 2: Further Characterization of the IL-6R-Fc-IL-35 Heterodimeric Protein

Size-exclusion chromatography (SEC) was performed with the IL-6R-Fc-IL-35 heterodimeric protein. The appearance of a single peak by SEC, with an absorbance wavelength of 210 nm, indicated that there is likely only a single species of protein present, which was intended from using charge polarized linker domains (Fc-alpha and Fc-beta) in the two constructs (FIG. 16A). Interestingly, SEC with an absorbance wavelength of 280 nm showed a second, lower molecular weight band (FIG. 16B).

IL-6R-Fc-IL-35 heterodimeric protein was then used in an IL-6 SINK Assay. Here, the ability of the IL-6R-Fc-IL-35 heterodimeric protein to sequester IL6 was tested. Cultures of DS-1 cells, a B cell line that is dependent on

exogenous IL6 for survival, was incubated with the IL-6R-Fc-IL-35 heterodimeric protein and in the presence of exogenous IL6. When the DS-1 cells are not exposed to IL6, cell death results. Thus, these experiments were conducted to determine if the IL-6R-Fc-IL-35 heterodimeric protein can sequester IL6 and lead to DS-1 cell death.

DS-1 cells were cultured in the presence of IL-6R-Fc-IL-35 at increasing molar ratios to IL-6 for 24 hours. Cell death was measured by caspase 3/7 activity (with a luciferase readout).

FIG. 17 shows that IL-6R-Fc-IL-35 heterodimeric protein (identified as Lot '00 and Lot '48) are able to induce cell death in DS-1 cells. Indeed, depending on the lot used, the heterodimeric protein showed between 7 and 281 times greater sequestering of IL-6 than Tocilizumab (an anti-human IL-6 receptor monoclonal antibody which blocks DS-1's binding to IL6) depending on the lot used.

The functionality of IL-6R-Fc-IL-35 heterodimeric protein was then tested. IL-35 has been reported to induce an atypical regulatory phenotype in CD4 T Cells, which is characterized by little or no FoxP3 production coupled with production of IL-35. Additionally, IL-35 is known to turn off TGF-β and IL-10 production.

Here, magnetically enriched human naïve CD4 T cells were isolated from a single donor and activated with αCD3/αCD28 beads and cultured for 5 days in the presence of the indicated agent (shown in FIG. 18). Total mRNA was isolated and RT-qPCR performed.

FIG. 18 shows that the IL-6R-Fc-IL-35 heterodimeric protein (identified as HdA '00) induces IL-35 (which is a dimer of EB13 and IL12A) production. Surprisingly, the heterodimeric protein also increases production of FoxP3. Moreover, the IL-6R-Fc-IL-35 heterodimeric protein was permissive for cell proliferation, unlike the other treated agents. Although, IL-35 is known to turn off TGF-β and IL-10 production, the IL-6R-Fc-IL-35 heterodimeric protein resulted in detectable levels of IL-10 production (2X over control). Finally, the heterodimeric protein has no notable impact on IL-6 production.

Example 3: Construction and Characterization of the IL-21R-Fc-IL-35 Heterodimeric Protein

Constructs encoding an IL21r-Alpha-IL12a chain and an IL2rg-Beta-IL27B chain were dual transfected into CHO cells, followed by purification of the secreted protein using protein A. When an IL21r-Alpha-IL12a chain and an IL2rg-Beta-IL27B chain are combined (within a cell or *in vitro*), they form a heterodimeric protein referred to herein as IL-21R-Fc-IL-35. (FIG. 19A).

Western blots were performed on expressed heterodimeric proteins. These revealed, under denaturing and deglycosylated conditions, bands corresponding to the predicted molecular weights of the IL21r-Alpha-IL12a chain and the IL2rg-Beta-IL27B chain (FIG. 19B).

Size-exclusion chromatography (SEC) was performed with the IL-21R-Fc-IL-35 heterodimeric protein. The appearance of a single peak by SEC indicated that there is likely only a single species of protein present, which was intended from using charge polarized linker domains (Fc-alpha and Fc-beta) in the two constructs (FIG. 20).

In these experiments, an illustrative charge polarized core domain (negative – positive, *i.e.,* "alpha core domain") sequence is provided by SEQ ID NO: 16 and an illustrative alpha core domain comprising knob in hole mutations

is provided in SEQ ID NO: 24. An illustrative charge polarized core domain (positive – negative, *i.e.,* "beta core domain") sequence is provided by SEQ ID NO: 17 and an illustrative beta core domain comprising knob in hole mutations is provided in SEQ ID NO: 25.

The sequences of the components of illustrative polypeptide chains used in this example are set forth in SEQ ID NO: 26 for the extracellular domain of IL-21r, in SEQ ID NO: 27 for the extracellular domain of IL2RG, in SEQ ID NO: 20 for the IL-12a, and in SEQ ID NO: 21 for the IL-27b. An illustrative IL21r-Alpha-IL12a chain had the sequence set forth in SEQ ID NO: 28, and an illustrative IL2rg-Beta-IL27B chain had the sequence set forth in SEQ ID NO: 29.

In alternate embodiment, an IL-21R-Fc-IL-35 heterodimeric protein can comprise an IL2rg-Alpha-IL27B chain (SEQ ID NO: 36) and an IL21r-Beta-IL12a chain (SEQ ID NO: 37).

Example 4: Construction and Characterization of the IFNγR-Fc-IL-35 Heterodimeric Protein

Constructs encoding an IFNgR-Alpha-IL12a chain and an IFNGR2-Beta-IL27B chain were dual transfected into CHO cells, followed by purification of the secreted protein using protein A. When an IFNgR-Alpha-IL12a chain and an IFNGR2-Beta-IL27B chain are combined (within a cell or *in vitro*), they form a heterodimeric protein referred to herein as IFNγR-Fc-IL-35.

Western blots were performed on the IFNγR-Fc-IL-35 heterodimeric protein comprising the IFNgR-Alpha-IL12a chains and the hIFNGR2-Beta-IL27B chain probed with an antibody indicated below each blot. These revealed, under denaturing and deglycosylated conditions, bands corresponding to the predicted molecular weights of the IFNgR-Alpha-IL12a and the hIFNGR2-Beta-IL27B (FIG. 21). Bands noted in yellow highlighting are non-specific bands.

Size-exclusion chromatography (SEC) was performed with the IFNγR-Fc-IL-35 heterodimeric protein. The appearance of a single peak by SEC indicated that there is likely only a single species of protein present, which was intended from using charge polarized linker domains (Fc-alpha and Fc-beta) in the two constructs (FIG. 22).

In these experiments, an illustrative charge polarized core domain (negative – positive, *i.e.,* "alpha core domain") sequence is provided by SEQ ID NO: 16 and an illustrative alpha core domain comprising knob in hole mutations is provided in SEQ ID NO: 24. An illustrative charge polarized core domain (positive – negative, *i.e.,* "beta core domain") sequence is provided by SEQ ID NO: 17 and an illustrative beta core domain comprising knob in hole mutations is provided in SEQ ID NO: 25.

The sequences of the components of illustrative polypeptide chains used in this example are set forth in SEQ ID NO: 30 for the extracellular domain of IFNgR, in SEQ ID NO: 31 for the extracellular domain of IFNGR2, in SEQ ID NO: 20 for the IL-12a, and in SEQ ID NO: 21 for the IL-27b. An illustrative IFNgR-Alpha-IL12a chain had the sequence set forth in SEQ ID NO: 32, and an illustrative IFNGR2-Beta-IL27B chain had the sequence set forth in SEQ ID NO: 33.

In alternate embodiment, an IFNγR-Fc-IL-35 heterodimeric protein can comprise an IFNGR2-Alpha-IL27B chain (SEQ ID NO: 39) and an IFNgR-Beta-IL12a chain (SEQ ID NO: 38).

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 heterodimeric protein comprising a first polypeptide chain and a second polypeptide chain, wherein

the first polypeptide chain comprises a first subunit of a first protein at the amino terminus linked by a first charge polarized core domain to a first subunit of a second protein at the carboxy terminus; and

the second polypeptide chain comprises a second subunit of the first protein at the amino terminus linked by a second charge polarized core domain to a second subunit of the second protein at the carboxy terminus; and

wherein the first polypeptide chain and the second polypeptide chain forms a heterodimer through electrostatic interactions between positively charged amino acid residues and negatively charged amino acid residues on the first and second charge polarized core domains.

2. The heterodimeric protein of claim 1, wherein the first and/or second charge polarized core domain comprises a polypeptide linker, optionally selected from a flexible amino acid sequence, IgG hinge region, or antibody sequence.

3. The heterodimeric protein of claim 2, wherein the linker is a synthetic linker, optionally PEG.

4. The heterodimeric protein of claim 2, wherein the linker comprises the hinge-CH2-CH3 Fc domain derived from IgG1, optionally human IgG1.

5. The heterodimeric protein of claim 2, wherein the linker comprises the hinge-CH2-CH3 Fc domain derived from IgG4, optionally human IgG4.

6. The heterodimeric protein of any one of the above claims, wherein the first and/or second charge polarized core domain further comprise peptides having positively and/or negatively charged amino acid residues at the amino and carboxy terminus of the charge polarized core domain.

7. The heterodimeric protein of claim 6, wherein the peptide comprising positively charged amino acid residues may include one or more of amino acids selected from His, Lys, and Arg.

8. The heterodimeric protein of claim 7, wherein the peptide comprising positively charged amino acid residues comprises a sequence selected from $Y_nX_nY_nX_nY_n$ (where X is a positively charged amino acid such as arginine, histidine or lysine and Y is a spacer amino acid such as serine or glycine) (SEQ ID NO: 1), $YY_nXX_nYY_nXX_nYY_n$ (where X is a positively charged amino acid such as arginine, histidine or lysine and Y is a spacer amino acid such as serine or glycine) (SEQ ID NO: 3), and $Y_nX_nCY_nX_nY_n$ (where X is a positively charged amino acid such as arginine, histidine or lysine and Y is a spacer amino acid such as serine or glycine) (SEQ ID NO: 5).

9. The heterodimeric protein of claim 8, wherein the peptide comprising positively charged amino acid residues comprises the sequence RKGGKR (SEQ ID NO: 11) or GSGSRKGGKRGS (SEQ ID NO: 12).

10. The heterodimeric protein of claim 6, wherein the peptide comprising negatively charged amino acid residues may include one or more amino acids selected from Asp and Glu.

11. The heterodimeric protein of claim 9, wherein the peptide comprising negatively charged amino acid residues comprises a sequence selected from $Y_nZ_nY_nZ_nY_n$ (where Z is a negatively charged amino acid such as aspartic acid or glutamic acid and Y is a spacer amino acid such as serine or glycine) (SEQ ID NO: 2), $YY_nZZ_nYY_nZZ_nYY_n$ (where Z is a negatively charged amino acid such as aspartic acid or glutamic acid and Y is a spacer amino acid such as serine or glycine) (SEQ ID NO: 4), and $Y_nZ_nCY_nZ_nY_n$ (where Z is a negatively charged amino acid such as aspartic acid or glutamic acid and Y is a spacer amino acid such as serine or glycine) (SEQ ID NO: 6).

12. The heterodimeric protein of claim 8, wherein the peptide comprising positively charged amino acid residues comprises the sequence DEGGED (SEQ ID NO: 13) or GSGSDEGGEDGS (SEQ ID NO: 14).

13. The heterodimeric protein of any one of the above claims, wherein the first and/or second charge polarized core domain comprises one or more amino acid changes for promoting heterodimerization *via* icreased hydrogen bonding and/or van der Waals forces.

14. The heterodimeric protein of claim 13, wherein the one or more amino acid changes creates a knob in hole motif.

15. The heterodimeric protein of claim 14, wherein the knob in hole motif is formed by one or more amino acid changes that replaces one or more tyrosine (Y) residues with one or more threonine (T) residues in the first charge polarized core domain comprises and/or is formed by one or more amino acid changes that replaces one or more threonine (T) residues with one or more tyrosine (Y) residues in the second charge polarized core domain.

