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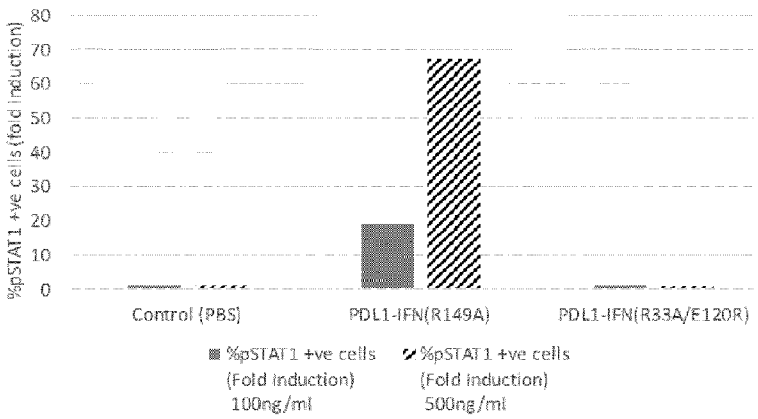
(54) Title: TARGETED THERAPEUTIC AGENTS AND USES THEREOF

FIG. 5



(57) Abstract: The present invention relates, in part, to chimeric proteins that find use in various immunotherapies. Particularly, the present invention provides targeted therapeutic agents that modulate the immune system for the treatment of diseases such as cancer.

WO 2017/134302 A2

TARGETED THERAPEUTIC AGENTS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Nos. 62/291,769, filed February 5, 2016; 62/335,880, filed May 13, 2016; 62/411,805, filed October 24, 2016; 62/291,772, filed February 5, 2016; 62/291,774, filed February 5, 2016; 62/335,965, filed May 13, 2016; 62/291,776, filed February 5, 2016; 62/335,968, filed May 13, 2016; 62/335,979, filed May 13, 2016; 62/336,030, filed May 13, 2016, 62/353,607, filed June 23, 2016; and 62/291,779, filed February 5, 2016, the entire contents of all of which are herein incorporated by reference.

FIELD

The present invention relates, in part, to targeted therapeutic agents and their use in the treatment of diseases such as cancer.

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: ORN-021PC_Sequence_listing; date recorded: February 1, 2017; file size: 231 KB).

BACKGROUND

In contrast to conventional cancer therapies such as chemotherapy and radiation, immunotherapies provide the advantage of cell specificity against cancer cells. Specifically, immunotherapies directly target cancer cells by inducing, enhancing, or suppressing a patient's own immune response. As such, methods for enhancing the efficacy of immune-based therapies can be clinically beneficial. However, despite impressive patient responses to agents targeting the immune costimulatory and coinhibitory molecules, including, for example, clinical trials that led to the approval of YERVOY, KEYTRUDA, and OPDIVO, immunotherapies such as checkpoint inhibition therapy still fails in the overwhelming majority of patients. Further still, many immunotherapies are complicated by side effects that significantly narrows a patient's therapeutic window for treatment and makes the patient more susceptible to other diseases.

Accordingly, there remains a need for improved immunotherapeutic agents that can provide targeted therapy against cancers while causing minimal side effects.

SUMMARY

In some aspects, the present invention relates to chimeric proteins having one targeting moiety which has a recognition domain (*e.g.* antigen recognition domains, including without limitation, various antibody formats, inclusive of single-domain antibodies) which specifically bind to a target (*e.g.* antigen, receptor) of interest. In various embodiments, the targeting moiety has a recognition domain that bind to a checkpoint protein selected

from programmed cell death protein 1 (PD-1), PD-1 ligand 1 (PD-L1; also known as B7-H1 and CD274), and PD-1 ligand 2 (PD-L2; also known as B7-DC and CD273).

In various embodiments, the chimeric protein further comprises a modified (*e.g.* mutant) signaling agent (for instance, an immune-modulating agent). In various embodiments, the modified (*e.g.* mutant) signaling agent has one or more modifications (*e.g.* mutations) that provide improved safety as compared to an unmodified (*e.g.* wild type) signaling agent. In various embodiments, the modified signaling agent is selected from one or more of an interleukin, interferon, and tumor necrosis factor. In an embodiment, the modified signaling agent is human interferon-α2. In another embodiment, the modified signaling agent is human TNF-α. In a further embodiment, the modified signaling agent is human IL-1β.

In some embodiments, the targeting moiety and the modified (*e.g.* mutant) signaling agent are connected by one or more linkers.

In various embodiments, the present chimeric proteins find use in the treatment of various diseases or disorders such as cancer, infections, immune disorders, and autoimmune diseases, and the present invention encompasses various methods of treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows C57BL/6 mice were inoculated subcutaneously (50 µl) with $6x10^5$ B16mCD20cl1 melanoma tumor cells. Perilesional treatment with 120 µg of an anti-PD-L1 VHH (120 µl) or a fusion of an anti-PD-L1 VHH to human IFNalpha, Q124R mutant was started when tumors reached a size of ± 10 mm^2 as measured by caliper. The curves are in the same order (top to bottom) as in the figure legend identifying the treatment types (*e.g.* PBS is the top curve, anti-PD-L1 is the middle curve, and the bottom curve is the fusion of an anti-PD-L1 VHH to human IFNalpha, Q124R mutant).

FIG. 2 shows, in panels A-G, safety data that correspond with the efficacy data of FIG. 1. In panel A, the weight of mice is shown for all treatment types. In panels B-G, all of the data is presented as histograms in sets of three which are, from left to right: PBS, anti-PD-L1 VHH, and fusion of an anti-PD-L1 VHH to human IFNalpha, Q124R. Shown are white blood cell counts ("wbc") and lymphocytes count ("ly") (panel B), neutrophil count ("ne") and monocyte count ("mo") (panel C); red blood cell count ("rbc") and hemoglobin ("hb") (panel D); hemocrit ("hct"), mean corpuscular volume ("mcv"), mean corpuscular hemoglobin ("mch"), mean corpuscular hemoglobin concentration ("mchc") (panel E); pitted red blood cells ("pit") (panel F); and mean platelet volume ("mpv") (panel G).

FIG. 3 shows the effect of a serial dilution of anti-human PD-1 VHHs, anti-human PD-L1 VHHs, anti-mouse PD-L1 VHHs or irrelevant VHHs when tested in a PD1/PD-L1 plate-binding assay. Average ± standard deviation of triplicate measurements are plotted. At the 10,000 point, the curves from top to bottom are: anti-mouse PD-L1 VHHs, irrelevant VHHs, anti-human PD-1 VHHs, and anti-human PD-L1 VHHs.

FIG. 4 shows an experiment in which MDA-MB-321 cells were stimulated with a serial dilution anti-PD-L1/attenuated IFN chimeras and stained for phospho STAT1. Data are plotted as mean fluorescent intensities (MFI). The top curve is anti-human PD-L1 VHH/human IFN R149A.

FIG. 5 shows a human dendritic cell pSTAT1 signaling assay. Chimeras studied were anti-human PD-L1 VHH/human IFN R149A and anti-human PD-L1 VHH/human IFN R33A/E120R. Two doses of the agents were studied: 100 ng/ml and 500 ng/ml. PBS was the control and the data are expressed as a fold change of the percentage of $pSTAT1^+$ dendritic cells (data is an average of a triplicate data set).

DETAILED DESCRIPTION

The present invention provides chimeric proteins with a signaling agent bearing therapeutically beneficial mutations and a targeting moiety that targets the signaling agent to sites in need of therapeutic action (*e.g.* a tumor cell) or that are appropriate for therapeutic action (*e.g.* an immune cell). The present invention provides pharmaceutical compositions comprising the chimeric proteins and their use in the treatment of various diseases. Administration of the chimeric proteins and pharmaceutical compositions of the invention achieves significantly reduced side effects compared to the wild type signaling agent.

Targeting Moieties

In various embodiments, the present chimeric protein has one targeting moiety directed against an immune checkpoint protein. In various embodiments, the immune checkpoint protein is selected from programmed cell death protein 1 (PD-1), PD-1 ligand 1 (PD-L1; also known as B7-H1 and CD274), and PD-1 ligand 2 (PD-L2; also known as B7-DC and CD273).

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

In some embodiments, the targeting moiety comprises antibody derivatives or formats. In some embodiments, the targeting moiety of the present chimeric protein is a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a shark heavy-chain-only antibody (VNAR), a microprotein (cysteine knot protein, knottin), a DARPin; a Tetranectin; an Affibody; a Transbody; an Anticalin; an AdNectin; an Affilin; a Microbody; a peptide aptamer; an alterase; a plastic antibody; a phylomer; a stradobody; a maxibody; an

evibody; a fynomer, an armadillo repeat protein, a Kunitz domain, an avimer, an atrimer, a probody, an immunobody, a triomab, a troybody; a pepbody; a vaccibody, a UniBody; Affimers, a DuoBody, a Fv, a Fab, a Fab', a $F(ab')_2$, a peptide mimetic molecule, or a synthetic molecule, as described in US Patent Nos. or Patent Publication Nos. US 7,417,130, US 2004/132094, US 5,831,012, US 2004/023334, US 7,250,297, US 6,818,418, US 2004/209243, US 7,838,629, US 7,186,524, US 6,004,746, US 5,475,096, US 2004/146938, US 2004/157209, US 6,994,982, US 6,794,144, US 2010/239633, US 7,803,907, US 2010/119446, and/or US 7,166,697, the contents of which are hereby incorporated by reference in their entireties. *See* also, Storz MAbs. 2011 May-Jun; 3(3): 310–317.

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

In various embodiments, the present chimeric protein has one targeting moiety directed against PD-1. PD-1 is expressed on a large proportion of tumor-infiltrating lymphocytes from many different tumor types. For example, PD-1 is highly expressed on CD4+ and CD8+ T cells. PD-1 is also expressed on other activated non-T lymphocytes, including B cells and natural killer (NK) cells. In some embodiments, the chimeric protein comprises a targeting moiety directed against PD-1 and a modified IFN-α comprising one or more mutations as described herein (*e.g.* the human IFN-α2 mutation described herein). In some embodiments, the chimeric protein comprises: a targeting moiety directed against PD-1 and a modified TNF-α comprising one or more mutations as described herein. In some embodiments, the chimeric protein comprises: a targeting moiety directed against PD-1 and a modified IL-1β comprising one or more mutations as described herein.

In various embodiments, the present chimeric protein has a targeting moiety directed against PD-1. In some embodiments, the chimeric protein has a targeting moiety which selectively bind a PD-1 polypeptide. In some embodiments, the chimeric protein comprises an antibody, an antibody derivative or format, a peptide or polypeptide, or a fusion protein that selectively binds a PD-1 polypeptide.

In an embodiment, the targeting moiety comprises the anti-PD-1 antibody pembrolizumab (aka MK-3475, KEYTRUDA), or fragments thereof. Pembrolizumab and other humanized anti-PD-1 antibodies are disclosed in Hamid, et al. (2013) New England Journal of Medicine 369 (2): 134-44, US 8,354,509, and WO 2009/114335, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, pembrolizumab or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising the amino acid sequence of:

QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNF
NEKFKNRVTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTTVTVSS
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS

GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG
NVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 1);

and/or a light chain comprising the amino acid sequence of:

EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLES
GVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 2).

In an embodiment, the targeting moiety comprises the anti-PD-1 antibody, nivolumab (aka BMS-936558, MDX-1106, ONO-4538, OPDIVO), or fragments thereof. Nivolumab (clone 5C4) and other human monoclonal antibodies that specifically bind to PD-1 are disclosed in US 8,008,449 and WO 2006/121168, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, nivolumab or an antigen-binding fragment thereof comprises a heavy chain comprising the amino acid sequence of:

QVQLVESGGG VVQPGRSLRL DCKASGITFS NSGMHWVRQA PGKGLEWVAV IWYDGSKRYY
ADSVKGRFTI SRDNSKNTLF LQMNSLRAED TAVYYCATND DYWGQGTLVT VSSASTKGPS
VFPLAPCSRS TSESTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS
VVTVPSSSLG TKTYTCNVDH KPSNTKVDKR VESKYGPPCP PCPAPEFLGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS QEDPEVQFNW YVDGVEVHNA KTKPREEQFN STYRVVSVLT
VLHQDWLNGK EYKCKVSNKG LPSSIEKTIS KAKGQPREPQ VYTLPPSQEE MTKNQVSLTC
LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SRLTVDKSRW QEGNVFSCSV
MHEALHNHYT QKSLSLSLGK (SEQ ID NO: 3);

and/or a light chain comprising the amino acid sequence of:

EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA
RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ SSNWPRTFGQ GTKVEIKRTV AAPSVFIFPP
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC (SEQ ID NO: 4).