16. The heterodimeric protein of claim 14 or claim 15, wherein the knob in hole motif is formed by one or more amino acid changes that replaces one or more tyrosine (Y) residues with one or more threonine (T) residues in the second charge polarized core domain comprises and/or is formed by one or more amino acid changes that replaces one or more threonine (T) residues with one or more tyrosine (Y) residues in the first charge polarized core domain.

17. The heterodimeric protein of any one of claims 13 to 16, wherein one or both of the charge polarized core domains comprise one or more effector and complement silencing substitutions selected from L234A and L235A (LALA) , and optionally, P329G.

18. The heterodimeric protein of any one of claims 13 to 17, wherein one or both of the charge polarized core domains comprise one or more half-life extension mutations selected from M252Y, S254T, and T256E.

19. The heterodimeric protein of any one of the above claims, wherein the first protein is selected from Table 1.

20. The heterodimeric protein of any one of the above claims, wherein the second protein is selected from Table 1.

21. The heterodimeric protein of any one of claims 1-20, wherein the first and/or second protein is selected from a cytokine, a growth factor, and/or a hormone.

22. The heterodimeric protein of claim 21, wherein the first and/or second protein is an interleukin.

23. The heterodimeric protein of claim 22, wherein the first and/or second protein is IL-35 comprising the IL12α and IL27β subunits.

24. The heterodimeric protein of any one of the above claims, wherein the first and/or second protein is selected from a receptor for a cytokine, a growth factor, and/or a hormone.

25. The heterodimeric protein of claim 24, wherein the first and/or second protein is a receptor for an interleukin.

26. The heterodimeric protein of claim 25, wherein the first and/or second protein is IL6 receptor comprising the IL6Rα and gp130 subunits.

27. The heterodimeric protein of claim 25, wherein the first and/or second protein is IL21 receptor comprising the IL21r and IL2rg subunits.

28. The heterodimeric protein of claim 25, wherein the first and/or second protein is IL21 receptor comprising the IFNgR and IFNGR2 subunits.

29. The heterodimeric protein of any one of the above claims, wherein the heterodimeric protein is capable of both (i) reducing or eliminating an immune inhibitory signal and (ii) increasing or activating an immune stimulatory signal.

30. The heterodimeric protein of any one of the above claims, wherein the heterodimeric protein is capable of increasing a ratio of effector T cells to regulatory T cells.

31. The heterodimeric protein of any one of the above claims, wherein the heterodimeric protein is capable of increasing and/or preventing a decrease in a sub-population of cytotoxic T cells; effector memory T cells; central memory T cells; $CD8^+$ stem cell memory effector cells; TH1 effector T-cells; TH2 effector T cells; TH9 effector T cells; TH17 effector T cells; and/or effector T cells secreting IL-2, IL-4 and/or IFN-γ.

32. A pharmaceutical composition, comprising the heterodimeric protein of any one of the above claims.

33. An expression vector, comprising a nucleic acid encoding the first and/or second polypeptide chains of the heterodimeric protein of any one of the above claims.

34. The expression vector of claim 33, wherein the expression vector is a mammalian expression vector.

35. The expression vector of claim 34, wherein the expression vector comprises DNA or RNA.

36. A host cell, comprising the expression vector of any one of claims 33-35.

37. A method of treating cancer, comprising administering to a subject in need thereof an effective amount of a pharmaceutical composition of claim 32 to a subject in need thereof.

38. A method of treating an autoimmune disease or disorder, comprising administering an effective amount of a pharmaceutical composition of claim 32 to a subject in need thereof.

39. A method of modulating a patient's immune response, comprising administering an effective amount of a pharmaceutical composition of claim 32 to a subject in need thereof.

40. The method of any one of claims 37-39, wherein the patient's T cells are activated by the extracellular domain having an immune stimulatory signal.

41. The method of any one of claims 37-39, wherein the patient has a tumor and one or more tumor cells are prevented from transmitting an immunosuppressive signal activated by the extracellular domain having an immune inhibitory signal.

42. The method of claim 38, wherein the autoimmune disease or disorder is selected from rheumatoid arthritis, systemic lupus erythematosus, diabetes mellitus, ankylosing spondylitis, Sjögren's syndrome, inflammatory bowel diseases (*e.g.,* colitis ulcerosa, Crohn's disease), multiple sclerosis, sarcoidosis, psoriasis, Grave's disease, Hashimoto's thyroiditis, psoriasis, hypersensitivity reactions (*e.g.*, allergies, hay fever, asthma, and acute edema cause type I hypersensitivity reactions), and vasculitis.

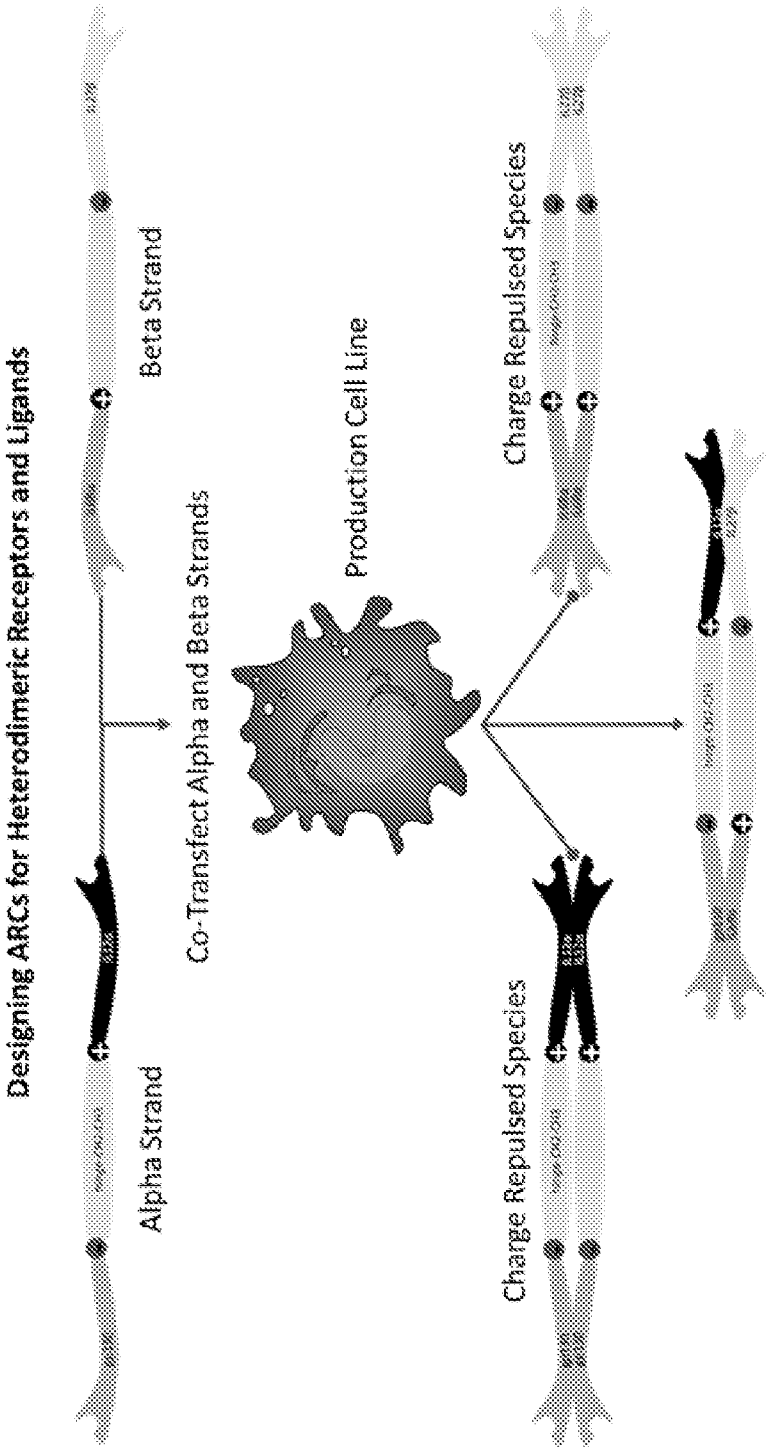

Designing ARCs for Heterodimeric Receptors and Ligands
Alpha Strand
Beta Strand
Co-Transfect Alpha and Beta Strands
Production Cell Line
Charge Repulsed Species
Charge Repulsed Species

FIG. 1

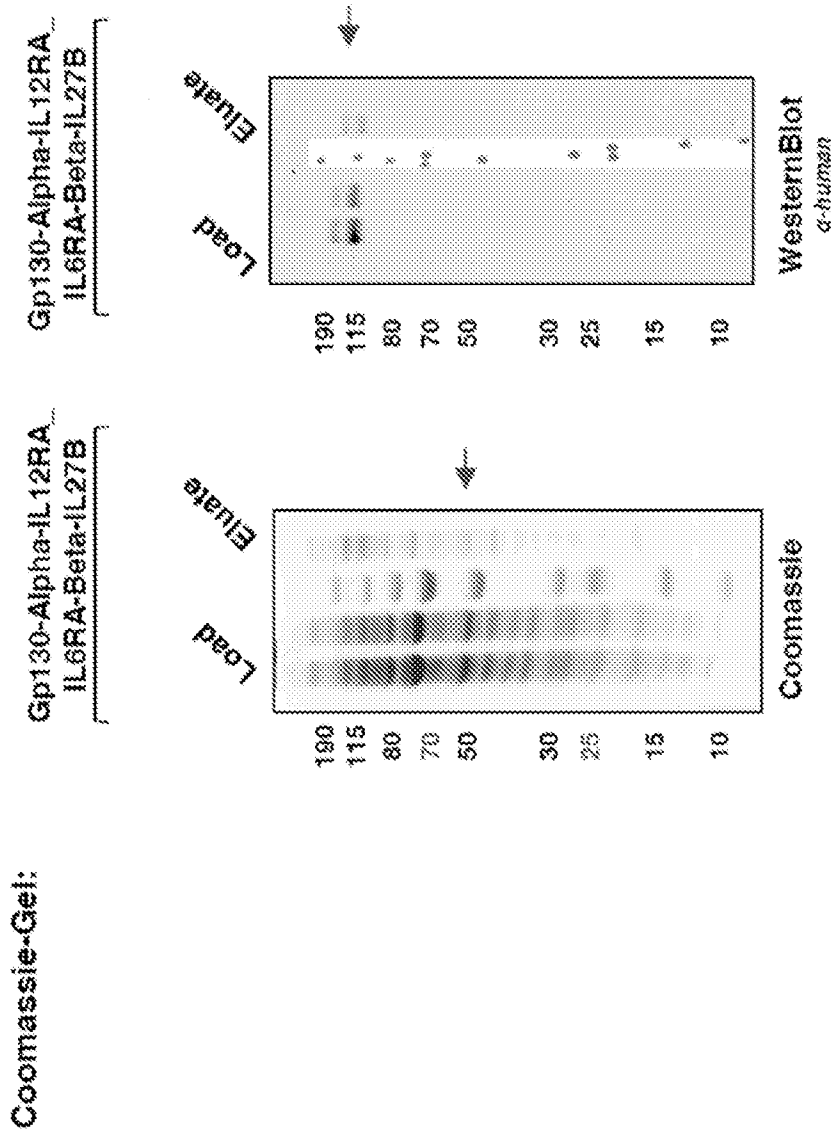

Coomassie-Gel:
Gp130-Alpha-IL12RA_
IL6RA-Beta-IL27B
Load
Eluate
190
115
80
70
50
30
25
15
10
Coomassie
Gp130-Alpha-IL12RA_
IL6RA-Beta-IL27B
Load
Eluate
190
115
80
70
50
30
25
15
10
WesternBlot
α-human