In an embodiment, the targeting moiety comprises the anti-PD-1 antibody pidilizumab (aka CT-011, hBAT or hBAT-1), or fragments thereof. Pidilizumab and other humanized anti-PD-I monoclonal antibodies are disclosed in US 2008/0025980 and WO 2009/101611, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, the anti-PD-1 antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a light chain variable regions comprising an amino acid sequence selected from SEQ ID NOS: 15-18 of US 2008/0025980:

SEQ ID No: 15 of US 2008/0025980 (SEQ ID NO: 5):

EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYRTSNLASGVPSR
FSGSGSGTDFTLTINSLQPEDFATYYCQQRSSFPLTFGGGTKLEIK;

SEQ ID No: 16 of US 2008/0025980 (SEQ ID NO: 6):

EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWFQQKPGKAPKLWIYRTSNLASGVPSR
FSGSGSGTDYTLTINSLQPEDFATYYCQQRSSFPLTFGGGTKLEIK;

SEQ ID No: 17 of US 2008/0025980 (SEQ ID NO: 7):

EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWFQQKPGKAPKLWIYRTSNLASGVPSR
FSGSGSGTDYCLTINSLQPEDFATYYCQQRSSFPLTFGGGTKLEIK;

SEQ ID No: 18 of US 2008/0025980 (SEQ ID NO: 8):

EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWFQQKPGKAPKLWIYRTSNLASGVPSR
FSGSGSGTSYCLTINSLQPEDFATYYCQQRSSFPLTFGGGTKLEIK;

and/or a heavy chain comprising an amino acid sequence selected from SEQ ID NOS: 20-24 of US 2008/0025980:

SEQ ID No: 20 of US 2008/0025980 (SEQ ID NO: 9):

QVQLVQSGSELKKPGASVKISCKASGYSFSNYGMNWVRQAPGQGLQWMGWINTDSGESTY
AEEFKGRFVFSLDTSVSTAYLQITSLTAEDTGMYFCAKVGYDALDYWGQGTLVTVSS;

SEQ ID No: 21 of US 2008/0025980 (SEQ ID NO: 10):

QVQLVQSGSELKKPGASVKISCKASGYTFTNYGMNWVRQAPGQGLQWMGWINTDSGESTY
AEEFKGRFVFSLDTSVSTAYLQITSLTAEDTGMYFCAKVGYDALDYWGQGTLVTVSS;

SEQ ID No: 22 of US 2008/0025980 (SEQ ID NO: 11):

QVQLVQSGSELKKPGASVKISCKASGYTFTNYGMNWVRQAPGQGLQWMGWINTDSGESTY
AEEFKGRFVFSLDTSVNTAYLQITSLTAEDTGMYFCVRVGYDALDYWGQGTLVTVSS;

SEQ ID No: 23 of US 2008/0025980 (SEQ ID NO: 12):

QIQLVQSGSELKKPGASVKISCKASGYTFTNYGMNWVRQAPGQGLQWMGWINTDSGESTY
AEEFKGRFVFSLDTSVNTAYLQITSLTAEDTGMYFCVRVGYDALDYWGQGTLVTVSS;

SEQ ID No: 24 of US 2008/0025980 (SEQ ID NO: 13):

QIQLVQSGSELKKPGASVKISCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTDSGESTY
AEEFKGRFAFSLDTSVNTAYLQITSLNAEDTGMYFCVRVGYDALDYWGQGTLVTVSS.

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

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

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

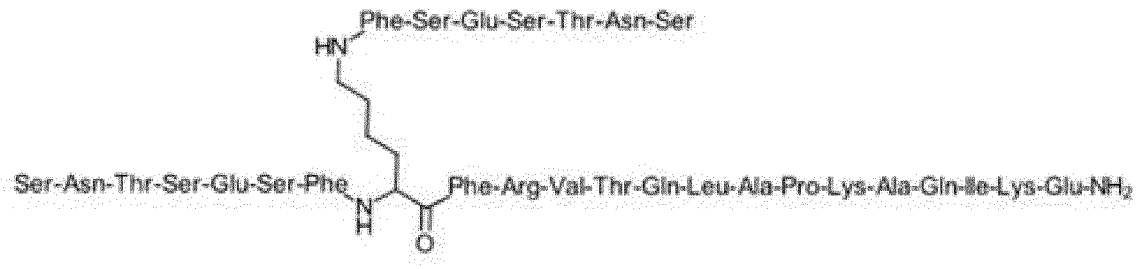
LFTVTVPKELYIIEHGSNVTLECNFDTGSHVNLGAITASLQKVENDTSPHRERATLLEEQ
LPLGKASFHIPQVQVRDEGQYQCIIIYGVAWDYKYLTLKVKASYRKINTHILKVPETDEV
ELTCQATGYPLAEVSWPNVSVPANTSHSRTPEGLYQVTSVLRLKPPPGRNFSCVFWNTHV
RELTLASIDLQSQMEPRTHPTWLLHIFIPFCIIAFIFIATVIALRKQLCQKLYSSKDTTK
RPVTTTKREVNSAI (SEQ ID NO: 14)

and/or the B7-DC fusion protein which comprises SEQ ID NO:83 of WO2010/027827:

MIFLLLMLSLELQLHQIAALFTVTVPKELYIIEHGSNVTLECNFDTGSHVNLGAITASLQ
KVENDTSPHRERATLLEEQLPLGKASFHIPQVQVRDEGQYQCIIIYGVAWDYKYLTLKVK
ASYRKINTHILKVPETDEVELTCQATGYPLAEVSWPNVSVPANTSHSRTPEGLYQVTSVL
RLKPPPGRNFSCVFWNTHVRELTLASIDLQSQMEPRTHPTWEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 15)

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

SNTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 16)



or: SNTSESF-NH
SNTSESFKFRVTQLAPKAQIKE-NH2

.

In an embodiment, the targeting moiety comprises the anti-PD-1 antibody 1E3, or fragments thereof, as disclosed in US 2014/0044738, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 1E3 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

EVQLQQSGPV LVKPGASVKM SCKASGYTFT DYYMNWVKQS HGKSLEWIGN

INPYNGGTTY NQKFKGKATL TVDKSSRTAY MEINSLTSED SAVYYCARGR
IYDGSLDYWG QGTALTVSS (SEQ ID NO: 17);

and/or a light chain variable region comprising the amino acid sequence of:

DIQMTQFPSS LCASQGGKVT VTCKASQDIN NYMAWYQHKP GKGPRLLIHY
TSTLLSGIPS RFSGSGSGRD YSFSISNLEP EDIATYYCLQ YDNLWTFGGG
TKLEIK (SEQ ID NO: 18).

In an embodiment, the targeting moiety comprises the anti-PD-1 antibody 1E8, or fragments thereof, as disclosed in US 2014/0044738, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 1E8 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

QVQLQQSGAE LAKPGASVRL SCKASGYTFT NYWMHWVKQR PGQGLEWIGH
INPSSGFTTY NQNFKDKATL TADKSSNTAY MQLSSLTYED SAVYFCARED
YDVDYWGQGT TLTVSS (SEQ ID NO: 19);

and/or a light chain variable region comprising the amino acid sequence of:

DIVMTQSQKF MSTSVGDRVS VTCKASQSVD TNVAWYQQKP GQSPKALIFS
ASYRYSGVPD RFTGSGSGTD FTLTINSVQS EDLAEYFCQQ YNSYPYTFGS
GTKLEIK (SEQ ID NO: 20).

In an embodiment, the targeting moiety comprises the anti-PD-1 antibody 1H3, or fragments thereof, as disclosed in US 2014/0044738, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 1H3 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

EVQLVESGGG LVKPGGSLKL SCAASGFTFS DYGMHWVRQA PEKGLEWVAY
ISSGSYTIYY TDTVKGRFTI SRDNAKNTLF LQMTSLRSED TAMYYCARRG
YGSFYEYYFD YWGQGTTLTV SS (SEQ ID NO: 21);

and/or light chain variable region comprising the amino acid sequence of:

QIVLTQSPAL MSASPGEKVT MTCSASSSVS YMYWYQQKPR SSPKPWIYLT
SNLASGVPAR FSGSGSGTSY SLTISSMEAE DAATYYCQQW SSNPFTFGSG
TKLEIK (SEQ ID NO: 22).

In an embodiment, the targeting moiety comprises a VHH directed against PD-1 as disclosed, for example, in US 8,907,065 and WO 2008/071447, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, the VHHs against PD-1 comprise SEQ ID NOS: 347-351 of US 8,907,065:

SEQ ID No: 347 of US 8,907,065 (SEQ ID NO: 23):

EVQLVESGGGLVQAGKSLRLSCAASGSIFSIHAMGWFRQAPGKEREFVAA
ITWSGGITYYEDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCAADR
AESSWYDYWGQGTQVTVSS;

SEQ ID No: 348 of US 8,907,065 (SEQ ID NO: 24):

EVQLVESGGGLVQAGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAV
ITWSGGITYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCAGDK
HQSSWYDYWGQGTQVTVSS;

SEQ ID No: 349 of US 8,907,065 (SEQ ID NO: 25):

EVQLVESGGGLVQAGGSLRLSCAASGSISSIHAMGWFRQAPGKEREFVAA
ITWSGGITYYADSLKGRFTISRDNAKNTGYLQMNSLKPEDTAIYYCAADR
AQSSWYDYWGQGTQVTVSS;

SEQ ID No: 350 of US 8,907,065 (SEQ ID NO: 26):

EVQLVESGGGLVQAGGSLGLSCAASGSIFSINAMAWFRQAPGKEREFVAL
ISWSGGSTYYEDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCAADR
VDSNWYDYWGQGTQVTVSS;

SEQ ID No: 351 of US 8,907,065 (SEQ ID NO: 27):

EVQLVESGGGLVQAGGSLRLSCAASGRAFSSGTMGWFRRAPGKEREFVA
SIPWSGGRIYYADSVKGRFTISRDNAQNTVYLQMNSLKPEDTAVYYCAVK
ERSTGWDFASWGQCTQVTVSS.

In an embodiment, the targeting moiety comprises any one of the anti-PD-1 antibodies, or fragments thereof, as disclosed in US2011/0271358 and WO2010/036959, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NOS: 25-29 of US2011/0271358:

SEQ ID No: 25 of US2011/0271358 (SEQ ID NO: 28):

QVQLVQSGAELKQPGASVKMSCKASGYSFTSSWIHWVKQAPGQGLEWIGYIYPSTGFTEY
NQKFKDRATLTADKSTSTAYMELSSLRSEDSAVYYCARWRDSSGYHAMDYWGQGTSVTVS
S;

SEQ ID No: 26 of US2011/0271358 (SEQ ID NO: 29):

QVQLVQSGAEVKQPGASVKMSCKASGYSFTSSWIHWVKQAPGQGLEWIGYIYPSTGFTEY
NQKFKDRATLTADKSTSTAYMELSSLRSEDTAVYY3/d10CARWRDSSGYHAMDYWGQGTSVTVS
S;

SEQ ID No: 27 of US2011/0271358 (SEQ ID NO: 30):

QVQLVQSGHEVKQPGASVKMSCKASGYSFTSSWIHWVKQAPGQGLEWIGYIYPSTGFTEY
NQKFKDRATLTADKSTSTAYMELSSLRSEDTAVYYCARWRDSSGYHAMDYWGQGTLVTVS
S;

SEQ ID No: 28 of US2011/0271358 (SEQ ID NO: 31):

QVQLVQSGHEVKQPGASVKMSCKASGYSFTSSWIHWVRQAPGQGLEWIGYIYPSTGFTEY
NQKFKDRATLTADKSTSTAYMELSSLRSEDTAVYYCARWRDSSGYHAMDYWGQGTLVTVS
S;

SEQ ID No: 29 of US2011/0271358 (SEQ ID NO: 32):

QVQLVQSGHEVKQPGASVKVSCKASGYSFTSSWIHWVRQAPGQGLEWIGYIYPSTGFTEY
NQKFKDRATITADKSTSTAYMELSSLRSEDTAVYYCARWRDSSGYHAMDYWGQGTLVTVS
S;

and/or a light chain comprising an amino acid sequence selected from SEQ ID NOS: 30-33 of US2011/0271358:

SEQ ID No: 30 of US2011/0271358 (SEQ ID NO: 33):

DIVLTQSPASLTLSPGQRLTISCRASQSVSTSGYSYMHWYQQKPDQSPKLLIKFGSNLES
GIPARFSGSGSGTDFTLTISSLEEEDFATYYCQHSWEIPYTFGQGTKLEIK;

SEQ ID No: 31 of US2011/0271358 (SEQ ID NO: 34):

DIVLTQSPATLSLSPGQRLTISCRASQSVSTSGYSYMHWYQQKPDQSPKLLIKFGSNLES
GIPARFSGSGSGTDFTLTISSLEPEDFATYYCQHSWEIPYTFGQGTKLEIK;

SEQ ID No: 32 of US2011/0271358 (SEQ ID NO: 35):

EIVLTQSPATLSLSPGQRLTISCRASQSVSTSGYSYMHWYQQKPDQSPKLLIKFGSNLES
GIPARFSGSGSGTDFTLTISSLEPEDFATYYCQHSWEIPYTFGQGTKLEIK;

SEQ ID No: 33 of US2011/0271358 (SEQ ID NO: 36):

DIVLTQSPATLSLSPGQRLTISCRASQSVSTSGYSYMHWYQQKPDQSPKLLIKFGSNLES
GIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQHSWEIPYTFGQGTKLEIK.

In various embodiments, the present chimeric protein comprises an antibody directed against PD-1, or an antibody fragment thereof, selected from TSR-042 (Tesaro, Inc.), REGN2810 (Regeneron Pharmaceuticals, Inc.), PDR001 (Novartis Pharmaceuticals), and BGB-A317 (BeiGene Ltd.)

In various embodiments, the present chimeric protein has one targeting moiety directed against PD-L1. In some embodiments, the chimeric protein has one targeting moiety which selectively bind a PD-L1 polypeptide. In some embodiments, the chimeric protein include an antibody, an antibody derivative or format, a peptide or polypeptide, or a fusion proteins that selectively binds a PD-L1 polypeptide.

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody MEDI4736 (aka durvalumab), or fragments thereof. MEDI4736 is selective for PD-L1 and blocks the binding of PD-L1 to the PD-1 and CD80 receptors. MEDI4736 and antigen-binding fragments thereof for use in the methods provided herein comprises a heavy chain and a light chain or a heavy chain variable region and a light chain variable region. The sequence of MEDI4736 is disclosed in WO/2016/06272, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, MEDI4736 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising the amino acid sequence of:

EVQLVESGGG LVQPGGSLRL SCAASGFTFS RYWMSWVRQA PGKGLEWVAN
IKQDGSEKYY VDSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCAREG
GWFGELAFDY WGQGTLVTVS SASTKGPSVF PLAPSSKSTS GGTAALGCLV
KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ
TYICNVNHKP SNTKVDKRVE PKSCDKTHTC PPCPAPEFEG GPSVFLFPPK
PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPASIEKTI SKAKGQPREP
QVYTLPPSRE EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP
VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG
K (SEQ ID NO: 37);

and/or a light chain comprising the amino acid sequence of:

EIVLTQSPGT LSLSPGERAT LSCRASQRVS SSYLAWYQQK PGQAPRLLIY
DASSRATGIP DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSLPWTFG
QGTKVEIKRT VAAPSVFIFP PSDEQLKSGT ASVVCLLNNF YPREAKVQWK
VDNALQSGNS QESVTEQDSK DSTYSLSSTL TLSKADYEKH KVYACEVTHQ
GLSSPVTKSF NRGEC (SEQ ID NO: 38).

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

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWVANIKQDGSEKYY
VDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAREGGWFGELAFDYWGQGTLVTVS
S (SEQ ID NO: 39);

and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:3 of WO/2016/06272:

EIVLTQSPGTLSLSPGERATLSCRASQRVSSSYLAWYQQKPGQAPRLLIYDASSRATGIP
DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSLPWTFGQGTKVEIK (SEQ ID NO: 40)

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody atezolizumab (aka MPDL3280A, RG7446), or fragments thereof. In illustrative embodiments, atezolizumab or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising the amino acid sequence of:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRF
TISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSSASTKGPSVFPLAPSSKST
SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41);

and/or a light chain comprising the amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC (SEQ ID NO: 42).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody avelumab (aka MSB0010718C), or fragments thereof. In illustrative embodiments, avelumab or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising the amino acid sequence of:

EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYIMMWVRQA PGKGLEWVSS
IYPSGGITFY ADTVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARIK
LGTVTTVDYW GQGTLVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK
DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT
YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
(SEQ ID NO: 43);

and/or a light chain comprising the amino acid sequence of:

QSALTQPASV SGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLMI
YDVSNRPSGV SNRFSGSKSG NTASLTISGL QAEDEADYYC SSYTSSSTRV
FGTGTKVTVL GQPKANPTVT LFPPSSEELQ ANKATLVCLI SDFYPGAVTV
AWKADGSPVK AGVETTKPSK QSNNKYAASS YLSLTPEQWK SHRSYSCQVT
HEGSTVEKTV APTECS (SEQ ID NO: 44).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody BMS-936559 (aka 12A4, MDX-1105), or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, BMS-936559 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

QVQLVQSGAEVKKPGSSVKVSCKTSGDTFSTYAISWVRQAPGQGLEWMGGIIPIFGKAHY
AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYFCARKFHFVSGSPFGMDVWGQGTTVT
VSS (SEQ ID NO: 45);

and/or a light chain variable region comprising the amino acid sequence of:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPA
RFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPTFGQGTKVEIK (SEQ ID NO: 46).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 3G10, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 3G10 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYGFSWVRQAPGQGLEWMGWITAYNGNTNY
AQKLQGRVTMTTDTSTSTVYMELRSLRSDDTAVYYCARDYFYGMDVWGQGTTVTVSS (SEQ ID
NO: 47);

and/or a light chain variable region comprising the amino acid sequence of:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLVWYQQKPGQAPRLLIYDASNRATGIPA
RFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPRTFGQGTKVEIK (SEQ ID NO: 48).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 10A5, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 10A5 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDVHWVRQAPGQRLEWMGWLHADTGITKF
SQKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCARERIQLWFDYWGQGTLVTVSS (SEQ ID
NO: 49);

and/or a light chain variable region comprising the amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK (SEQ ID NO: 50).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 5F8, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 5F8 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

QVQLVQSGAEVKKPGSSVKVSCKVSGGIFSTYAINWVRQAPGQGLEWMGGIIPIFGTANH
AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDQGIAAALFDYWGQGTLVTVSS (SEQ ID
NO: 51);

and/or a light chain variable region comprising the amino acid sequence of:

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIP
DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK (SEQ ID NO: 52).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 10H10, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 10H10 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

EVQLVESGGGLVQPGRSLRLSCAVSGFTFDDYVVHWVRQAPGKGLEWVSGISGNSGNIGY
ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAVPFDYWGQGTLVTVSS (SEQ ID NO:
53);

and/or a light chain variable region comprising the amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK (SEQ ID NO: 54).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 1B12, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 1B12 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

QVQLVQSGAEVKKPGSSVKVSCKTSGDTFSSYAISWVRQAPGQGLEWMGGIIPIFGRAHY
AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYFCARKFHFVSGSPFGMDVWGQGTTVT
VSS (SEQ ID NO: 55);

and/or a light chain variable region comprising the amino acid sequence of:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPA
RFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPTFGQGTKVEIK (SEQ ID NO: 56).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 7H1, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 7H1 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

QVQLVQSGAEVKKPGSSVKVSCKTSGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGKAHY
AQKFQGRVTITADESTTTAYMELSSLRSEDTAVYYCARKYDYVSGSPFGMDVWGQGTTVT
VSS (SEQ ID NO: 57);

and/or a light chain variable region comprising the amino acid sequence of:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPA
RFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPTFGQGTKVEIK (SEQ ID NO: 58).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 11E6, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 11E6 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAINWVRQAPGQGLEWMGGIIPIFGSANY
AQKFQDRVTITADESTSAAYMELSSLRSEDTAVYYCARDSSGWSRYYMDVWGQGTTVTVS
S (SEQ ID NO: 59);

and/or a light chain variable region comprising the amino acid sequence of:

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIP
DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPFGGGTKVEIK (SEQ ID NO: 60).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 12B7, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 12B7 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

QVQLVQSGAEVKEPGSSVKVSCKASGGTFNSYAISWVRQAPGQGLEWMGGIIPLFGIAHY
AQKFQGRVTITADESTNTAYMDLSSLRSEDTAVYYCARKYSYVSGSPFGMDVWGQGTTVT
VSS (SEQ ID NO: 61);

and/or a light chain variable region comprising the amino acid sequence of:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPA
RFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPTFGQGTRLEIK (SEQ ID NO: 62).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 13G4, or fragments thereof, as disclosed in US 2013/0309250 and WO2007/005874, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 13G4 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

EVQLVESGGGLVQPGRSLRLSCAASGITFDDYGMHWVRQAPGKGLEWVSGISWNRGRIEY
ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKGRFRYFDWFLDYWGQGTLVTVS
S (SEQ ID NO: 63);

and/or a light chain variable region comprising the amino acid sequence of:

AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYPFTFGPGTKVDIK (SEQ ID NO: 64).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 1E12, or fragments thereof, as disclosed in US 2014/0044738, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 1E12 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

EVKLQESGPS LVKPSQTLSL TCSVTGYSIT SDYWNWIRKF PGNKLEYVGY
ISYTGSTYYN PSLKSRISIT RDTSKNQYYL QLNSVTSEDT ATYYCARYGG
WLSPFDYWGQ GTTLTVSS (SEQ ID NO: 65);

and/or a light chain variable region comprising the amino acid sequence of:

DIVMTQSHKL MSTSVGDRVS ITCKASQDVG TAVAWYQQKP GQSPKLLIYW
ASTRHTGVPD RFTGSGSGTD FTLTISNVQS EDLADYFCQQ DSSYPLTFGA
GTKVELK (SEQ ID NO: 66).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 1F4, or fragments thereof, as disclosed in US 2014/0044738, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 1F4 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

EVQLQESGPG LVAPSQSLSI TCTVSGFSLT TYSINWIRQP PGKGLEWLGV
MWAGGGTNSN SVLKSRLIIS KDNSKSQVFL KMNSLQTDDT ARYYCARYYG
NSPYYAIDYW GQGTSVTVSS (SEQ ID NO: 67);

and/or a light chain variable region comprising the amino acid sequence of:

DIVTTQSHKL MSTSVGDRVS ITCKASQDVG TAVAWYQQKP GQSPKLLIYW
ASTRHTGVPD RFTGSGSGTD FTLTISNVQS EDLADYFCQQ DSSYPLTFGA
GTKVELK (SEQ ID NO: 68).

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

EVKLQESGPS LVKPSQTLSL TCSVTGYSII SDYWNWIRKF PGNKLEYLGY
ISYTGSTYYN PSLKSRISIT RDTSKNQYYL QLNSVTTEDT ATYYCARRGG
WLLPFDYWGQ GTTLTVSS (SEQ ID NO: 69);

and/or a light chain variable region comprising the amino acid sequence of:

DIVMTQSPSS LAVSVGEKVS MGCKSSQSLL YSSNQKNSLA WYQQKPGQSP
KLLIDWASTR ESGVPDRFTG SGSGTDFTLT ISSVKAEDLA VYYCQQYYGY
PLTFGAGTKL ELK (SEQ ID NO: 70).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 3B6, or fragments thereof, as disclosed in US 2014/0044738, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 3B6 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

EVKLQESGPS LVKPGASVKL SCKASGYTFT SYDINWVKQR PGQGLEWIGW
IFPRDNNTKY NENFKGKATL TVDTSSTTAY MELHSLTSED SAVYFCTKEN
WVGDFDYWGQ GTTLTLSS (SEQ ID NO: 71);

and/or a light chain variable region comprising the amino acid sequence of:

DIVMTQSPAI MSASPGEKVT MTCSASSSIR YMHWYQQKPG TSPKRWISDT
SKLTSGVPAR FSGSGSGTSY ALTISSMEAE DAATYYCHQR SSYPWTFGGG
TKLEIK (SEQ ID NO: 72).

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 3D10, or fragments thereof, as disclosed in US 2014/0044738 and WO2012/145493, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 3D10 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

EVQLQQSGPD LVTPGASVRI SCQASGYTFP DYYMNWVKQS HGKSLEWIGD
IDPNYGGTTY NQKFKGKAIL TVDRSSSTAY MELRSLTSED SAVYYCARGA
LTDWGQGTSL TVSS (SEQ ID NO: 73);

and/or a light chain variable region comprising the amino acid sequence of:

QIVLSQSPAI LSASPGEKVT MTCRASSSVS YIYWFQQKPG SSPKPWIYAT
FNLASGVPAR FSGSGSGTSY SLTISRVETE DAATYYCQQW SNNPLTFGAG
TKLELK (SEQ ID NO: 74).