FIG. 2

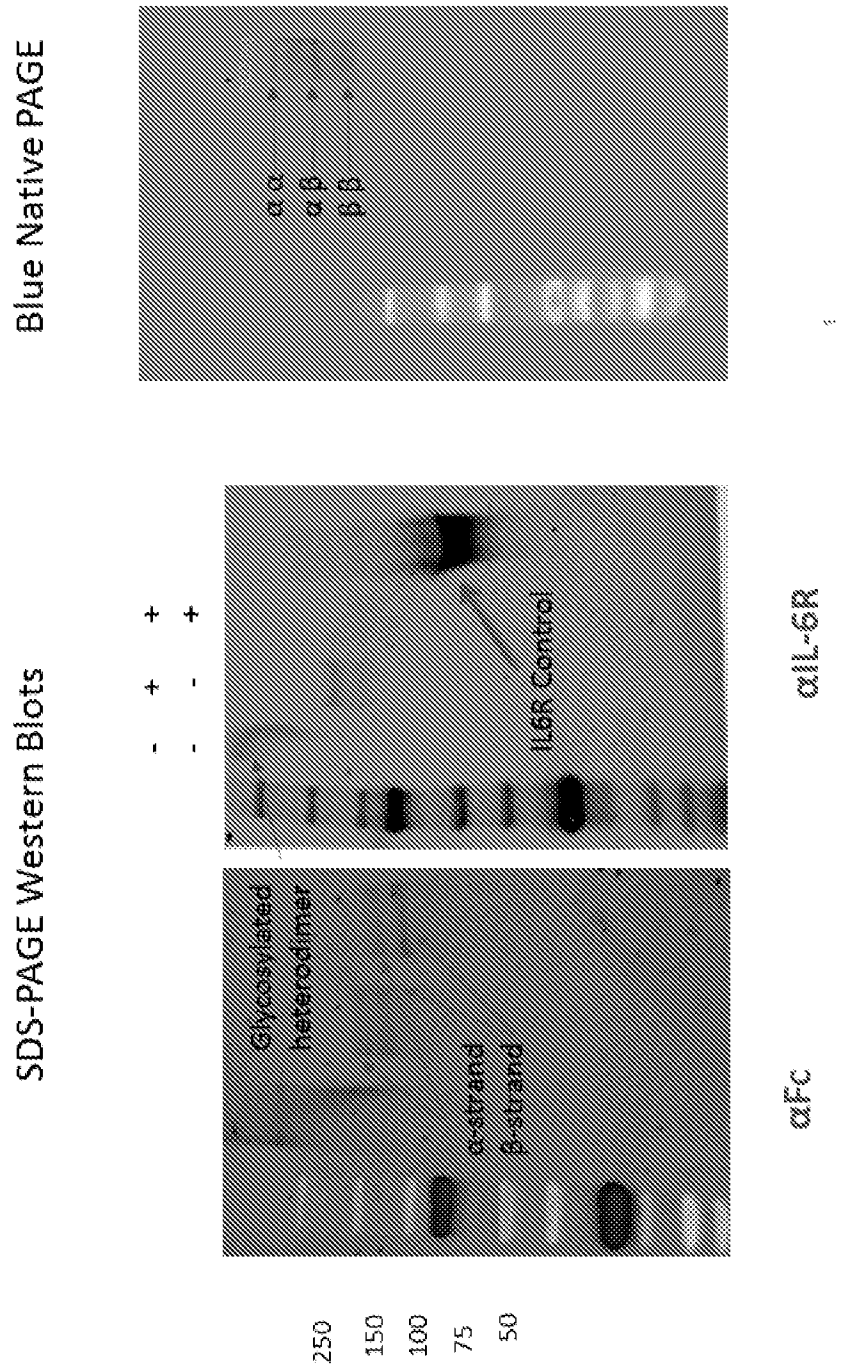
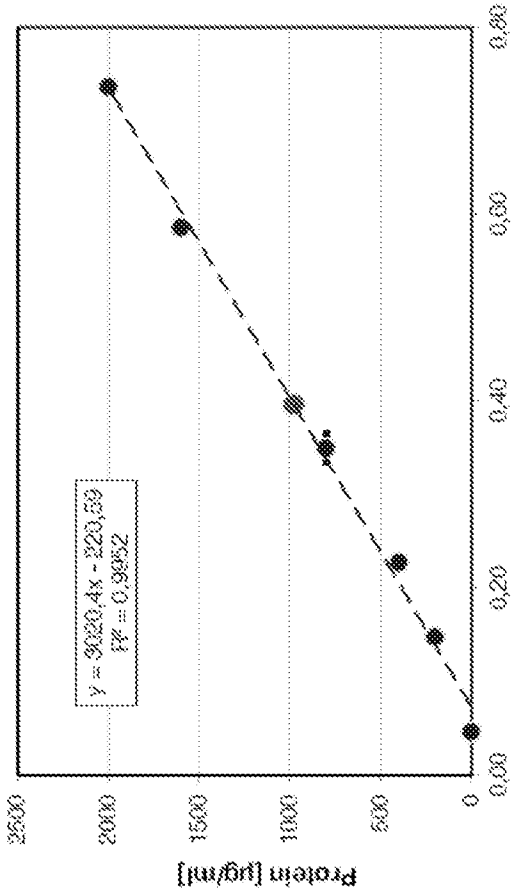

Blue Native PAGE
α α
α β
β β
SDS-PAGE Western Blots
- + + +
- + - -
Glycosylated heterodimer
α-strand
β-strand
IL6R Control
250
150
100
75
50
αFc
αIL-6R

FIG. 3



Sample	Abs at 280 nm	Stdev	Protein [µg/ml]	Stdev
Gp130-Alpha-IL12RA_IL6RA-Beta-IL27B	0,306	0,003	976,6	7,9

FIG. 4

FIG. 5

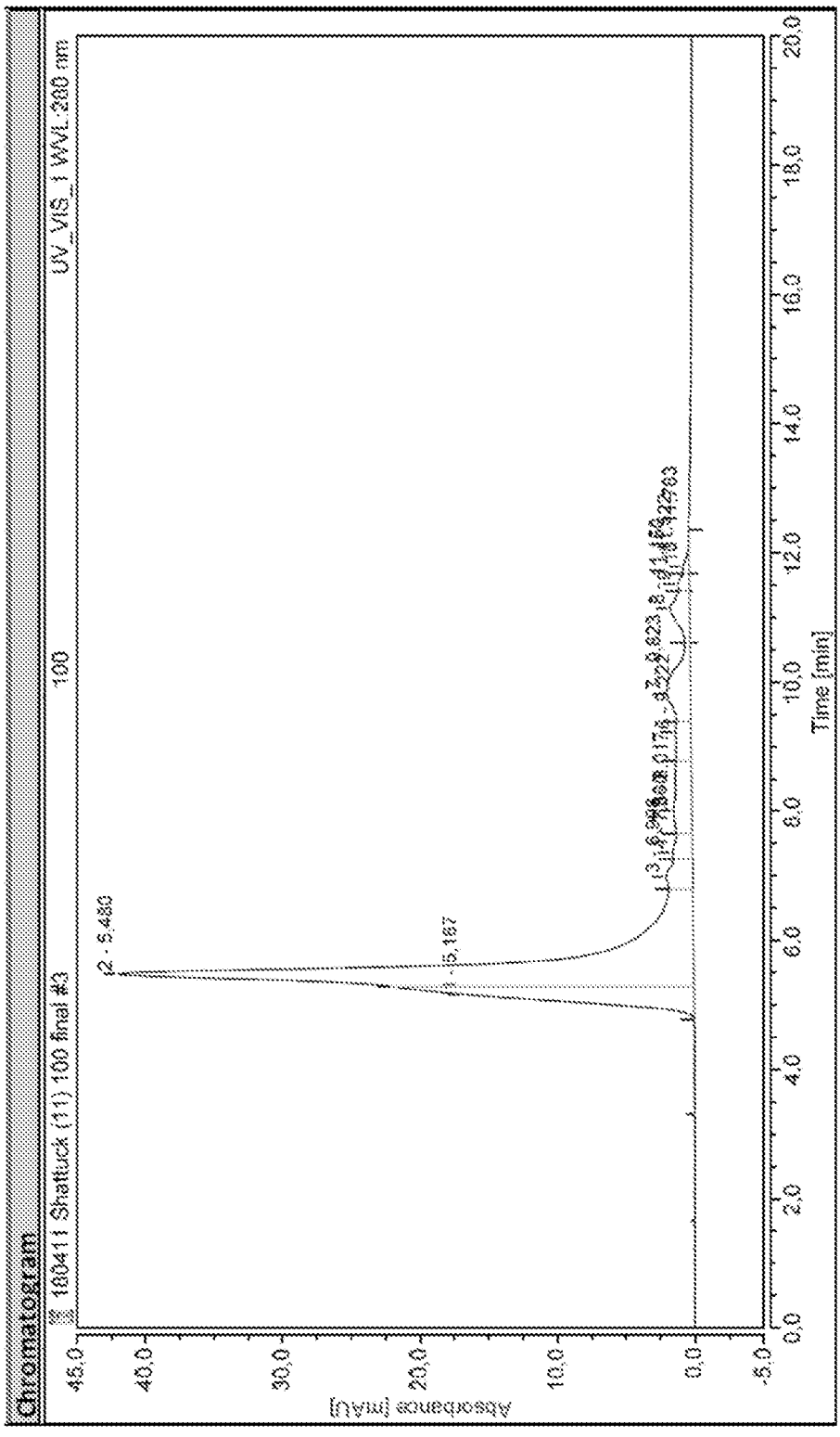

Chromatogram
180411 Shattuck (11) 100 final #3
UV_VIS_1 WVL:280 nm
100
2 - 5.480
1 - 15.187
Absorbance [mAU]
Time [min]
45.0
40.0
30.0
20.0
10.0
0.0
-5.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0

FIG. 6

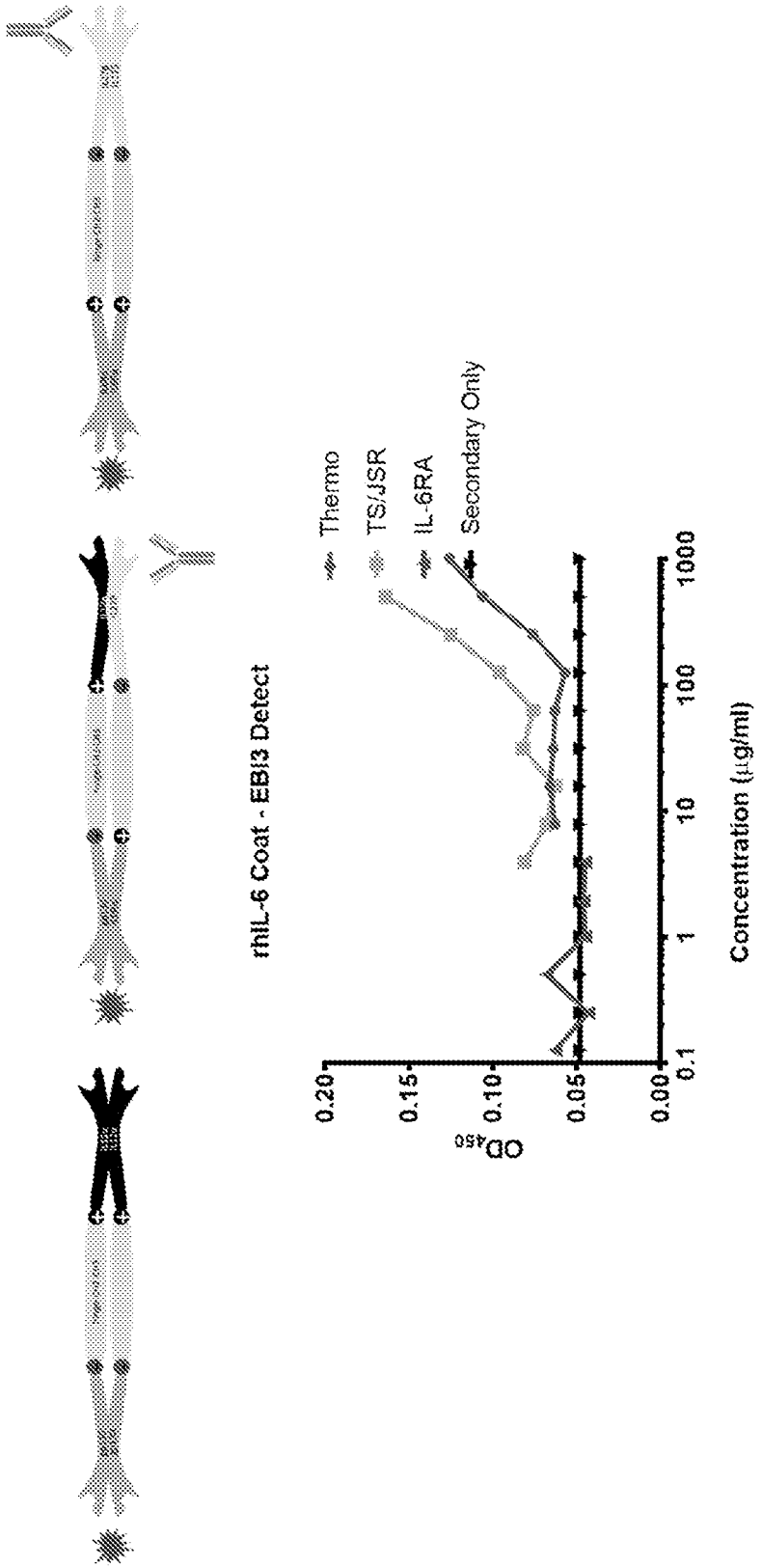

rhIL-6 Coat - EBI3 Detect
Thermo
TS/JSR
IL-6RA
Secondary Only
OD450
0.20
0.15
0.10
0.05
0.00
0.1
1
10
100
1000
Concentration (µg/ml)