In an embodiment, the targeting moiety comprises any one of the anti-PD-L1 antibodies disclosed in US2011/0271358 and WO2010/036959, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID Nos: 34-38 of US2011/0271358:

SEQ ID No: 34 of US2011/0271358 (SEQ ID NO: 75):

EVQLVQSGPELKKPGASVKMSCKASGYTFTSYVMHWVKQAPGQRLEWIGYVNPFNDGTKY
NEMFKGRATLTSDKSTSTAYMELSSLRSEDSAVYYCARQAWGYPWGQGTLVTVSS;

SEQ ID No: 35 of US2011/0271358 (SEQ ID NO: 76):

EVQLVQSGAEVKKPGASVKMSCKASGYTFTSYVMHWVKQAPGQRLEWIGYVNPFNDGTKY
NEMFKGRATLTSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS;

SEQ ID No: 36 of US2011/0271358 (SEQ ID NO: 77):

EVQLVQSGAEVKKPGASVKMSCKASGYTFTSYVMHWVRQAPGQRLEWIGYVNPFNDGTKY
NEMFKGRATLTSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS;

SEQ ID No: 37 of US2011/0271358 (SEQ ID NO: 78):

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVMHWVRQAPGQRLEWIGYVNPFNDGTKY
NEMFKGRATLTSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS;

SEQ ID No: 38 of US2011/0271358 (SEQ ID NO: 79):

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVMHWVRQAPGQRLEWIGYVNPFNDGTKY
NEMFKGRATITSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS;

and/or a light chain comprising an amino acid sequence selected from SEQ ID Nos: 39-42 of US2011/0271358:

SEQ ID No: 39 of US2011/0271358 (SEQ ID NO: 80):

DIVLTQSPASLALSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDS
GVPSRFSGSGSGTDFTLTINSLEEEDAAMYFCQQSRRVPYTFGQGTKLEIK;

SEQ ID No: 40 of US2011/0271358 (SEQ ID NO: 81):

DIVLTQSPATLSLSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDS
GVPSRFSGSGSGTDFTLTINSLEAEDAAMYFCQQSRRVPYTFGQGTKLEIK;

SEQ ID No: 41 of US2011/0271358 (SEQ ID NO: 82):

EIVLTQSPATLSLSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDS
GVPSRFSGSGSGTDFTLTINSLEAEDAAMYFCQQSRRVPYTFGQGTKLEIK;

SEQ ID No: 42 of US2011/0271358 (SEQ ID NO: 83):

DIVLTQSPATLSLSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDS
GVPSRFSGSGSGTDFTLTINSLEAEDAATYFCQQSRRVPYTFGQGTKLEIK.

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 2.7A4, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 2.7A4 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

SEQ ID No: 2 of WO 2011/066389 (SEQ ID NO: 84):

EVQLVESGGGLVKPGGSLRLSCAASGFTFSTYSMNWVRQAPGKGLEWVSSISSSGDYIYY
ADSVKGRFTISRDNAKNSLFLQMNSLKAEDTAVYYCARDLVTSMVAFDYWGQGTLVTVSS;

and/or a light chain variable region comprising the amino acid sequence of:

SEQ ID No: 7 of WO 2011/066389 (SEQ ID NO: 85):

SYELTQPPSVSVSPGQAARITCSGDALPQKYVFWYQQKSGQAPVLVIYEDSKRPSGIPER
FSGSSSGTMATLTISGAQVEDEADYYCYSTDRSGNHRVFGGGTRLTVL.

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 2.9D10, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 2.9D10 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

SEQ ID No: 12 of WO 2011/066389 (SEQ ID NO: 86):

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGGEQYY
VDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDWNYGYYDMDVWGQGTTVTVSS;

and/or a light chain variable region comprising the amino acid sequence of:

SEQ ID No: 17 of WO 2011/066389 (SEQ ID NO: 87):

EIVLTQSPGTLSLSPGERATLSCRASQSVSSNYLAWFQQKPGQAPRLLIFGTSSRATGIP
DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSIFTFGPGTKVDIK.

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 2.14H9, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 2.14H9 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

SEQ ID No: 22 of WO 2011/066389 (SEQ ID NO: 88):

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWVANIKQDGSEKYY
VDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAREGGWFGELAFDYWGQGTLVTVS
S;

and/or a light chain variable region comprising the amino acid sequence of:

SEQ ID No: 27 of WO 2011/066389 (SEQ ID NO: 89):

EIVLTQSPGTLSLSPGERATLSCRASQRVSSSYLAWYQQKPGQAPRLLIYDASSRATGIP
DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSLPWTFGQGTEVEIK.

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 2.20A8, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 2.20A8 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

SEQ ID No: 32 of WO 2011/066389 (SEQ ID NO: 90):

EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSAIRGSGGSTYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDLHYDSSGYLDYWGQGTLVTVS
S;

and/or a light chain variable region comprising the amino acid sequence of:

SEQ ID No: 37 of WO 2011/066389 (SEQ ID NO: 91):

DIQMTQSPSSVSASVGDRVTITCRASQGIRSWLAWYQQKPGKAPKLLIYAISRLQSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQANSFPLTFGGGTKVEIK.

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 3.15G8, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 3.15G8 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

SEQ ID No: 42 of WO 2011/066389 (SEQ ID NO: 92):

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGGEKYY
VDSVKGRFTISRDNAKNSLFLQMNSLRAEDTAVYYCARVQLYSDYFDYWGQGTLVTVSS;

and/or a light chain variable region comprising the amino acid sequence of:

SEQ ID No: 47 of WO 2011/066389 (SEQ ID NO: 93):

DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKSGKAPKLLIYAASGLQSGVPS
RFSGSGSGTDFTLTISSLQPEDLATYYCQQSHSLPPTFGQGTKVEIK.

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 3.18G1, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, the entire disclosures of which are hereby

incorporated by reference. In illustrative embodiments, 3.18G1 or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

SEQ ID No: 52 of WO 2011/066389 (SEQ ID NO: 94):

EVQLLESGGDLVQPGGSLRLSCAASGFTFNSYAMSWVRQAPGKGLEWVSTISGSGGFTFS
ADSVKGRFTISRDNSKNTLFLQMNSLRVEDSAVYSCAKVLVGFNNGCWDYWGQGTLVTVS
S;

and/or a light chain variable region comprising the amino acid sequence of:

SEQ ID No: 57 of WO 2011/066389 (SEQ ID NO: 95):

SYVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPER
FSGSNSGNTATLTISRVEAGDEADYYCQVWDSSNDHVVFGGGTKLTVL.

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 2.7A4OPT, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, and US2014/0356353, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 2.7A4OPT or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

SEQ ID No: 62 of WO 2011/066389 (SEQ ID NO: 96):

EVQLVESGGGLVKPGGSLRLSCAASGFTFSTYSMNWVRQAPGKGLEWVSSISSSGDYIYY
ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDLVTSMVAFDYWGQGTLVTVSS;

and/or a light chain variable region comprising the amino acid sequence of:

SEQ ID No: 67 of WO 2011/066389 (SEQ ID NO: 97):

SYELTQPPSVSVSPGQTARITCSGDALPQKYVFWYQQKSGQAPVLVIYEDSKRPSGIPER
FSGSSSGTMATLTISGAQVEDEADYYCYSTDRSGNHRVFGGGTKLTVL.

In an embodiment, the targeting moiety comprises the anti-PD-L1 antibody 2.14H9OPT, or fragments thereof, as disclosed in WO 2011/066389, US8,779,108, and US2014/0356353, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, 2.14H9OPT or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain variable region comprising the amino acid sequence of:

SEQ ID No: 72 of WO 2011/066389 (SEQ ID NO: 98):

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWVANIKQDGSEKYY
VDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAREGGWFGELAFDYWGQGTLVTVS
S;

and/or a light chain variable region comprising the amino acid sequence of:

SEQ ID No: 77 of WO 2011/066389 (SEQ ID NO: 99):

EIVLTQSPGTLSLSPGERATLSCRASQRVSSSYLAWYQQKPGQAPRLLIYDASSRATGIP
DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSLPWTFGQGTKVEIK.

In an embodiment, the targeting moiety comprises any one of the anti-PD-L1 antibodies disclosed in WO2016/061142, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID Nos: 18, 30, 38, 46, 50, 54, 62, 70, and 78 of WO2016/061142:

SEQ ID No: 18 of WO2016/061142 (SEQ ID NO: 100):

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMYWVRQATGQGLEWMGRIDPNSGSTKY
NEKFKNRFTISRDDSKNTAYLQMNSLKTEDTAVYYCARDYRKGLYAMDYWGQGTTVTVSS;

SEQ ID No: 30 of WO2016/061142 (SEQ ID NO: 101):

EVQLVQSGAEVKKPGATVKISCKVSGYTFTSYWMYWVRQATGQGLEWMGRIDPNSGSTKY
NEKFKNRVTITADKSTSTAYMELSSLRSEDTAVYYCARDYRKGLYAMDYWGQGTTVTVSS;

SEQ ID No: 38 of WO2016/061142 (SEQ ID NO: 102):

EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMYWVRQAPGQGLEWMGRIDPNSGSTKY
NEKFKNRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDYRKGLYAMDYWGQGTTVTVSS;

SEQ ID No: 46 of WO2016/061142 (SEQ ID NO: 103):

EVQLVQSGAEVKKPGATVKISCKVSGYTFTSYWMYWIRQSPSRGLEWLGRIDPNSGSTKY
NEKFKNRLTISKDTSKNQVVLTMTNMDPVDTATYYCARDYRKGLYAMDYWGQGTTVTVSS;

SEQ ID No: 50 of WO2016/061142 (SEQ ID NO: 104):

EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMYWIRQPPGKGLEWIGRIDPNSGSTKY
NEKFKNRVTITADKSTSTAYMELSSLRSEDTAVYYCARDYRKGLYAMDYWGQGTTVTVSS;

SEQ ID No: 54 of WO2016/061142 (SEQ ID NO: 105):

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMYWIRQSPSRGLEWLGRIDPNSGSTKY
NEKFKNRFTISRDDSKNTAYLQMNSLKTEDTAVYYCARDYRKGLYAMDYWGQGTTVTVSS;

SEQ ID No: 62 of WO2016/061142 (SEQ ID NO: 106):

EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMYWVRQARGQRLEWIGRIDPNSGSTKY
NEKFKNRLTISKDTSKNQVVLTMTNMDPVDTATYYCARDYRKGLYAMDYWGQGTTVTVSS;

SEQ ID No: 70 of WO2016/061142 (SEQ ID NO: 107):

QITLKESGPTLVKPTQTLTLTCTFSGYTFTSYWMYWVRQAPGKGLEWVSRIDPNSGSTKY

NEKFKNRVTITADKSTSTAYMELSSLRSEDTAVYYCARDYRKGLYAMDYWGQGTTVTVSS;

SEQ ID No: 78 of WO2016/061142 (SEQ ID NO: 108):

EVQLVQSGAEVKKPGATVKISCKVSGYTFTSYWMYWVRQARGQRLEWIGRIDPNSGSTKY
NEKFKNRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDYRKGLYAMDYWGQGTTVTVSS;

and/or a light chain comprising an amino acid sequence selected from SEQ ID Nos: 22, 26, 34, 42, 58, 66, 74, 82, and 86 of WO2016/061142:

SEQ ID No: 22 of WO2016/061142 (SEQ ID NO: 109):

DIVMTQTPLSLPVTPGEPASISCKASQDVGTAVAWYLQKPGQSPQLLIYWASTRHTGIPA
RFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNSYPLTFGQGTKVEIK;

SEQ ID No: 26 of WO2016/061142 (SEQ ID NO: 110):

DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYLQKPGQSPQLLIYWASTRHTGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPLTFGQGTKVEIK;

SEQ ID No: 34 of WO2016/061142 (SEQ ID NO: 111):

EIVLTQSPDFQSVTPKEKVTITCKASQDVGTAVAWYLQKPGQSPQLLIYWASTRHTGVPD
RFSGSGSGTDFTLKISRVEAEDVGVYYCQQYNSYPLTFGQGTKVEIK;

SEQ ID No: 42 of WO2016/061142 (SEQ ID NO: 112):

EIVLTQSPDFQSVTPKEKVTITCKASQDVGTAVAWYLQKPGQSPQLLIYWASTRHTGVPS
RFSGSGSGTDFTFTISSLQPEDIATYYCQQYNSYPLTFGQGTKVEIK.

SEQ ID No: 58 of WO2016/061142 (SEQ ID NO: 113):

EIVLTQSPATLSLSPGERATLSCKASQDVGTAVAWYLQKPGQSPQLLIYWASTRHTGIPP
RFSGSGYGTDFTLTINNIESEDAAYYFCQQYNSYPLTFGQGTKVEIK;

SEQ ID No: 66 of WO2016/061142 (SEQ ID NO: 114):

DVVMTQSPLSLPVTLGQPASISCKASQDVGTAVAWYQQKPGQAPRLLIYWASTRHTGVPS
RFSGSGSGTEFTLTISSLQPDDFATYYCQQYNSYPLTFGQGTKVEIK;

SEQ ID No: 74 of WO2016/061142 (SEQ ID NO: 115):

DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGQAPRLLIYWASTRHTGVPS
RFSGSGSGTDFTFTISSLQPEDIATYYCQQYNSYPLTFGQGTKVEIK;

SEQ ID No: 82 of WO2016/061142 (SEQ ID NO: 116):

AIQLTQSPSSLSASVGDRVTITCKASQDVGTAVAWYLQKPGQSPQLLIYWASTRHTGVPS
RFSGSGSGTDFTFTISSLEAEDAATYYCQQYNSYPLTFGQGTKVEIK;

SEQ ID No: 86 of WO2016/061142 (SEQ ID NO: 117):

EIVLTQSPDFQSVTPKEKVTITCKASQDVGTAVAWYQQKPGQAPRLLIYWASTRHTGVPS
RFSGSGSGTEFTLTISSLQPDDFATYYCQQYNSYPLTFGQGTKVEIK.