FIG. 7

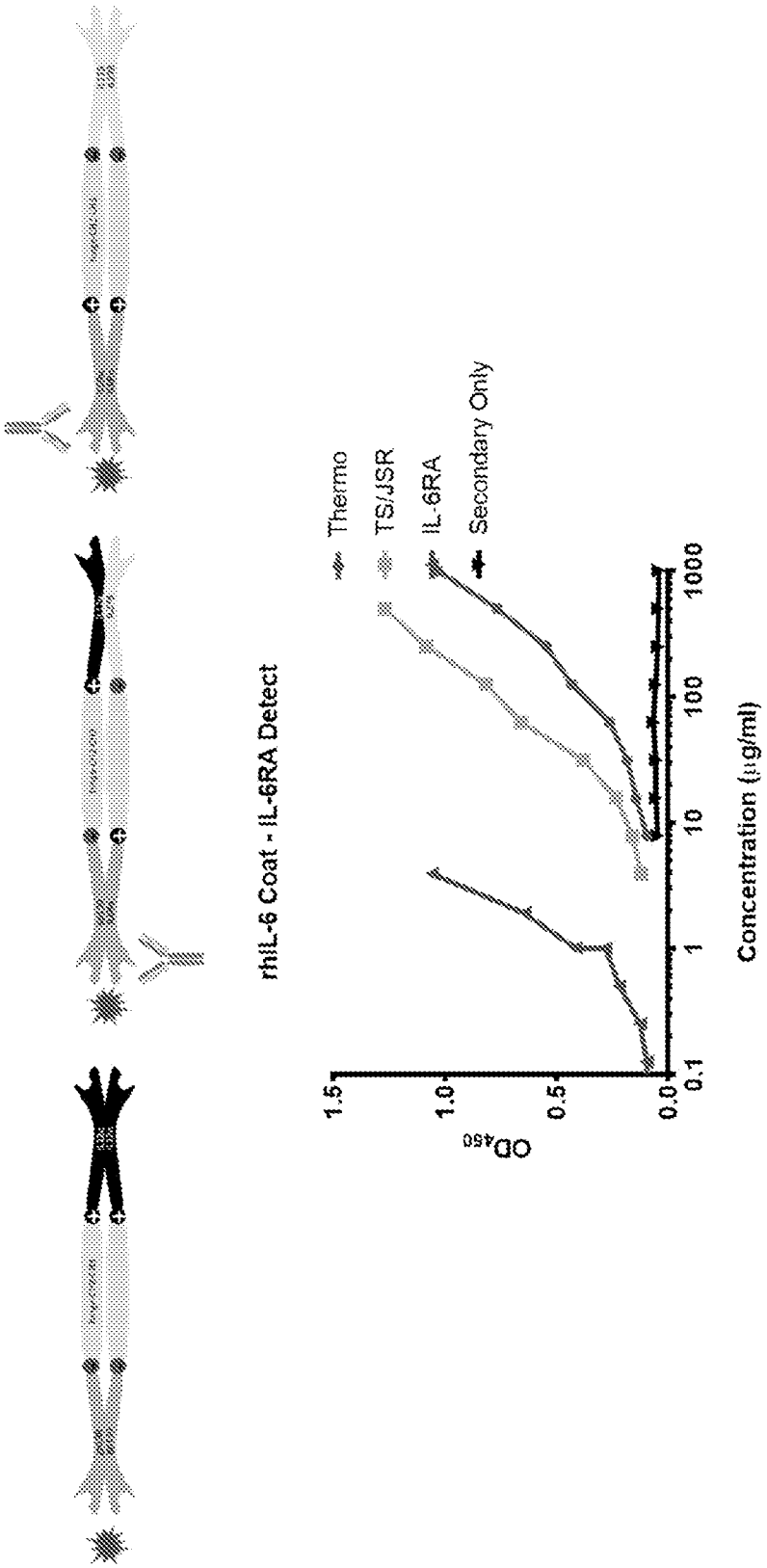

rhIL-6 Coat - IL-6RA Detect
Thermo
TS/JSR
IL-6RA
Secondary Only
OD450
1.5
1.0
0.5
0.0
0.1
1
10
100
1000
Concentration (µg/ml)

FIG. 8

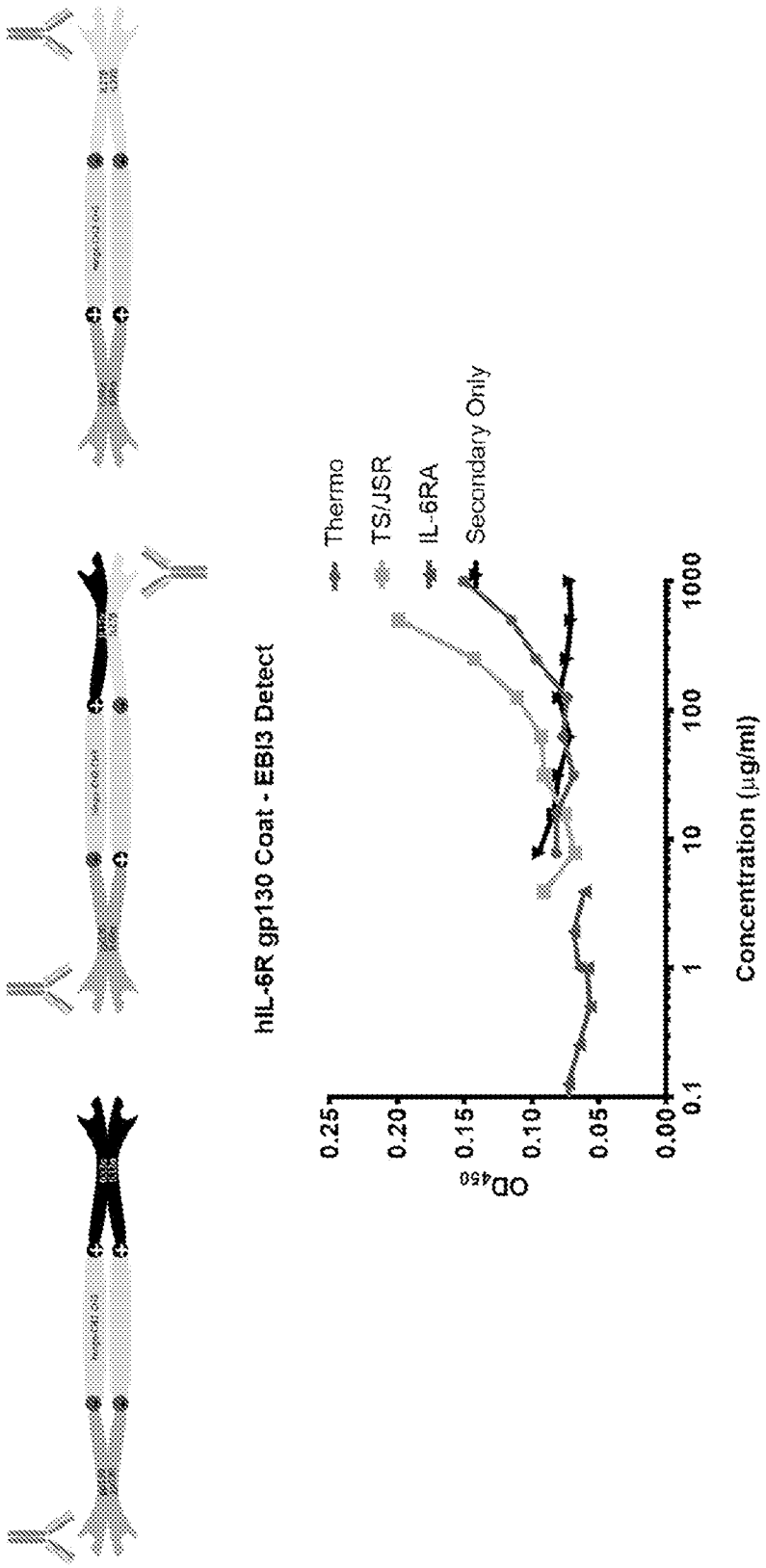

hIL-6R gp130 Coat - EBI3 Detect
Thermo
TS/JSR
IL-6RA
Secondary Only
OD450
0.25
0.20
0.15
0.10
0.05
0.00
0.1
1
10
100
1000
Concentration (µg/ml)

FIG. 9

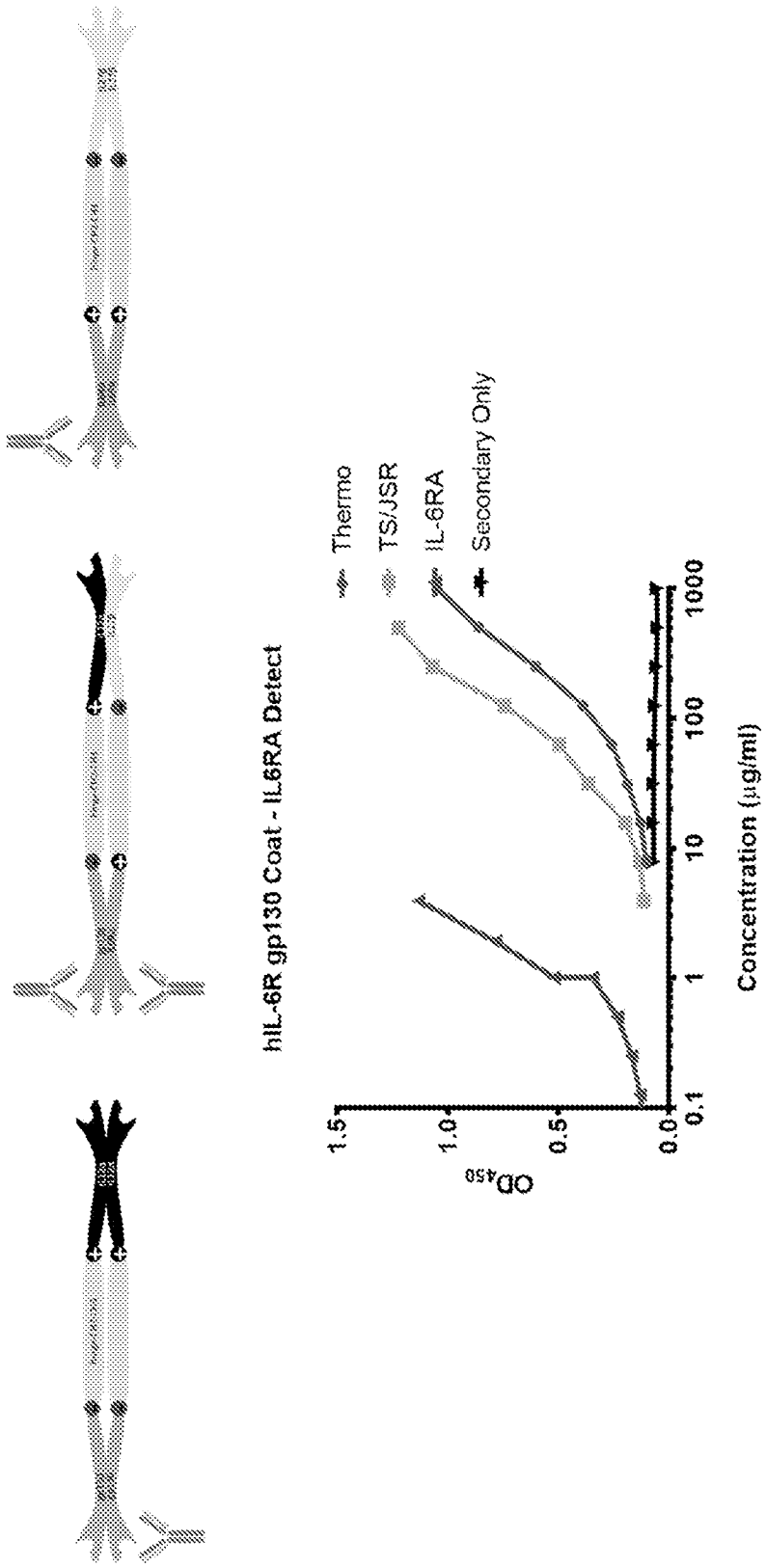

hIL-6R gp130 Coat - IL6RA Detect
Thermo
TS/JSR
IL-6RA
Secondary Only
OD450
1.5
1.0
0.5
0.0
0.1
1
10
100
1000
Concentration (µg/ml)

FIG. 10

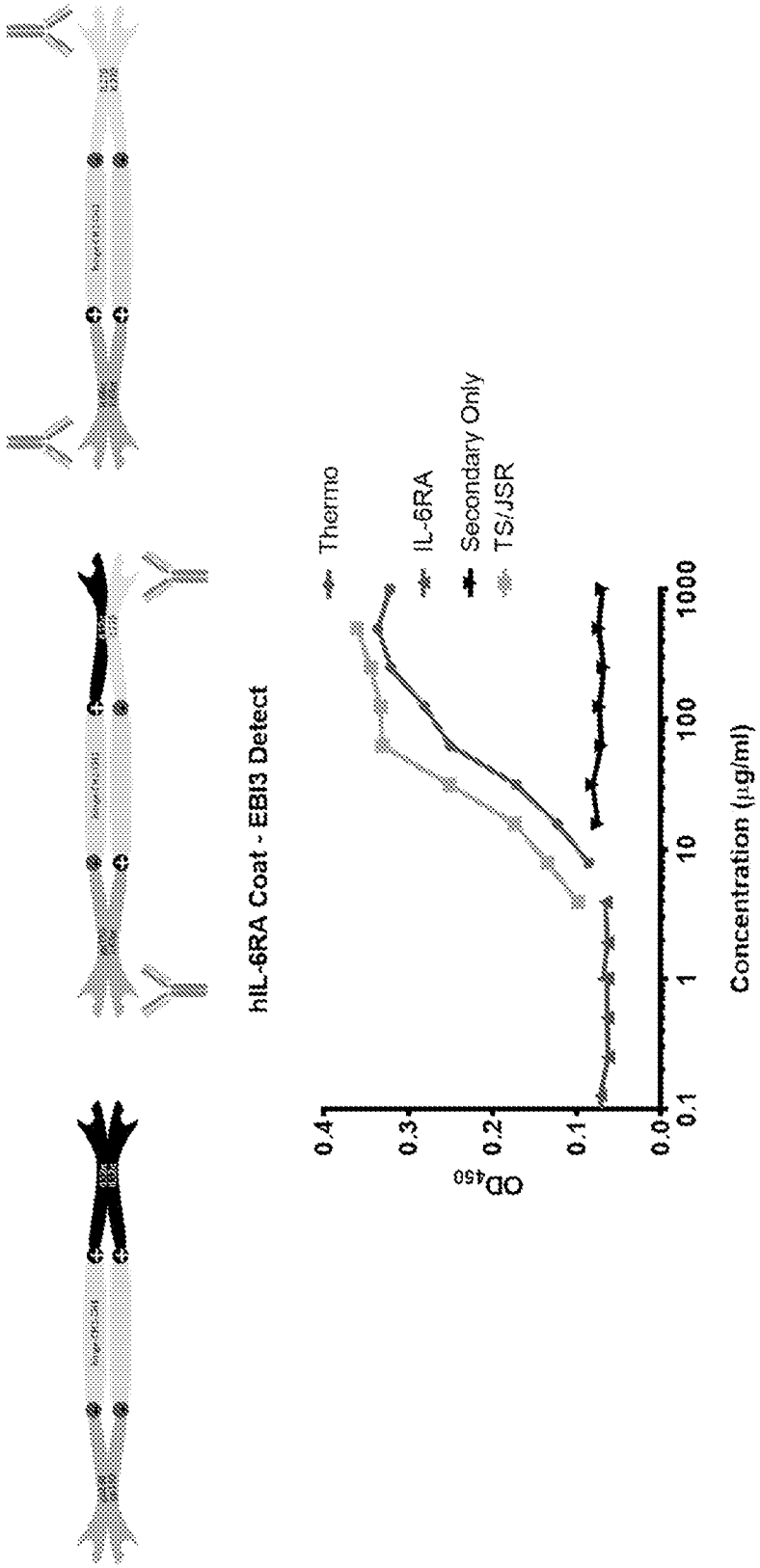

hIL-6RA Coat - EBI3 Detect
Thermo
IL-6RA
Secondary Only
TS/JSR
OD450
0.4
0.3
0.2
0.1
0.0
0.1
1
10
100
1000
Concentration (µg/ml)

FIG. 11

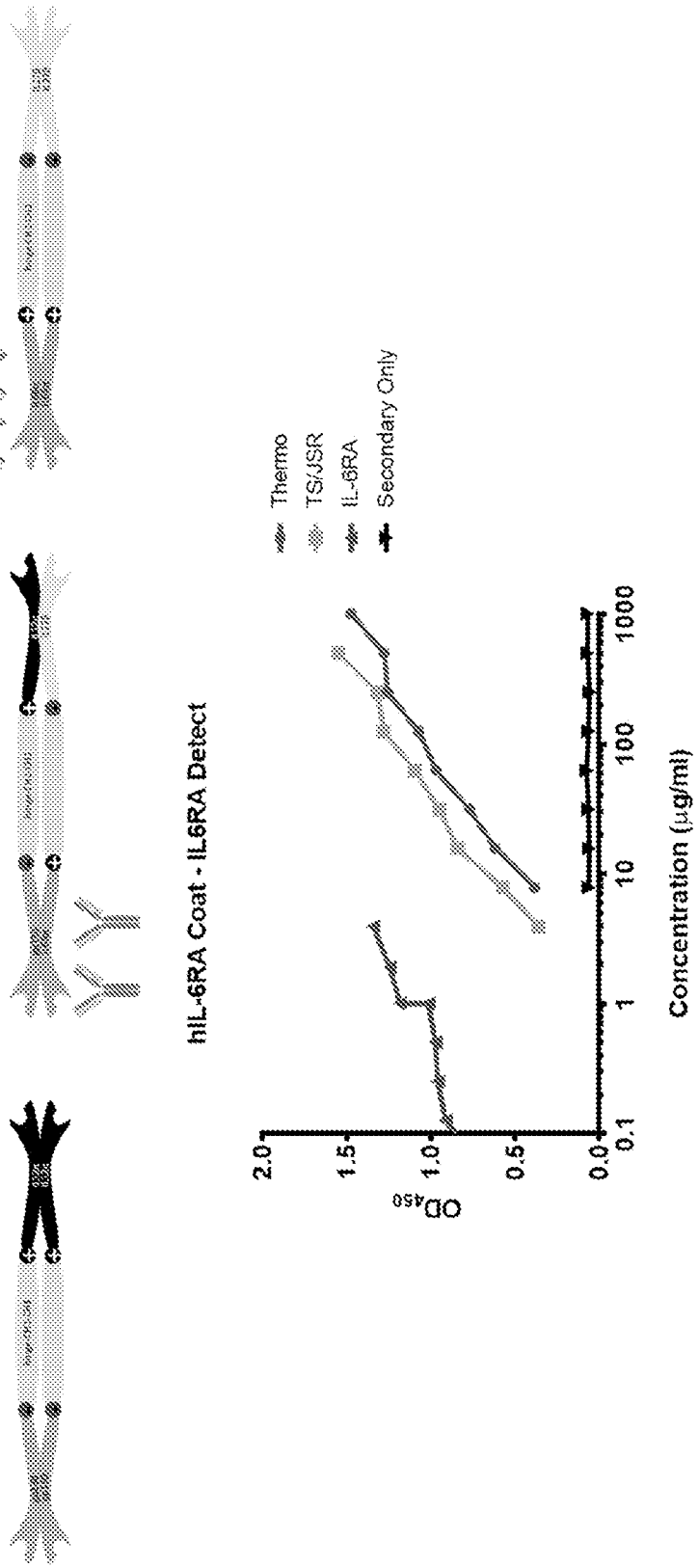

hIL-6RA Coat - IL6RA Detect
Thermo
TS/JSR
IL-6RA
Secondary Only
OD450
2.0
1.5
1.0
0.5
0.0
0.1
1
10
100
1000
Concentration (µg/ml)