In an embodiment, the targeting moiety comprises any one of the anti-PD-L1 antibodies disclosed in WO2016/022630, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID Nos: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, and 46 of WO2016/022630:

SEQ ID No: 2 of WO2016/022630 (SEQ ID NO: 118):

EVKLVESGGGLVKPGGSLKLSCAASGFIFRSYGMSWVRQTPEKRLEWVASISSGGSTYYP
DSVKGRFTISRDNARNILYLQMSSLRSEDTAMYDCARGYDSGFAYWGQGTLVTVSE;

SEQ ID No: 6 of WO2016/022630 (SEQ ID NO: 119):

EVKLVESGGGLVKPGGSLKLSCAASGFTFRSYGMSWVRQTPEKRLEWVASISSGGTTYYP
DSVKGRFIISRDNARNILYLQMSSLRSEDTAMYYCAKGYDSGFAYWGQGTLVIVSA;

SEQ ID No: 10 of WO2016/022630 (SEQ ID NO: 120):

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTTYGVHWVRQSPGKGLEWLGVIWRGVTTDYN
AAFMSRLTITKDNSKSQVFFKMNSLQANDTAIYYCARLGFYAMDYWGQGTSVTVSS;

SEQ ID No: 14 of WO2016/022630 (SEQ ID NO: 121):

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTSYGVHWVRQSPGKGLEWLGVIWSGGVTDYN
AAFISRLSISKDNSKSQVFFKMNSLQANDTAIYYCARLGFYAMDYWGQGTSVTVSS;

SEQ ID No: 18 of WO2016/022630 (SEQ ID NO: 122):

EVKLFESGGGLVQPGGSLKLSCVASGFDFSTYWMHWVRQAPGQGLEWIGQINPDSTTINY
APSLKDRFIISRDNAKNTLFLQMSKVRSEDTALYYCAKPGDYGYDFDCWGQGTTLTVSS;

SEQ ID No: 22 of WO2016/022630 (SEQ ID NO: 123):

EVQLQESGPSLVKPSQTLSLTCSVTGDSITSGYWNWIRKFPGNKLEYMGYISYSGSTYYN
PSLKSRISITRDTSKNQYYLQLNSVTTEDTATYYCARSLLWFSTGFAYWGQGTLVTVSA;

SEQ ID No: 26 of WO2016/022630 (SEQ ID NO: 124):

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTSYGVHWVRQSPGKGLEWLGVIWSGGITDYN
AAFKSRLSISKDNSKSQVFFKMNSLQANDTAIYFCARLGFYAMDYWGQGTSVTVSS;

SEQ ID No: 30 of WO2016/022630 (SEQ ID NO: 125):

EVKLVESGGGLVKPGGSLKLSCAASGFTFRSYGMSWARQIPEKRLEWVASISSGGTTYYL
GSVQGRFTISRDNARNILYLQMSSLRSEDTAMYYCARGYDAGFAYWGQGTLVSVSE;

SEQ ID No: 34 of WO2016/022630 (SEQ ID NO: 126):

EVQLQESGPSLVKPSQTLSLTCSVTGDSITSGYWTWIRKFPGNKLEYMGYISYTGSTYYN
PSLKSRISISRDTSKSQYYLQLNSVTTEDTATYYCARQRDWLGFAYWGQGTLVTVSA;

SEQ ID No: 38 of WO2016/022630 (SEQ ID NO: 127):

EEKLVESGGGLVKPGGSLKLSCAASGFSFSSYGMSWVRQTPEKRLEWVASISSGGSIYYP
DSVKGRFTISRDNARNILYLQMSSLRSEDTAMYYCARGYDAGFAFWGQGTLVTASA;

SEQ ID No: 42 of WO2016/022630 (SEQ ID NO: 128):

QITLKESGPTLVKPTQTLTLTCTVSGFSLSTYGVHWIRQPPGKALEWLGVIWRGVTTDYN
AAFMSRLTITKDNSKNQVVLTMNNMDPVDTATYYCARLGFYAMDYWGQGTLVTVSS;

SEQ ID No: 46 of WO2016/022630 (SEQ ID NO: 129):

EVQLVESGGGLVKPGGSLRLSCAASGFIFRSYGMSWVRQAPGKGLEWVASISSGGSTYYP
DSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYDCARGYDSGFAYWGQGTLVTVSS;

and/or a light chain comprising an amino acid sequence selected from SEQ ID Nos: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 of WO2016/022630:

SEQ ID No: 4 of WO2016/022630 (SEQ ID NO: 130):

DIVLTQSPASLAVSLGQRATISCRASQSVSTSSSSFMHWYQQKPGQPPKLLIKYASNLES
GVPARFSGSGSGTDFTLNIHPVEEEDTATYYCQHSWEIPYTFGGGTKLEIKR;

SEQ ID No: 8 of WO2016/022630 (SEQ ID NO: 131):

DIVLTQSPPSLAVSLGQRATISCRASQSVSTSSSSYMHWYQQKPGQPPKLLIKYASNLES
GVPARFSGSGSGTDFTLNIHPVEEEDTATYYCQHSWEIPYTFGGGTKLEIK;

SEQ ID No: 12 of WO2016/022630 (SEQ ID NO: 132):

SIVMTQTPKFLLVSAGDRVTITCKASQSVSNDVAWYQQKPGQSPKLLIYYAANRYTGVPD
RFTGSGYGTDFTFTISIVQAEDLAVYFCQQDYTSPYTFGGGTKLEIK;

SEQ ID No: 16 of WO2016/022630 (SEQ ID NO: 133):

SIVMTQTPKFLLVSAGDRVTITCKASQSVSNDVGWYQQKPGQSPKLLIYYASNRYSGVPD
RFTGSGYGTDFTFTISTVQAEDLAVYFCQQDYTSPYTFGGGTKLEIK;

SEQ ID No: 20 of WO2016/022630 (SEQ ID NO: 134):

DVLMTQTPLYLPVSLGDQASISCRSSQIIVHSNANTYLEWFLQKPGQSPKLLIYKVSNRF
SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPYTFGGGTKLEIK;

SEQ ID No: 24 of WO2016/022630 (SEQ ID NO: 135):

QIVLTQSPAIMSASPGEKVTLTCSASSSVSSSYLYWNQQKPGSSPKVWIYNTSNLASGVP
ARFSGSGSGTSYSLTISSMEAEDAASYFCHQWRSYPPTLGAGTKLELK;

SEQ ID No: 28 of WO2016/022630 (SEQ ID NO: 136):

QIVLTQSPAIMSASPGEKVTMTCSANSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPAR
FSGSGSGTSYSLTISSMGAEDAATYYCQQWSSNPWTFGGGTKLEIK;

SEQ ID No: 32 of WO2016/022630 (SEQ ID NO: 137):

DIVLTQSPASLAVSLGQRATISCRASQSVSTSSYSYMHWYQQKPGQPPKLLIKYASNLES
GVPARFSGSGSGTDFTLNIHPVEEEDTATYYCQNSWEIPYTFGGGTKLEIK;

SEQ ID No: 36 of WO2016/022630 (SEQ ID NO: 138):

DIVMTQTPSSLAVSLGEKVTMSCKSSQSLLYSSNQKNSLAWYQQKPGQSPKLLIYWASNR
ESGVPDRFTGSSSGTDFTLTISSVKAEDLAVYYCQQYYSYPLTFGAGTKLELK;

SEQ ID No: 40 of WO2016/022630 (SEQ ID NO: 139):

DIVLTQSPASLAVSLGQRATISCRASQSVSTSSYSYVHWYQQKPGQPPKLLIKYASNLES
GVPARFSGSGSGTDFTLNIHPVEEEDTATYYCQHSWEIPYTFGGGTKLEIK;

SEQ ID No: 44 of WO2016/022630 (SEQ ID NO: 140):

DIQMTQSPSSLSASVGDRVTITCKASQSVSNDVAWYQQKPGKAPKLLIYYAANRYTGVPD
RFSGSGYGTDFTFTISSLQPEDIATYFCQQDYTSPYTFGQGTKLEIK;

SEQ ID No: 48 of WO2016/022630 (SEQ ID NO: 141):

DIVLTQSPASLAVSPGQRATITCRASQSVSTSSSSFMHWYQQKPGQPPKLLIKYASNLES
GVPARFSGSGSGTDFTLTINPVEANDTANYYCQHSWEIPYTFGQGTKLEIK.

In an embodiment, the targeting moiety comprises any one of the anti-PD-L1 antibodies disclosed in WO2015/112900, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID Nos: 38, 50, 82, and 86 of WO 2015/112900:

SEQ ID No: 38 of WO2015/112900 (SEQ ID NO: 142):

EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWVRQATGQGLEWMGNIYPGTGGSNF
DEKFKNRVTITADKSTSTAYMELSSLRSEDTAVYYCTRWTTGTGAYWGQGTTVTVSS;

SEQ ID No: 50 of WO 2015/112900 (SEQ ID NO: 143):

EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWIRQSPSRGLEWLGNIYPGTGGSNF
DEKFKNRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRWTTGTGAYWGQGTTVTVSS;

SEQ ID No: 82 of WO 2015/112900 (SEQ ID NO: 144):

QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWMHWIRQSPSRGLEWLGNIYPGTGGSNF

DEKFKNRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRWTTGTGAYWGQGTTVTVSS;

SEQ ID No: 86 of WO 2015/112900 (SEQ ID NO: 145):

EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWVRQAPGQGLEWMGNIYPGTGGSNF
DEKFKNRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRWTTGTGAYWGQGTTVTVSS;

and/or a light chain comprising an amino acid sequence selected from SEQ ID Nos: 42, 46, 54, 58, 62, 66, 70, 74, and 78 of WO 2015/112900:

SEQ ID No: 42 of WO2015/112900 (SEQ ID NO: 146):

EIVLTQSPATLSLSPGERATLSCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
ESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQNDYSYPYTFGQGTKVEIK;

SEQ ID No: 46 of WO 2015/112900(SEQ ID NO: 147):

DIQMTQSPSSLSASVGDRVTITCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
ESGIPPRFSGSGYGTDFTLTINNIESEDAAYYFCQNDYSYPYTFGQGTKVEIK;

SEQ ID No: 54 of WO 2015/112900 (SEQ ID NO: 148):

EIVLTQSPATLSLSPGERATLSCKSSQSLLDSGNQKNFLTWYQQKPGKAPKLLIYWASTR
ESGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQNDYSYPYTFGQGTKVEIK;

SEQ ID No: 58 of WO 2015/112900 (SEQ ID NO: 149):

DIVMTQTPLSLPVTPGEPASISCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK;

SEQ ID No: 62 of WO 2015/112900 (SEQ ID NO: 150):

EIVLTQSPATLSLSPGERATLSCKSSQSLLDSGNQKNFLTWYQQKPGKAPKLLIYWASTR
ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK;

SEQ ID No: 66 of WO 2015/112900 (SEQ ID NO: 151):

EIVLTQSPDFQSVTPKEKVTITCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK;

SEQ ID No: 70 of WO 2015/112900 (SEQ ID NO: 152):

EIVLTQSPATLSLSPGERATLSCKSSQSLLDSGNQKNFLTWYQQKPGQAPRLLIYWASTR
ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK;

SEQ ID No: 74 of WO 2015/112900 (SEQ ID NO: 153):

DIQMTQSPSSLSASVGDRVTITCKSSQSLLDSGNQKNFLTWYLQKPGQSPQLLIYWASTR
ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK;

SEQ ID No: 78 of WO 2015/112900 (SEQ ID NO: 154):

DVVMTQSPLSLPVTLGQPASISCKSSQSLLDSGNQKNFLTWYQQKPGKAPKLLIYWASTR
ESGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQNDYSYPYTFGQGTKVEIK.

In an embodiment, the targeting moiety comprises any one of the anti-PD-L1 antibodies disclosed in WO 2010/077634 and US 8,217,149, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, the anti-PD-L1 antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain region comprising the amino acid sequence of:

SEQ ID No: 20 of WO 2010/077634 (SEQ ID NO: 155):

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYY
ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSA;

and/or a light chain variable region comprising the amino acid sequence of:

SEQ ID No: 21 of WO 2010/077634 (SEQ ID NO: 156):

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR.