FIG. 12

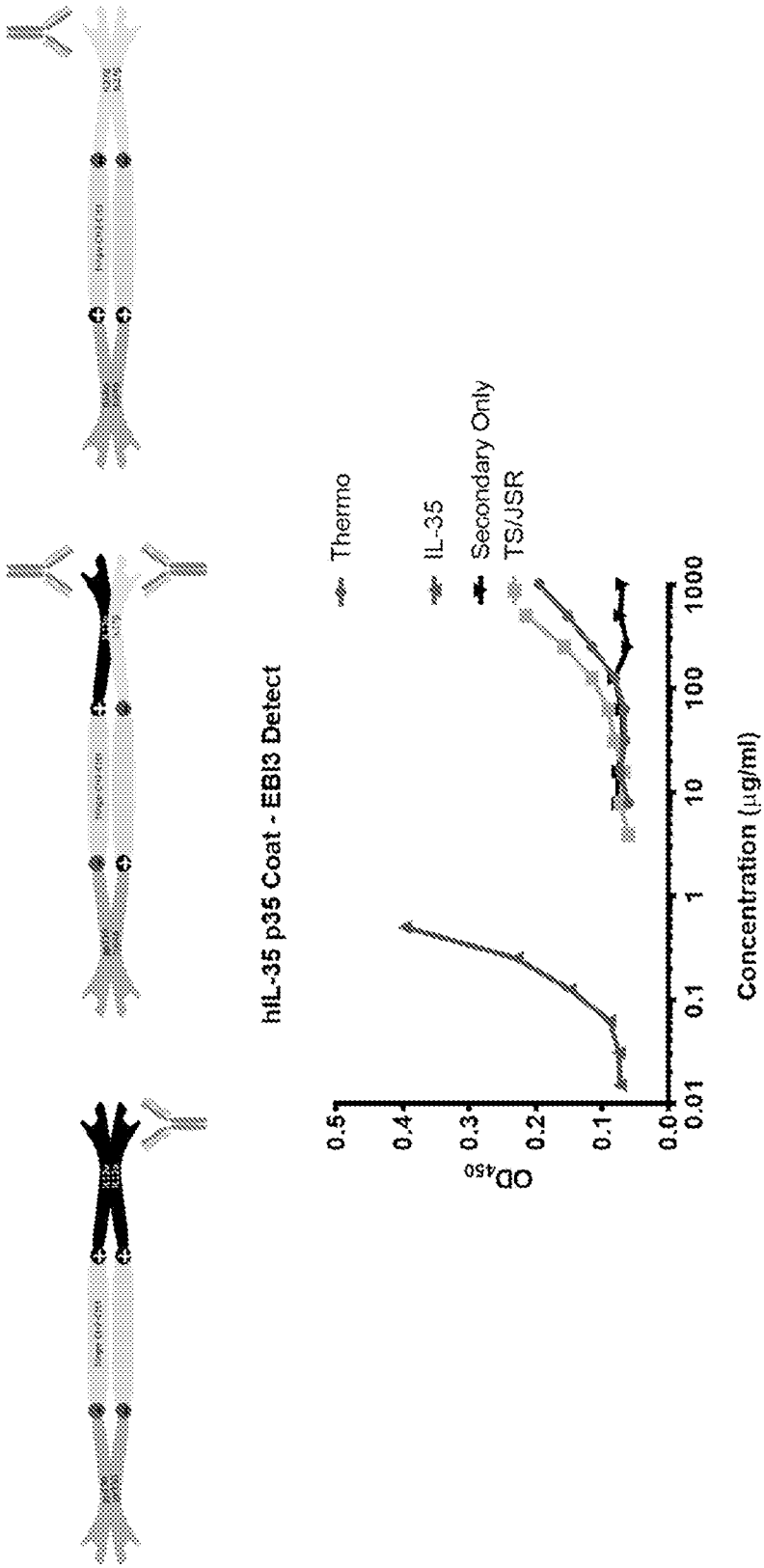

hIL-35 p35 Coat - EBI3 Detect
Thermo
IL-35
Secondary Only
TS/JSR
OD450
0.5
0.4
0.3
0.2
0.1
0.0
0.01
0.1
1
10
100
1000
Concentration (µg/ml)

FIG. 13

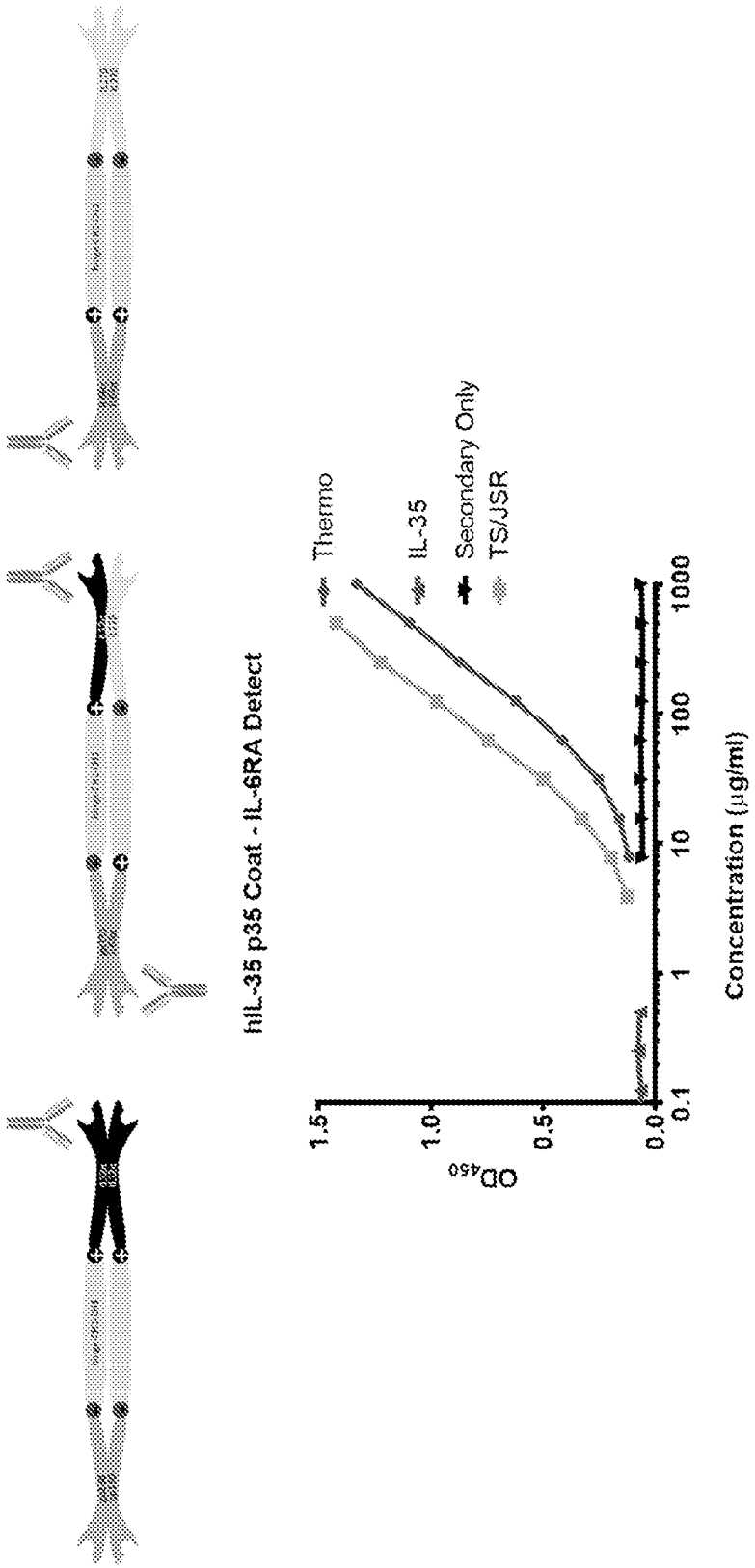

hIL-35 p35 Coat - IL-6RA Detect
Thermo
IL-35
Secondary Only
TS/JSR
OD450
1.5
1.0
0.5
0.0
0.1
1
10
100
1000
Concentration (µg/ml)

FIG. 14

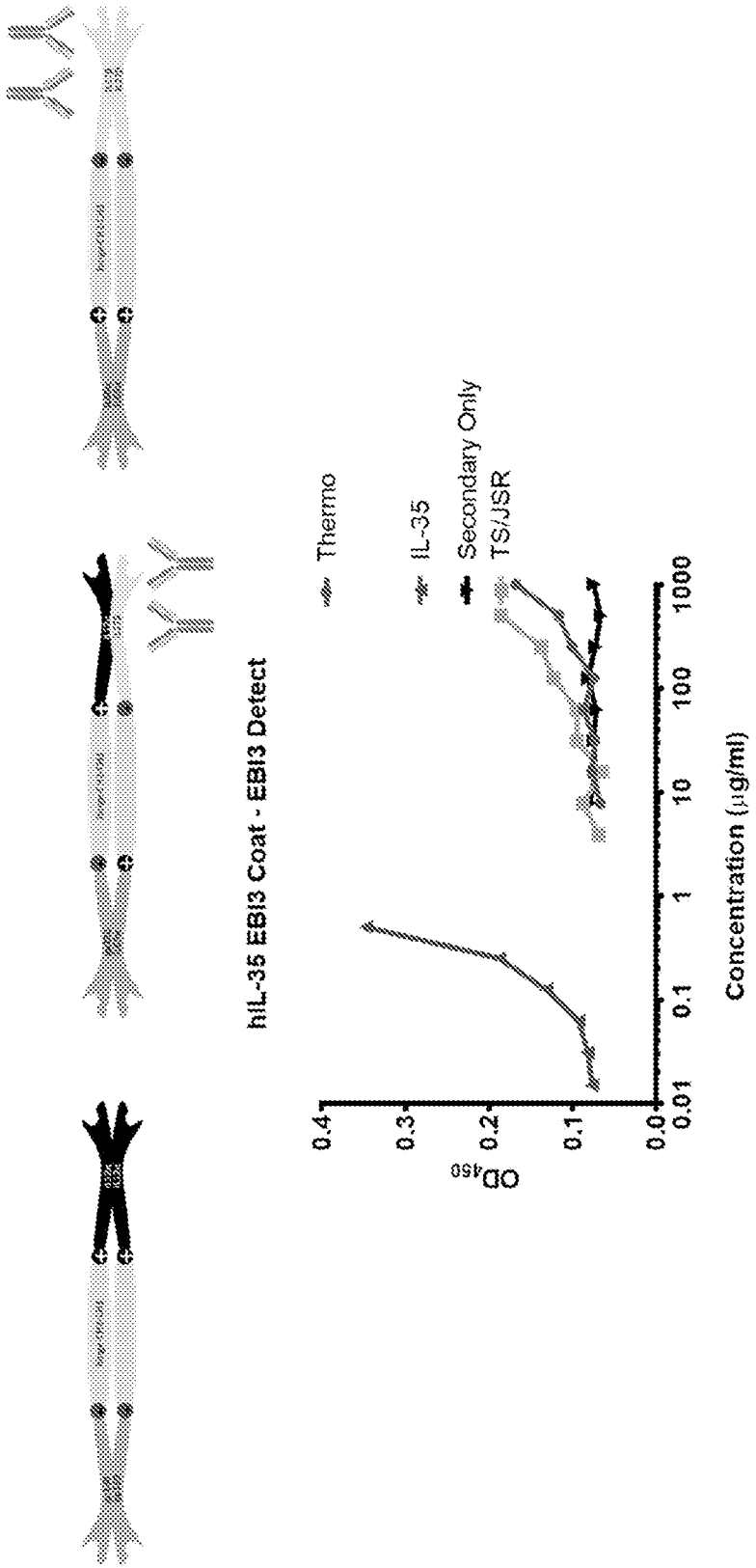

hIL-35 EBI3 Coat - EBI3 Detect
Thermo
IL-35
Secondary Only
TS/JSR
OD450
0.4
0.3
0.2
0.1
0.0
0.01
0.1
1
10
100
1000
Concentration (µg/ml)

FIG. 15

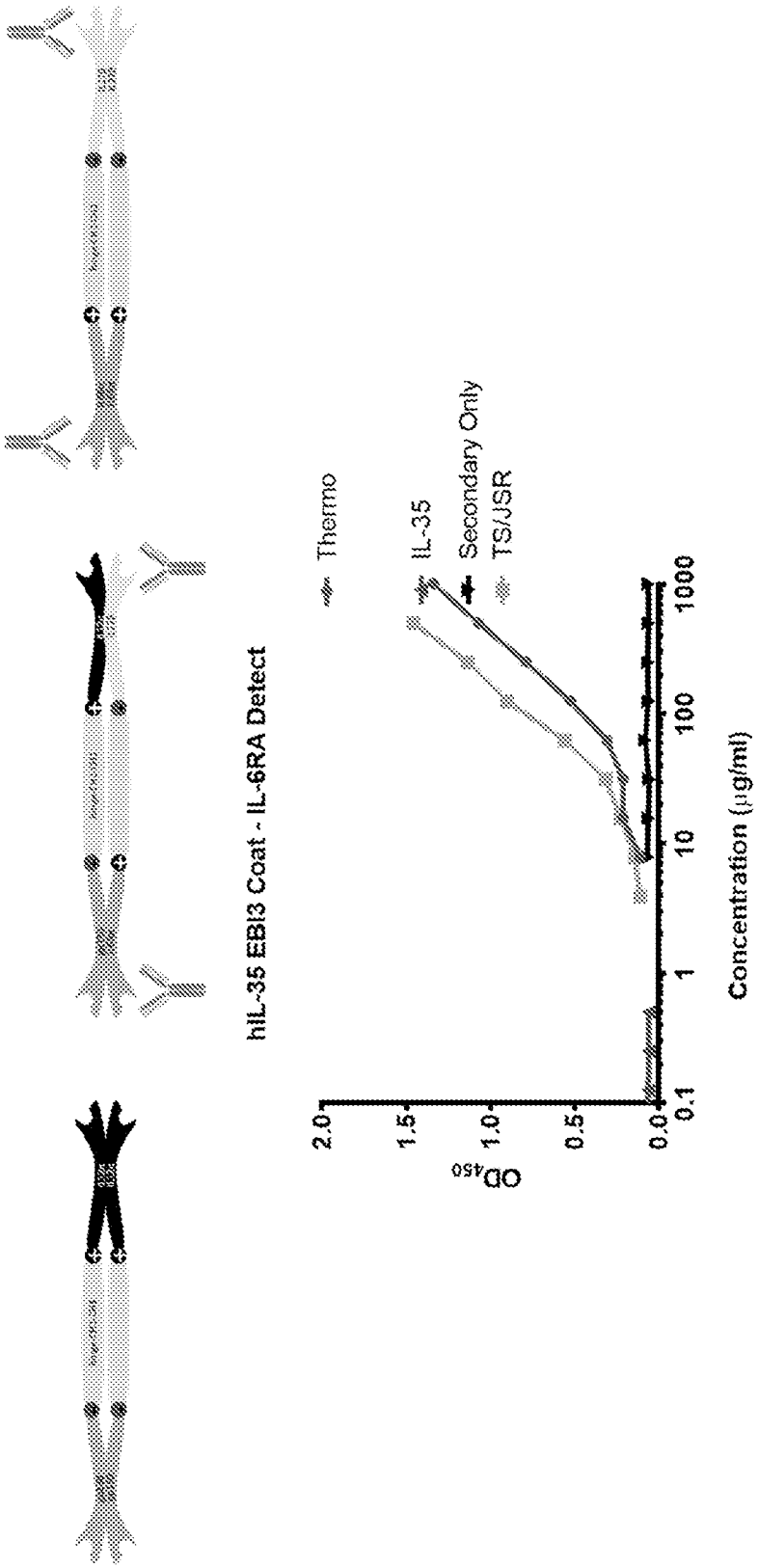

hIL-35 EBI3 Coat - IL-6RA Detect
Thermo
IL-35
Secondary Only
TS/JSR
OD450
2.0
1.5
1.0
0.5
0.0
0.1
1
10
100
1000
Concentration (µg/ml)

FIG. 16A

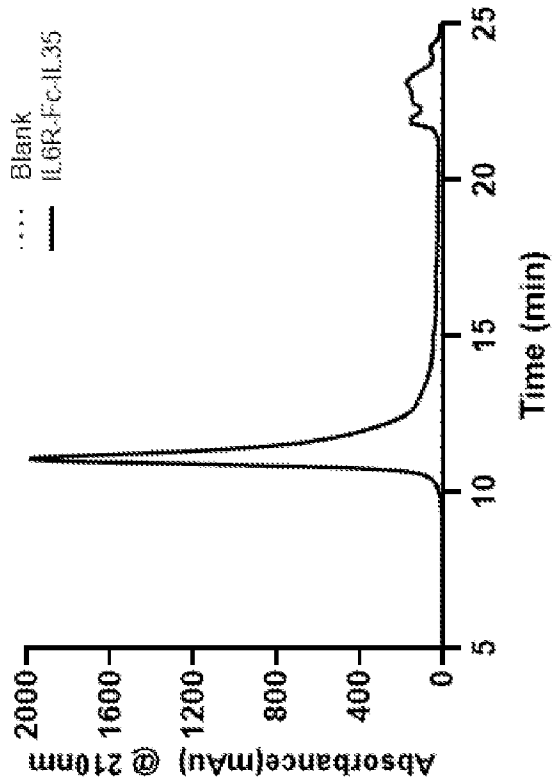


FIG. 16B

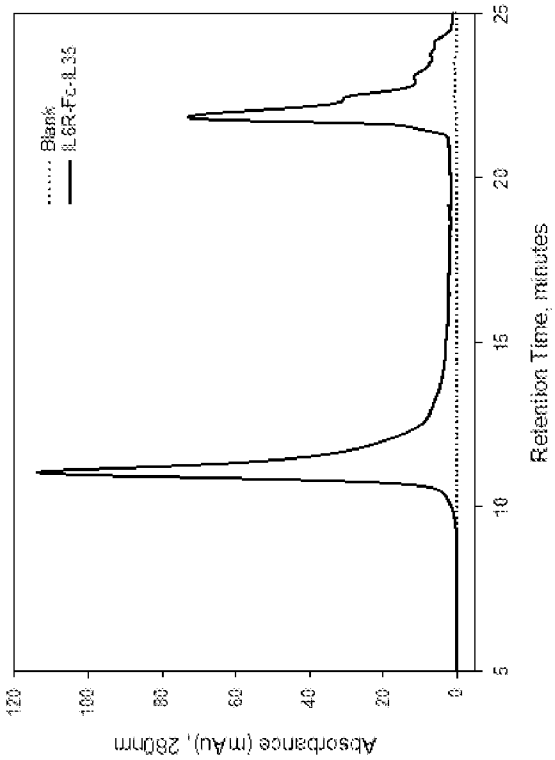


FIG. 17

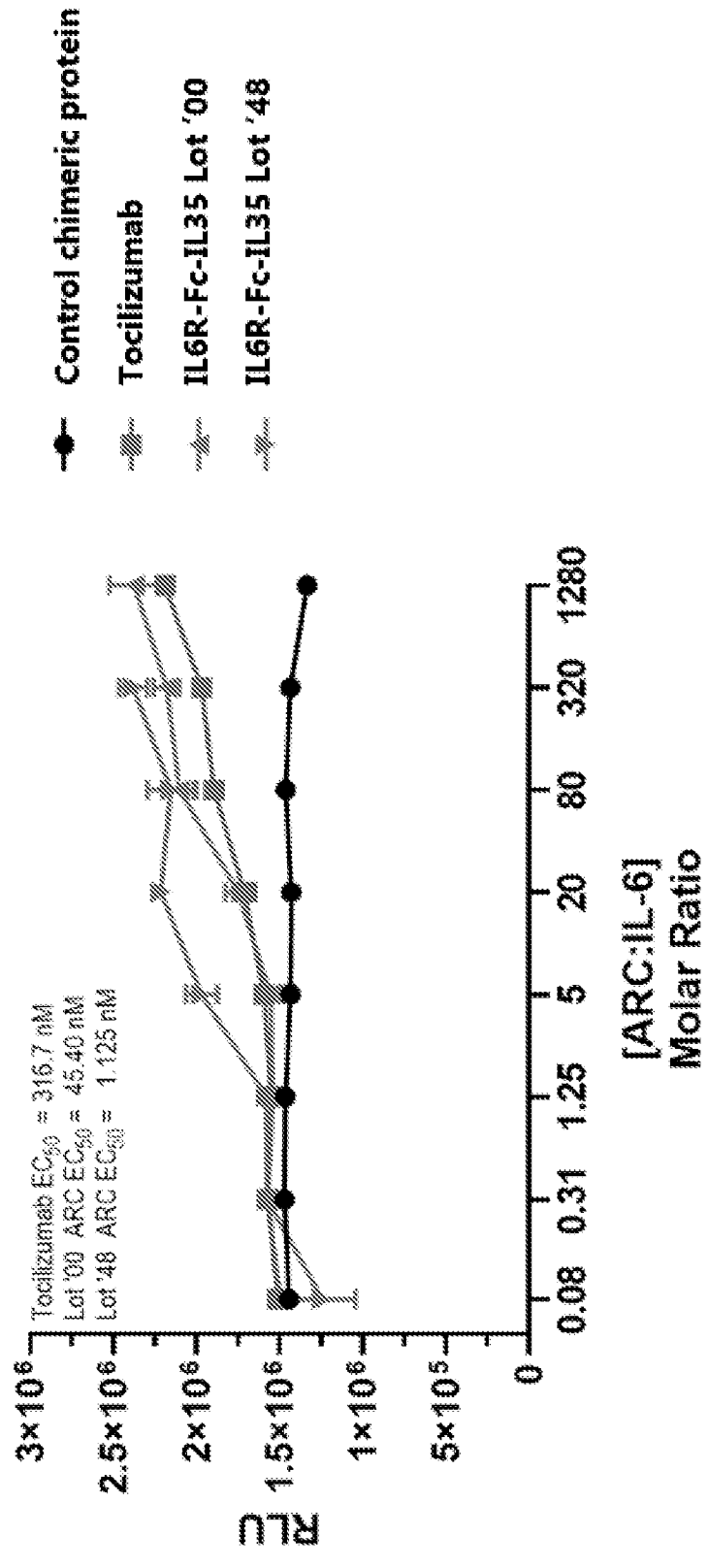

Control chimeric protein
Tocilizumab
IL6R-Fc-IL35 Lot '00
IL6R-Fc-IL35 Lot '48
Tocilizumab EC50 = 316.7 nM
Lot '00 ARC EC50 = 45.40 nM
Lot '48 ARC EC50 = 1.125 nM
RLU
3×10^6
2.5×10^6
2×10^6
1.5×10^6
1×10^6
5×10^5
0
0.08
0.31
1.25
5
20
80
320
1280
[ARC:IL-6]
Molar Ratio

FIG. 18

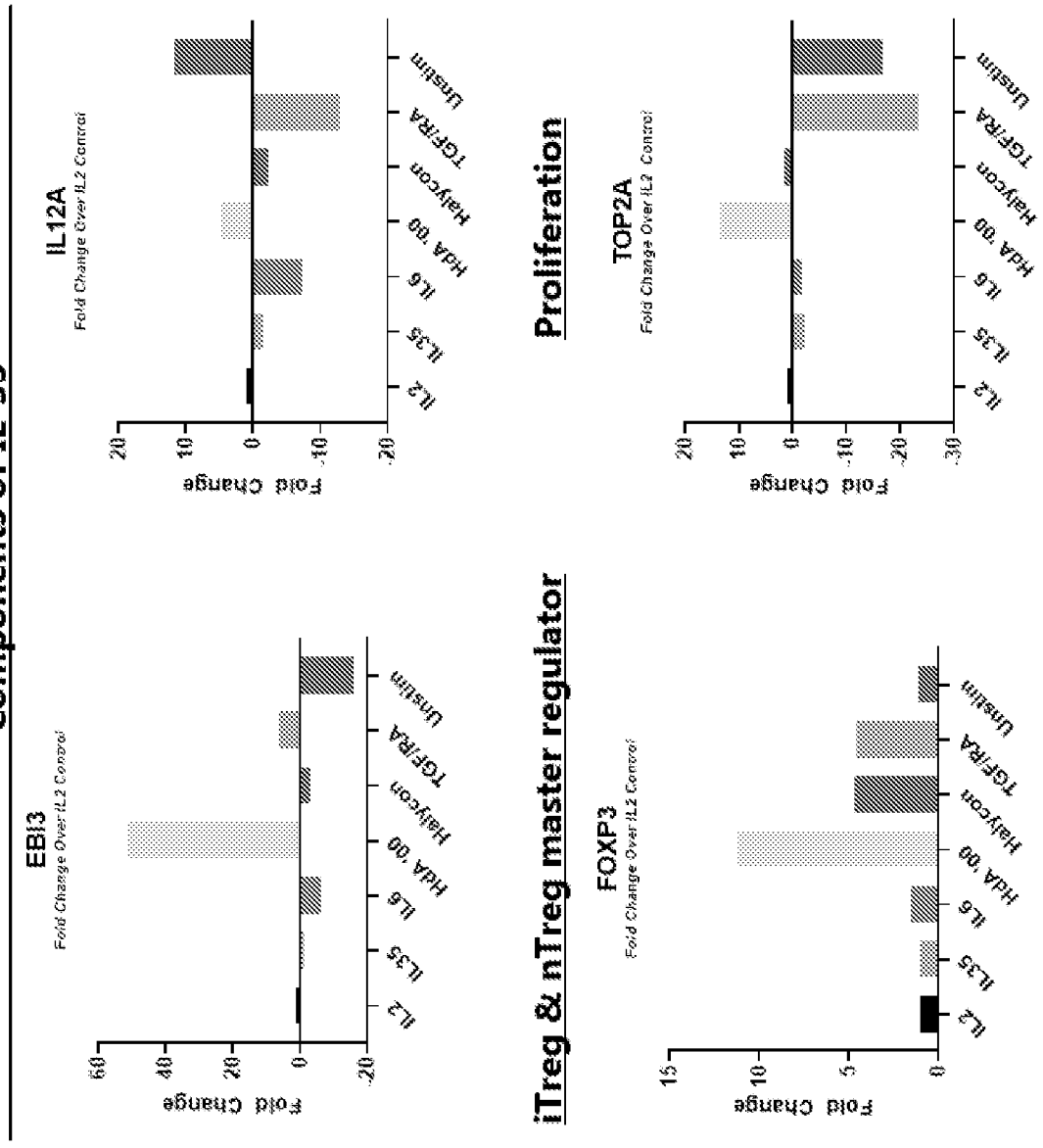

Components of IL-35
EBI3
Fold Change Over IL2 Control
Fold Change
60
40
20
0
-20
IL2
IL35
IL6
HDA '09
Halycon
TGFRA
Unstim
IL12A
Fold Change Over IL2 Control
Fold Change
20
10
0
-10
-20
IL2
IL35
IL6
HDA '09
Halycon
TGFRA
Unstim
iTreg & nTreg master regulator
FOXP3
Fold Change Over IL2 Control
Fold Change
15
10
5
0
IL2
IL35
IL6
HDA '09
Halycon
TGFRA
Unstim
Proliferation
TOP2A
Fold Change Over IL2 Control
Fold Change
20
10
0
-10
-20
-30
IL2
IL35
IL6
HDA '09
Halycon
TGFRA
Unstim