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

In an embodiment, the targeting moiety comprises a VHH directed against PD-L1 as disclosed, for example, in US 8,907,065 and WO 2008/071447, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, the VHHs against PD-L1 comprise SEQ ID NOS: 394-399 of US 8,907,065:

SEQ ID No: 394 of US 8,907,065 (SEQ ID NO: 157):

EVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAIGWFRQAPGKEREWASS
ISSSDGSTYYADSVKGRFTISRDNAKNTVFLQMNSLKPEDTAVYSCAASQ
APITIATMMKPFYDYWGQGTQVTVSS;

SEQ ID No: 395 of US 8,907,065 (SEQ ID NO: 158):

EVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAKCWFRQAPGKEREWVSC
ISSSDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYFCAARH
GGPLTVEYFFDYWGQGTQVTVSS;

SEQ ID No: 396 of US 8,907,065 (SEQ ID NO: 159):

EVQLVESGGGLVQPGGSLRLSCAASGFTFDYYAIGWFRQAPGKAREGVSC
ISGGDNSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCATGG
WKYCSGYDPEYIYWGQGTQVTVSS;

SEQ ID No: 397 of US 8,907,065 (SEQ ID NO: 160):

EVQLVESGGGLVQAGGSLRLSCAASGSTFSQYDVGWYRQAPGKQRELVA
FSSSGGRTIYPDSVKGRFTFSRDNTKNTVYLQMTSLKPEDTAVYYCKIDW
YLNSYWGQGTQVTVSS;

SEQ ID No: 398 of US 8,907,065 (SEQ ID NO: 161):

EVQLVESGGGLVQAGGSLRLSCAASGVDASNSAMGWYRQAPGKQREWVAR
ITGGGLIAYTDSVKGRFTISRDNAKSTVYLQMNSLEPEDTAVYYCNTINS
RDGWGQGTQVTVSS;

SEQ ID No: 399 of US 8,907,065 (SEQ ID NO: 162):

EVQLVESGGGLVQAGGSLTISCAASGITFSDSIVSWYRRARGKQREWVAG
ISNGGTTKYAESVLGRFTISRDNAKNNVYLQMNGLNPEDTAVYLCKVRQY
WGQGTQVTVSS.

In various embodiments, the present chimeric protein has one targeting moiety directed against PD-L2. In some embodiments, the chimeric protein include a targeting moiety which selectively bind a PD-L2 polypeptide. In some embodiments, the chimeric protein comprises an antibody, an antibody derivative or format, a peptide or polypeptide, or a fusion protein that selectively binds a PD-L2 polypeptide.

In an embodiment, the targeting moiety comprises a VHH directed against PD-L2 as disclosed, for example, in US 8,907,065 and WO 2008/071447, the entire disclosures of which are hereby incorporated by reference. In illustrative embodiments, the VHHs against PD-1 comprise SEQ ID Nos: 449-455 of US 8,907,065:

SEQ ID No: 449 of US 8,907,065 (SEQ ID NO: 163):

EVQLVESGGGLVQAGGSLRLSCAASESTVLINAMGWYRQAPGKQRELVAS
ISSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNADVY
PQDYGLGYVEGKVYYGHDYWGTGTLVTVSS;

SEQ ID No: 450 of US 8,907,065 (SEQ ID NO: 164):

EVQLVESGGGLVQAGGSLRLSCAASGSTFSNYVSNYAMGWGRQAPGTQ
RELVASISNGDTTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYY
CFEHQVAGLTWGQGTQVTVSS;

SEQ ID No: 451 of US 8,907,065 (SEQ ID NO: 165):

EVQLVESGGGLVQAGGSLRLSCVASGXALKIXVMGWYRQAPGKQRELV
AAITSGGRTNYSDSVKGRFTISGDNAXNTVYLQMNSLKSEDTAVYYCRE
WNSGYPPVDYWGQGTQVTVSS;

SEQ ID No: 452 of US 8,907,065 (SEQ ID NO: 166):

EVQLVESGGGLVQAGGSLRLSCAASGRTFSSGTMGWFRRAPGKEREFV
ASIPWSGGRTYYADSVKDRFTISRDNAQNTVFLQMNSLKPEDTAVYYCAF
KERSTGWDFASWGQGIQVTVSS;

SEQ ID No: 453 of US 8,907,065 (SEQ ID NO: 167):

EVQLVESGGGLVQTGGSLRLSCAASGFTLDYYGIGWFRQAPGKEREGVS
FISGSDGSTYYAESVKGRFTISRDKAKNTVYLQMNSLKPEDTAVYYCAAD
PWGPPSIATMTSYEYKHWGQGTQVTVSS;

SEQ ID No: 454 of US 8,907,065 (SEQ ID NO: 168):

EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYTMIWLRRAPGKGFEWV
STIDKDGNTNYVDSVKGRFAVSRDNTKNTLYLQMNSLKPEDTAMYYCTK
HGSSARGQGTRVTVSS;

SEQ ID No: 455 of US 8,907,065 (SEQ ID NO: 169):

EVQLVESGGGLVEPGGSLRLSCVASGFTFSSYDMSWVRQAPGKGLE
WVSTINSGGGITYRGSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYY
CENGGSSYRRGQGTQVTVSS.

In an embodiment, the targeting moiety comprises any one of the anti-PD-L2 antibodies disclosed in US2011/0271358 and WO2010/036959, the entire contents of which are hereby incorporated by reference. In illustrative embodiments, the antibody or an antigen-binding fragment thereof for use in the methods provided herein comprises a heavy chain comprising an amino acid sequence selected from SEQ ID Nos: 43-47 of US2011/0271358:

SEQ ID No: 43 of US2011/0271358 (SEQ ID NO: 170):

QVQLVQSGAELKKPGASVKMSCKASGYTFTGYTMHWVKQAPGQGLEWIGYINPRSGYTEY
NQKFKDRTTLTADKSTSTAYMELSSLRSEDSAVYYCARPWFAYWGQGTLVTVSS;

SEQ ID No: 44 of US2011/0271358 (SEQ ID NO: 171):

QVQLVQSGAEVKKPGASVKMSCKASGYTFTGYTMHWVKQAPGQGLEWIGYINPRSGYTEY
NQKFKDRTTLTADKSTSTAYMELSSLRSEDTAVYYCARPWFAYWGQGTLVTVSS;

SEQ ID No: 45 of US2011/0271358 (SEQ ID NO: 172):

QVQLVQSGAEVKKPGASVKMSCKASGYTFTGYTMHWVRQAPGQGLEWIGYINPRSGYTEY
NQKFKDRTTLTADKSTSTAYMELSSLRSEDTAVYYCARPWFAYWGQGTLVTVSS;

SEQ ID No: 46 of US2011/0271358 (SEQ ID NO: 173):

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYTMHWVRQAPGQGLEWIGYINPRSGYTEY
NQKFKDRTTLTADKSTSTAYMELSSLRSEDTAVYYCARPWFAYWGQGTLVTVSS;

SEQ ID No: 47 of US2011/0271358 (SEQ ID NO: 174):

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYTMHWVRQAPGQGLEWIGYINPRSGYTEY

NQKFKDRTTITADKSTSTAYMELSSLRSEDTAVYYCARPWFAYWGQGTLVTVSS;

and/or a light chain comprising an amino acid sequence selected from SEQ ID Nos: 48-51 of US2011/0271358:

SEQ ID No: 48 of US2011/0271358 (SEQ ID NO: 175):

DIVMTQSPASLTVTPGEKVTITCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYWASTR
ESGVPDRFTGSGSGTDFTLTISSLQAEDVAVYYCQNDYSYPLTFGQGTKLEIK;

SEQ ID No: 49 of US2011/0271358 (SEQ ID NO: 176):

DIVMTQSPASLSVTPGEKVTITCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYWASTR
ESGVPDRFTGSGSGTDFTLTISSLQAEDVAVYYCQNDYSYPLTFGQGTKLEIK;

SEQ ID No: 50 of US2011/0271358 (SEQ ID NO: 177):

DIVMTQSPAFLSVTPGEKVTITCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYWASTR
ESGVPDRFTGSGSGTDFTLTISSLQAEDVAVYYCQNDYSYPLTFGQGTKLEIK;

SEQ ID No: 51 of US2011/0271358 (SEQ ID NO: 178):

DIVMTQSPAFLSVTPGEKVTITCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYWASTR
ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDYSYPLTFGQGTKLEIK.

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

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

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

Modified Signaling Agents

In one aspect, the present invention provides a chimeric protein that includes a signaling agent (for instance, an immune-modulating agent). In various embodiments, the signaling agent is modified to have reduced activity. In some embodiments, the signaling agent is modified to have reduced affinity for one or more of its receptors which allows for attenuation of activity and/or prevents non-specific signaling or undesirable sequestration of the chimeric protein. In various embodiments, the activity of the modified signaling agent is restored in the context of the chimeric protein which further comprises a targeting moiety that targets specific cell types. Particularly, the activity of the modified signaling agent is restored towards targeted cells but not towards non-targeted cells. Accordingly, in various embodiments, the chimeric proteins of the invention offer therapeutic advantages such as enhanced specificity and efficacy at targeted cells as well as reduced side effects including lower systemic toxicity.

In various embodiments, the signaling agent is selected from modified versions of an immune-modulating agent, *e.g.* one or more of an interleukin, interferon, and tumor necrosis factor.

In some embodiments, the signaling agent is a modified version of an interleukin, including for example IL-1; IL-2; IL-3; IL-4; IL-5; IL-6; IL-7; IL-8; IL-9; IL-10; IL-11; IL-12; IL-13; IL-14; IL-15; IL-16; IL-17; IL-18; IL-19; IL-20; IL-21; IL-22; IL-23; IL-24; IL-25; IL-26; IL-27; IL-28; IL-29; IL-30; IL-31; IL-32; IL-33; IL-35; IL-36 or a fragment, variant, analogue, or family-member thereof.

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

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

In various embodiments, the signaling agent is a modified (*e.g.* mutant) form of the signaling agent having one or more mutations. In various embodiments, the mutations allow for the modified signaling agent to have one or more of attenuated activity such as one or more of reduced binding affinity, reduced endogenous activity, and reduced specific bioactivity relative to unmodified or unmutated, *i.e.* the wild type form of the signaling agent (*e.g.* comparing the same signaling agent in a wild type form versus a modified (*e.g.* mutant) form). In some embodiments, the mutations which attenuate or reduce binding or affinity include those mutations which substantially reduce or ablate binding or activity. In some embodiments, the mutations which attenuate or reduce binding or affinity are different than those mutations which substantially reduce or ablate binding or activity. Consequentially, in various embodiments, the mutations allow for the signaling agent to be more safe, e.g. have reduced systemic toxicity, reduced side effects, and reduced off-target effects relative to unmutated, *i.e.* wild type, signaling agent (*e.g.* comparing the same signaling agent in a wild type form versus a modified (*e.g.* mutant) form). As described herein, the signaling agent may have improved safety due to one of more modifications, *e.g.* mutations. In various embodiments, improved safety means that the present chimeric protein provides lower toxicity (e.g. systemic toxicity and/or tissue/organ-associated toxicities); and/or lessened or substantially eliminated side effects; and/or increased tolerability, lessened or substantially eliminated adverse events; and/or reduced or substantially eliminated off-target effects; and/or an increased therapeutic window.

In various embodiments, the signaling agent is modified to have one or more mutations that reduce its binding affinity or activity for one or more of its receptors. In some embodiments, the signaling agent is modified to have one or more mutations that substantially reduce or ablate binding affinity or activity for the receptors. In some embodiments, the activity provided by the wild type signaling agent is agonism at the receptor (*e.g.* activation of a cellular effect at a site of therapy). For example, the wild type signaling agent may activate its receptor. In such embodiments, the mutations result in the modified signaling agent to have reduced or ablated activating activity at the receptor. For example, the mutations may result in the modified signaling agent to deliver a reduced activating signal to a target cell or the activating signal could be ablated. In some embodiments, the activity provided by the wild type signaling agent is antagonism at the receptor (*e.g.* blocking or dampening of a cellular effect at a site of therapy). For example, the wild type signaling agent may antagonize or inhibit the receptor. In these embodiments, the mutations result in the modified signaling agent to have a reduced or ablated antagonizing activity at the receptor. For example, the mutations may result in the modified signaling agent to deliver a reduced inhibitory signal to a target cell or the inhibitory signal could be ablated. In various embodiments, the signaling agent is antagonistic due to one or more mutations, *e.g.* an agonistic signaling agent is converted to an antagonistic signaling agent (*e.g.* as described in WO 2015/007520, the entire contents of which are hereby incorporated by reference) and, such a converted signaling agent, optionally, also bears one or more mutations that reduce its binding affinity or activity for one or more of its receptors or that substantially reduce or ablate binding affinity or activity for one or more of its receptors. In some embodiments, the reduced affinity or activity at the receptor is restorable by attachment with the targeting moiety. In other embodiments, the reduced affinity or activity at the receptor is not substantially restorable by attachment with the targeting moiety.