FIG. 18 (Continued)

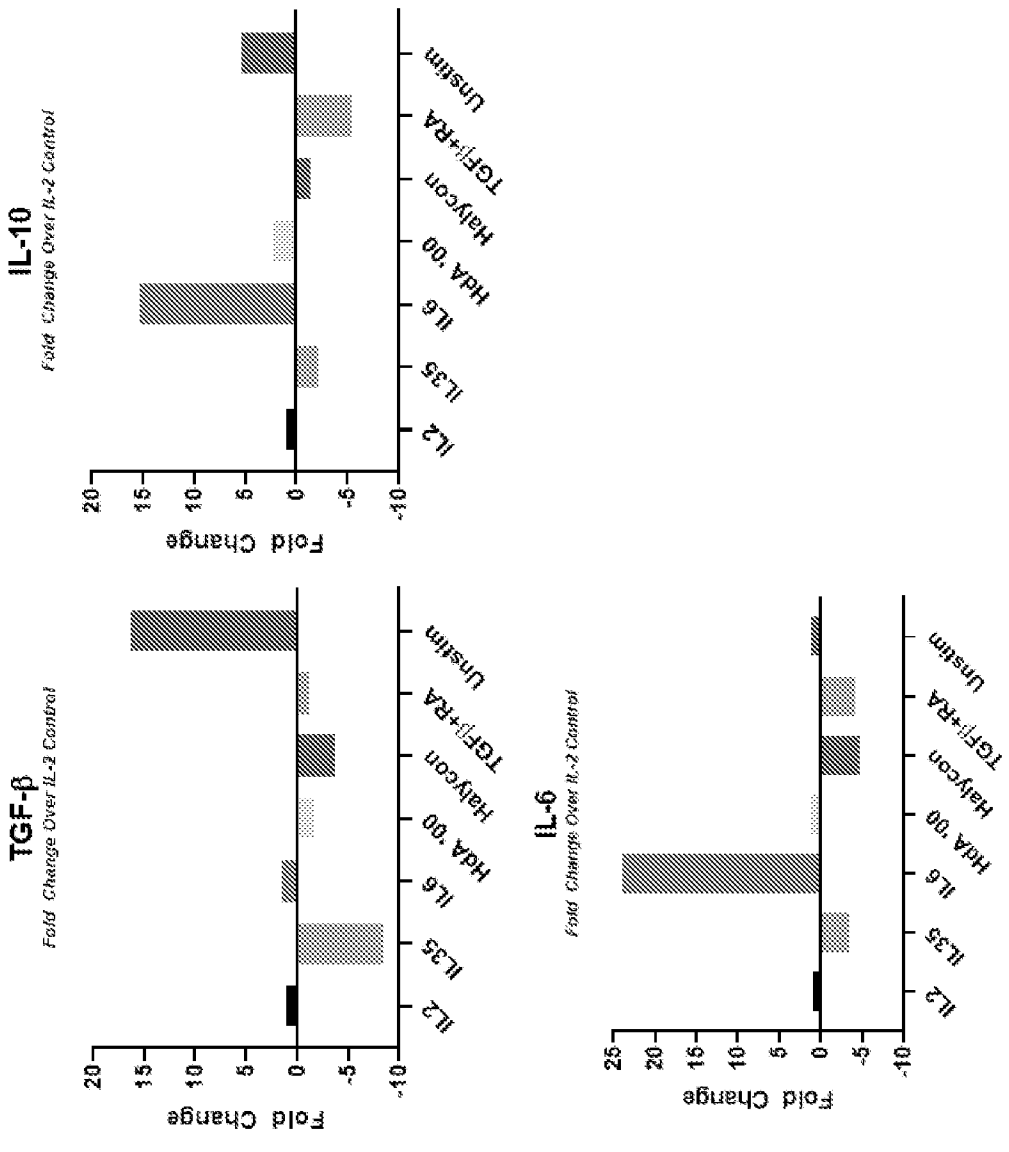


FIG. 19A

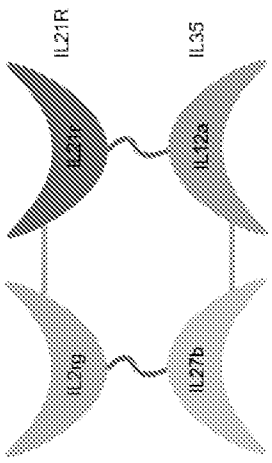


FIG. 19B

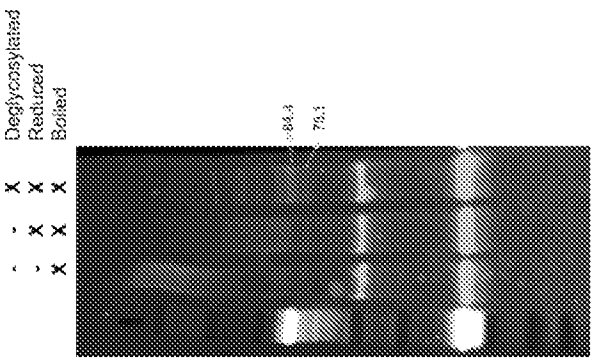


FIG. 20

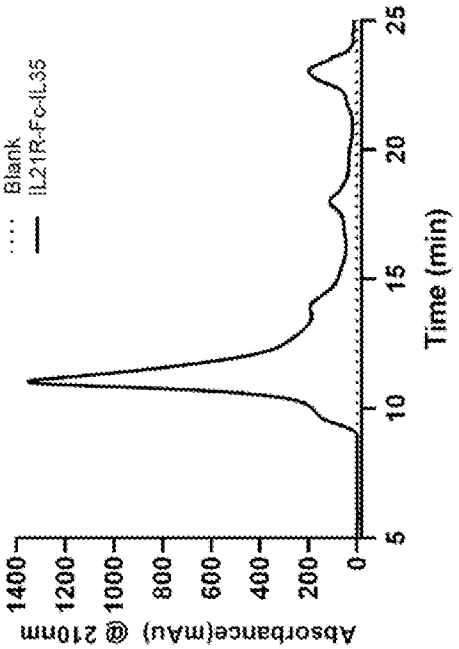


FIG. 21

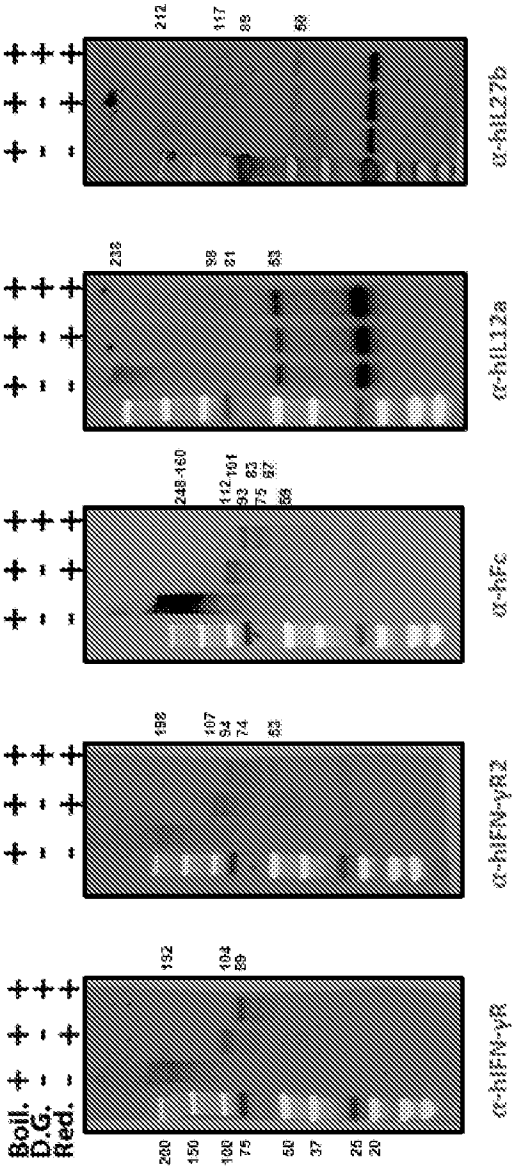
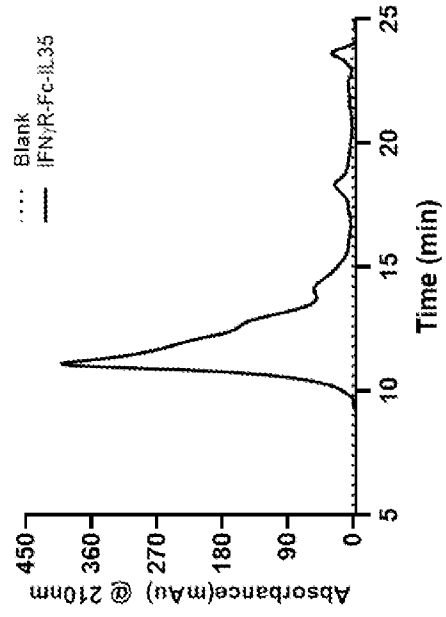

Boil.
D.G.
Red.
α-hIFN-γR
α-hIFN-γR2
α-hFc
α-hIL12a
α-hIL27b

FIG. 22


Blank
IFNγR-Fc-IL35
Absorbance(mAu) @ 210nm
Time (min)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/38451

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/00, A61K 38/02, A61K 39/395 (2019.01)
CPC - C07K 16/00, A61K 38/00, C07K 16/468, C07K 2317/526, C07K 2317/52

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.
Y	WO 1995/031480 A1 (SYNTHETIC PEPTIDES INCORPORATED) 23 November 1995 (23.11.1995) page 6, ln 6-17; page 8, ln 3-17; page 16, ln 3-12; page 22, ln 27-37; page 23, ln 1 -5; page 24, ln 19-30; Figure 3; claim 1; claim 3; claim 10	1-12
Y	US 2017/0095531 A1 (HEAT BIOLOGICS INC.) 6 April 2017 (06.04.2017) para [0031]; para [0089]-[0090]; para [0092]-[0093]; para [0095]; claim 1	1-12
Y	US 2002/0193570 A1 (GILLIES et al.) 19 December 2002 (19.12.2002) Abstract; claim 1	1-12
Y	US 2013/0330335 A1 (BREMEL et al.) 12 December 2013 (12.12.2013) para [0013]; para [0267]; SEQ ID NO: 404032	9
Y	US 2014/0356415 A1 (UNIVERSITY OF MARYLAND) 4 December 2014 (04.12.2014) para [0171]; Table 1; SEQ ID NO: 486	12

☐ 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
"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
3 September 2019	01 OCT 2019

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/38451

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.: 13-42
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.