In various embodiments, the chimeric proteins of the present invention reduce off-target effects because their signaling agents have mutations that weaken or ablate binding affinity or activity at a receptor. In various embodiments, this reduction in side effects is observed relative with, for example, the wild type signaling agents. In various embodiments, the signaling agent is active on target cells because the targeting moiety compensates for the missing/insufficient binding (*e.g.*, without limitation and/or avidity) required for substantial activation. In various embodiments, the modified signaling agent is substantially inactive *en route* to the site of therapeutic activity and has its effect substantially on specifically targeted cell types which greatly reduces undesired side effects.

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

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

In various embodiments, the modified signaling agent comprises one or more mutations that cause the signaling agent to have reduced, substantially reduced, or ablated affinity, *e.g.* binding (*e.g.* K_D) and/or activation (for instance, when the modified signaling agent is an agonist of its receptor, measurable as, for example, K_A and/or EC_{50}) and/or inhibition (for instance, when the modified signaling agent is an antagonist of its receptor, measurable as, for example, K_I and/or IC_{50}), for one or more of its receptors. In various embodiments, the reduced affinity at the signaling agent's receptor allows for attenuation of activity (inclusive of agonism or antagonism). In such embodiments, the modified signaling agent has about 1%, or about 3%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about

60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 10%-20%, about 20%-40%, about 50%, about 40%-60%, about 60%-80%, about 80%-100% of the affinity for the receptor relative to the wild type signaling agent. In some embodiments, the binding affinity is at least about 2-fold lower, about 3-fold lower, about 4-fold lower, about 5-fold lower, about 6-fold lower, about 7-fold lower, about 8-fold lower, about 9-fold lower, at least about 10-fold lower, at least about 15-fold lower, at least about 20-fold lower, at least about 25-fold lower, at least about 30-fold lower, at least about 35-fold lower, at least about 40-fold lower, at least about 45-fold lower, at least about 50-fold lower, at least about 100-fold lower, at least about 150-fold lower, or about 10-50-fold lower, about 50-100-fold lower, about 100-150-fold lower, about 150-200-fold lower, or more than 200-fold lower relative to the wild type signaling agent.

In embodiments wherein the chimeric protein has mutations that reduce binding at one receptor and substantially reduce or ablate binding at a second receptor, the attenuation or reduction in binding affinity of a modified signaling agent for one receptor is less than the substantial reduction or ablation in affinity for the other receptor. In some embodiments, the attenuation or reduction in binding affinity of a modified signaling agent for one receptor is less than the substantial reduction or ablation in affinity for the other receptor by about 1%, or about 3%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%. In various embodiments, substantial reduction or ablation refers to a greater reduction in binding affinity and/or activity than attenuation or reduction.

In various embodiments, the modified signaling agent comprises one or more mutations that reduce the endogenous activity of the signaling agent to about 75%, or about 70%, or about 60%, or about 50%, or about 40%, or about 30%, or about 25%, or about 20%, or about 10%, or about 5%, or about 3%, or about 1%, *e.g.*, relative to the wild type signaling agent

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

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

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

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

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

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

"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).

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

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

In some embodiments, the modified signaling agent comprises one or more mutations that cause the signaling agent to have reduced affinity and/or activity for a type I cytokine receptor, a type II cytokine receptor, and a receptor in the Tumor Necrosis Factor Receptor (TNFR) superfamily.

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

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

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

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

Mutant forms of interferon α such as IFN-α2 are known to the person skilled in the art. In an illustrative embodiment, the modified signaling agent is the allelic form IFN-α2a having the amino acid sequence of:

IFN-α2a (SEQ ID NO: 179):

CDLPQTHSLGSRRTLMLLAQMRKISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLH
EMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQGVGVTETPLMKED
SILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKE.

In an illustrative embodiment, the modified signaling agent is the allelic form IFN-α2b having the amino acid sequence of (which differs from IFN-α2a at amino acid position 23):

IFN-α2b (SEQ ID NO: 180):

CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLH
EMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQGVGVTETPLMKED
SILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKE.

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

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

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

In some embodiments, the IFN-α2 mutant comprises a mutation at position R120. In some embodiments, the IFN-α2 mutant comprises one or more mutations selected from R120E and R120E/K121E, as described in WO2015/007520 and WO2010/030671, the entire contents of which are hereby incorporated by reference. In such embodiments, said IFN-α2 mutant antagonizes wildtype IFN-α2 activity. In such embodiments, said mutant IFN-α2 has reduced affinity and/or activity for IFNAR1 while affinity and/or activity of IFNR2 is retained.

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

In some embodiments, the human IFN-α2 mutant comprises one or more mutations selected from, L15A, A19W, R22A, R23A, L26A, F27A, L30A, L30V, K31A, D32A, R33K, R33A, R33Q, H34A, D35A, Q40A, D114R, L117A, R120A, R125A , K134A, R144A, A145G, A145M, M148A, R149A, S152A, L153A, and N156A as disclosed in WO 2013/059885, the entire disclosures of which are hereby incorporated by reference. In some embodiments, the human IFN-α2 mutant comprises the mutations H57Y, E58N, Q61S, and/or L30A as disclosed in WO

2013/059885. In some embodiments, the human IFN-α2 mutant comprises the mutations H57Y, E58N, Q61S, and/or R33A as disclosed in WO 2013/059885. In some embodiments, the human IFN-α2 mutant comprises the mutations H57Y, E58N, Q61S, and/or M148A as disclosed in WO 2013/059885. In some embodiments, the human IFN-α2 mutant comprises the mutations H57Y, E58N, Q61S, and/or L153A as disclosed in WO 2013/059885. In some embodiments, the human IFN-α2 mutant comprises the mutations N65A, L80A, Y85A, and/or Y89A as disclosed in WO 2013/059885. In some embodiments, the human IFN-α2 mutant comprises the mutations N65A, L80A, Y85A, Y89A, and/or D114A as disclosed in WO 2013/059885.

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

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

In an embodiment, the modified signaling agent is TNF-α. TNF is a pleiotropic cytokine with many diverse functions, including regulation of cell growth, differentiation, apoptosis, tumorigenesis, viral replication, autoimmunity, immune cell functions and trafficking, inflammation, and septic shock. It binds to two distinct membrane receptors on target cells: TNFR1 (p55) and TNFR2 (p75). TNFR1 exhibits a very broad expression pattern whereas TNFR2 is expressed preferentially on certain populations of lymphocytes, Tregs, endothelial cells, certain neurons, microglia, cardiac myocytes and mesenchymal stem cells. Very distinct biological pathways are activated in response to receptor activation, although there is also some overlap. As a general rule, without wishing to be bound by theory, TNFR1 signaling is associated with induction of apoptosis (cell death) and TNFR2 signaling is associated with activation of cell survival signals (*e.g.* activation of NFkB pathway). Administration of TNF is systemically toxic, and this is largely due to TNFR1 engagement. However, it should be noted that activation of TNFR2 is also associated with a broad range of activities and, as with TNFR1, in the context of developing TNF based therapeutics, control over TNF targeting and activity is important.

In some embodiments, the modified signaling agent has reduced affinity and/or activity for TNFR1 and/or TNFR2. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for TNFR1 and/or TNFR2. TNFR1 is expressed in most tissues, and is involved in cell death signaling while, by contrast, TNFR2 is involved in cell survival signaling. Accordingly, in embodiments directed to methods of treating cancer, the modified signaling agent has reduced affinity and/or activity for TNFR1 and/or substantially reduced or ablated affinity and/or activity for TNFR2. In these embodiments, the chimeric proteins may be targeted to a cell for which apoptosis is desired, *e.g.* a tumor cell or a tumor vasculature endothelial cell. In embodiments directed to methods of promoting cell survival, for example, in neurogenesis for the treatment of

neurodegenerative disorders, the modified signaling agent has reduced affinity and/or activity for TNFR2 and/or substantially reduced or ablated affinity and/or activity for TNFR1. Stated another way, the present chimeric proteins, in some embodiments, comprise modified TNF-α agent that allows of favoring either death or survival signals.

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

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

In some embodiments, the chimera causes the death of auto-reactive T cells, *e.g.* by activation of TNFR2 and/or avoidance of TNFR1 (*e.g.* a modified TNF having reduced affinity and/or activity for TNFR2 and/or substantially reduced or ablated affinity and/or activity for TNFR1). Without wishing to be bound by theory these auto-reactive T cells, have their apoptosis/survival signals altered *e.g.* by NFkB pathway activity/signaling alterations.

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

In an embodiment, the wild type TNF-α has the amino acid sequence of:

TNF-α (SEQ ID NO: 181)

VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLV
VPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQ
RETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYF
GIIAL.

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

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

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

In some embodiments, the modified human TNF-α moiety has mutations that provide receptor selectivity as described in PCT/IB2016/001668, the entire contents of which are hereby incorporated by reference. In some embodiments, the mutations to TNF are TNF-R1 selective. In some embodiments, the mutations to TNF which are TNF-R1 selective are at one or more of positions R32, S86, and E146. In some embodiments, the mutations to TNF which are TNF-R1 selective are one or more of R32W, S86T, and E146K. In some embodiments, the mutations to TNF which are TNF-R1 selective are one or more of R32W, R32W/S86T, R32W/E146K and E146K. In some embodiments, the mutations to TNF are TNF-R2 selective. In some embodiments, the mutations to TNF which are TNF-R2 selective are at one or more of positions A145, E146, and S147. In some embodiments, the mutations to TNF which are TNF-R2 selective are one or more of A145T, A145R, E146D, and S147D. In some embodiments, the mutations to TNF which are TNF-R2 selective are one or more of A145R, A145T/S147D, and A145T/E146D/S147D.

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

In an embodiment, the wild type TNF-β has the amino acid sequence of:

TNF-β (SEQ ID NO: 182)

LPGVGLTPSAAQTARQHPKMHLAHSNLKPAAHLIGDPSKQNSLLWRANTD
RAFLQDGFSLSNNSLLVPTSGIYFVYSQVVFSGKAYSPKATSSPLYLAHEV
QLFSSQYPFHVPLLSSQKMVYPGLQEPWLHSMYHGAAFQLTQGDQLSTH
TDGIPHLVLSPSTVFFGAFAL.

In such embodiments, the modified TNF-β agent may comprise mutations at one or more amino acids at positions 106-113, which produce a modified TNF-β with reduced receptor binding affinity to TNFR2. In an

embodiment, the modified signaling agent has one or more substitution mutations at amino acid positions 106-113. In illustrative embodiments, the substitution mutations are selected from Q107E, Q107D, S106E, S106D, Q107R, Q107N, Q107E/S106E, Q107E/S106D, Q107D/S106E, and Q107D/S106D. In another embodiment, the modified signaling agent has an insertion of about 1 to about 3 amino acids at positions 106-113.

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

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

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

In an embodiment, the wild type TRAIL has the amino acid sequence of:

TRAIL

MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYFTNELKQMQDKYSK
SGIACFLKEDDSYWDPNDEESMNSPCWQVKWQLRQLVRKMILRTSEETIS
TVQEKQQNISPLVRERGPQRVAAHITGTRGRSNTLSSPNSKNEKALGRKIN
SWESSRSGHSFLSNLHLRNGELVIHEKGFYYIYSQTYFRFQEEIKENTKND
KQMVQYIYKYTSYPDPILLMKSARNSCWSKDAEYGLYSIYQGGIFELKEND
RIFVSVTNEHLIDMDHEASFFGAFLVG.

In such embodiments, the modified TRAIL agent may comprise a mutation at amino acid positions T127-R132, E144-R149, E155-H161, Y189-Y209, T214-1220,K224-A226, W231, E236-L239, E249-K251, T261-H264 and

H270-E271 (Numbering based on the human sequence, Genbank accession number NP _003801, version 10 NP _003801.1, GI: 4507593; see above).

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

In an embodiment, the wild type IL-1β has the amino acid sequence of:

IL-1 beta (mature form, wild type)

APVRSLNCTLRDSQQKSLVMSGPYELKALHLQGQDMEQQVVFSMSFVQG
EESNDKIPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFV
FNKIEINNKLEFESAQFPNWYISTSQAENMPVFLGGTKGGQDITDFTMQFV
SS (SEQ ID NO: 183).

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

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

In some embodiments, the modified IL-1 has reduced affinity and/or activity (*e.g.* antagonistic activity, *e.g.* natural antagonistic activity or antagonistic activity that is the result of one or more mutations, *see, e.g.,* WO 2015/007520, the entire contents of which are hereby incorporated by reference) for IL-1R1. In some embodiments, the modified IL-1 has substantially reduced or ablated affinity and/or activity for IL-1R2. In such embodiments, there is the IL-1/ IL-1R1 signaling is not restorable and prevention of loss of therapeutic chimeras at IL-R2 and therefore a reduction in dose of IL-1 that is required (*e.g.* relative to wild type or a chimera bearing

only an attenuation mutation for IL-R1). Such constructs find use in, for example, methods of treating autoimmune diseases, including, for example, suppressing the immune system.

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

In an embodiment, the modified signaling agent is IL-2. In such an embodiment, the modified signaling agent has reduced affinity and/or activity for IL-2Rα and/or IL-2Rβ and/or IL-2Rγ. In some embodiments, the modified signaling agent has reduced affinity and/or activity for IL-2Rβ and/or IL-2Rγ. In some embodiments, the modified signaling agent has substantially reduced or ablated affinity and/or activity for IL-2Rα. Such embodiments may be relevant for treatment of cancer, for instance when the modified IL-2 is agonistic at IL-2Rβ and/or IL-2Rγ. For instance, the present constructs may favor attenuated activation of $CD8^+$ T cells (which can provide an anti-tumor effect), which have IL2 receptors β and γ and disfavor T_{regs} (which can provide an immune suppressive, pro-tumor effect), which have IL2 receptors α, β, and γ. Further, in some embodiments, the preferences for IL-2Rβ and/or IL-2Rγ over IL-2Rα avoid IL-2 side effects such as pulmonary edema. Also, IL-2-based chimeras are

useful for the treatment of autoimmune diseases, for instance when the modified IL-2 is antagonistic (*e.g.* natural antagonistic activity or antagonistic activity that is the result of one or more mutations, *see, e.g.,* WO 2015/007520, the entire contents of which are hereby incorporated by reference) at IL-2Rβ and/or IL-2Rγ. For instance, the present constructs may favor attenuated suppression of $CD8^+$ T cells (and therefore dampen the immune response), which have IL2 receptors β and γ and disfavor T_{regs} which have IL2 receptors α, β, and γ. Alternatively, in some embodiments, the chimeras bearing IL-2 favor the activation of T_{regs}, and therefore immune suppression, and activation of disfavor of $CD8^+$ T cells. For instance, these constructs find use in the treatment of diseases or diseases that would benefit from immune suppression, *e.g.* autoimmune disorders.

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

In an embodiment, the wild type IL-2 has the amino acid sequence of:

IL-2 (mature form, wild type)

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATE
LKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMC
EYADETATIVEFLNRWITFCQSIISTLT (SEQ ID NO: 184).

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

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

In an embodiment, the modified signaling agent is IL-4. In such an embodiment, the modified signaling agent has reduced affinity and/or activity for type 1 and/or type 2 IL-4 receptors. In such an embodiment, the modified

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

In an embodiment, the wild type IL-4 has the amino acid sequence of:

IL-4 (mature form, wild type)

HKCDITLQEIIKTLNSLTEQKTLCTELTVTDIFAASKNTTEKETFCRAATVLRQ
FYSHHEKDTRCLGATAQQFHRHKQLIRFLKRLDRNLWGLAGLNSCPVKEA
NQSTLENFLERLKTIMREKYSKCSS (SEQ ID NO: 185).

In such embodiments, the modified IL-4 agent has one or more mutations at amino acids R121 (R121A, R121D, R121E, R121F, R121H, R121I, R121K, R121N, R121P, R121T, R121W), E122 (E122F), Y124 (Y124A, Y124Q, Y124R, Y124S, Y124T) and S125 (S125A). Without wishing to be bound by theory, it is believed that these modified IL-4 agents maintain the activity mediated by the type I receptor, but significantly reduces the biological activity mediated by the other receptors. See, for example, US Patent No. 6,433,157, the entire contents of which are hereby incorporated by reference.

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

In an embodiment, the wild type IL-6 has the amino acid sequence of:

IL-6 (mature form, wild type)

APVPPGEDSKDVAAPHRQPLTSSERIDKQIRYILDGISALRKETCNKSNMCE
SSKEALAENNLNLPKMAEKDGCFQSGFNEETCLVKIITGLLEFEVYLEYLQN
RFESSEEQARAVQMSTKVLIQFLQKKAKNLDAITTPDPTTNASLTTKLQAQN
QWLQDMTTHLILRSFKEFLQSSLRALRQM (SEQ ID NO: 186).

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

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

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

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

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

In an embodiment, the wild type IL-13 has the amino acid sequence of:

IL-13 (mature form, wild type)

SPGPVPPSTALRELIEELVNITQNQKAPLCNGSMVWSINLTAGMYCAALES
LINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLH
LKKLFREGRFN (SEQ ID NO: 187).

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

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

In an embodiment, the wild type IL-18 has the amino acid sequence of:

IL-18 (wild type)

MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENLESDYFGKLESKLSVIRNLN
DQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQPRGMAVTISVKC

EKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYE
GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNEDL (SEQ ID NO: 188).

In such embodiments, the modified IL-18 agent may comprise one or more mutations in amino acids or amino acid regions selected from Y37-K44, R49-Q54, D59-R63, E67-C74, R80, M87-A97, N127-K129, Q139-M149, K165-K171, R183 and Q190-N191, as described in WO/2015/007542, the entire contents of which are hereby incorporated by reference (numbering based on the human IL-18 sequence, Genbank accession number AAV38697, version AAV38697.1, GI: 54696650).

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

In an embodiment, the wild type IL-33 has the amino acid sequence of:

IL-33 (wild type)

MKPKMKYSTNKISTAKWKNTASKALCFKLGKSQQKAKEVCPMYFMKLRSG
LMIKKEACYFRRETTKRPSLKTGRKHKRHLVLAACQQQSTVECFAFGISGV
QKYTRALHDSSITGISPITEYLASLSTYNDQSITFALEDESYEIYVEDLKKDEK
KDKVLLSYYESQHPSNESGDGVDGKMLMVTLSPTKDFWLHANNKEHSVE
LHKCEKPLPDQAFFVLHNMHSNCVSFECKTDPGVFIGVKDNHLALIKVDSS
ENLCTENILFKLSET (SEQ ID NO: 189).

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

Linkers

In some embodiments, the present chimeric protein optionally comprises one or more linkers. In some embodiments, the present chimeric protein comprises a linker connecting the targeting moiety and the signaling agent. In some embodiments, the present chimeric protein comprises a linker within the signaling agent (*e.g.* in the case of single chain TNF, which can comprise two linkers to yield a trimer).

In some embodiments vectors encoding the present chimeric proteins linked as a single nucleotide sequence to any of the linkers described herein are provided and may be used to prepare such chimeric proteins.

In some embodiments, the linker length allows for efficient binding of a targeting moiety and the signaling agent to their receptors. For instance, in some embodiments, the linker length allows for efficient binding of one of the

targeting moieties and the signaling agent to receptors on the same cell as well as the efficient binding of the other targeting moiety to another cell. Illustrative pairs of cells are provided elsewhere herein.

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

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

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

The invention contemplates the use of a variety of linker sequences. In various embodiments, the linker may be derived from naturally-occurring multi-domain proteins or are empirical linkers as described, for example, in Chichili *et al.*, (2013), Protein Sci. 22(2):153-167, Chen *et al.*, (2013), Adv Drug Deliv Rev. 65(10):1357-1369, the entire contents of which are hereby incorporated by reference. In some embodiments, the linker may be designed using linker designing databases and computer programs such as those described in Chen *et al.*, (2013), Adv Drug Deliv Rev. 65(10):1357-1369 and Crasto *et al.*, (2000), Protein Eng. 13(5):309-312, the entire contents of which are hereby incorporated by reference. In various embodiments, the linker may be functional. For example, without limitation, the linker may function to improve the folding and/or stability, improve the expression, improve the pharmacokinetics, and/or improve the bioactivity of the present chimeric protein.

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

In some embodiments, a linker connects the two targeting moieties to each other and this linker has a short length and a linker connects a targeting moiety and a signaling agent this linker is longer than the linker

connecting the two targeting moieties. For example, the difference in amino acid length between the linker connecting the two targeting moieties and the linker connecting a targeting moiety and a signaling agent may be about 100, about 95, about 90, about 85, about 80, about 75, about 70, about 65, about 60, about 55, about 50, about 45, about 40, about 35, about 30, about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 12, about 11, about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, or about 2 amino acids.

In various embodiments, the linker is substantially comprised of glycine and serine residues (*e.g.* about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 95%, or about 97% glycines and serines). For example, in some embodiments, the linker is $(Gly_4Ser)_n$, where n is from about 1 to about 8, *e.g.* 1, 2, 3, 4, 5, 6, 7, or 8. In an embodiment, the linker sequence is GGSGGSGGGGSGGGGS (SEQ ID NO: 190). Additional illustrative linkers include, but are not limited to, linkers having the sequence LE, GGGGS (SEQ ID NO: 191), $(GGGGS)_n$ (n=1-4) (SEQ ID NO: 192), $(Gly)_8$ (SEQ ID NO: 193), $(Gly)_6$ (SEQ ID NO: 194), $(EAAAK)_n$ (n=1-3) (SEQ ID NO: 195), $A(EAAAK)_nA$ (n = 2-5) (SEQ ID NO: 196), AEAAAKEAAAKA (SEQ ID NO: 197), $A(EAAAK)_4ALEA(EAAAK)_4A$ (SEQ ID NO: 198), PAPAP (SEQ ID NO: 199), KESGSVSSEQLAQFRSLD (SEQ ID NO: 200), EGKSSGSGSESKST (SEQ ID NO: 201), GSAGSAAGSGEF (SEQ ID NO: 202), and $(XP)_n$, with X designating any amino acid, *e.g.*, Ala, Lys, or Glu. In various embodiments, the linker is GGS.

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

According to crystallographic studies, the immunoglobulin hinge region can be further subdivided functionally into three regions: the upper hinge region, the core region, and the lower hinge region. *See* Shin *et al.*, 1992 *Immunological Reviews* 130:87. The upper hinge region includes amino acids from the carboxyl end of C_{H1} to the first residue in the hinge that restricts motion, generally the first cysteine residue that forms an interchain disulfide bond between the two heavy chains. The length of the upper hinge region correlates with the segmental flexibility of the antibody. The core hinge region contains the inter-heavy chain disulfide bridges, and the lower hinge region joins the amino terminal end of the C_{H2} domain and includes residues in C_{H2}. *Id.* The core hinge region of wild-type human IgG1 contains the sequence Cys-Pro-Pro-Cys which, when dimerized by disulfide bond formation, results in a cyclic octapeptide believed to act as a pivot, thus conferring flexibility. In various embodiments, the present linker comprises, one, or two, or three of the upper hinge region, the core region, and the lower hinge region of any antibody (*e.g.*, of IgG, IgA, IgD, and IgE, inclusive of subclasses (*e.g.* IgG1, IgG2, IgG3, and IgG4, and IgA1 and IgA2)). The hinge region may also contain one or more glycosylation sites, which include a number of structurally distinct types of sites for carbohydrate attachment. For example, IgA1 contains five glycosylation sites within a 17-amino-acid segment of the hinge region, conferring resistance of the hinge region polypeptide to intestinal proteases, considered an advantageous property for a secretory immunoglobulin. In various embodiments, the linker of the present invention comprises one or more glycosylation sites. In various embodiments, the linker is a hinge-CH2-CH3 domain of a human IgG4 antibody.

If desired, the present chimeric protein can be linked to an antibody Fc region, comprising one or both of C_H2 and C_H3 domains, and optionally a hinge region. For example, vectors encoding the present chimeric proteins linked as a single nucleotide sequence to an Fc region can be used to prepare such polypeptides.

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

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

In various embodiments, the chimeric 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 chimeric 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 some embodiments, the chimeric protein may be fused or conjugated with an antibody or an antibody fragment such as an Fc fragment. For example, the chimeric protein may be fused to either the N-terminus or the C-terminus of the Fc domain of human immunoglobulin (Ig) G. In various embodiments, each of the individual chimeric 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.

Production of Chimeric Proteins

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

Nucleic acids encoding the chimeric protein of the invention can be incorporated (ligated) into expression vectors, which can be introduced into host cells through transfection, transformation, or transduction techniques. For example, nucleic acids encoding the chimeric protein of the invention can be introduced into host cells by retroviral transduction. Illustrative host cells are *E.coli* cells, Chinese hamster ovary (CHO) cells, human embryonic kidney 293 (HEK 293) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), and myeloma cells. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the chimeric protein of the invention. Accordingly, in various embodiments, the present invention provides expression vectors comprising nucleic acids that encode the chimeric protein of the invention. In various embodiments, the present invention additional provides host cells comprising such expression vectors.

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

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

Accordingly, in various embodiments, the present invention provides for a nucleic acid encoding a chimeric protein of the present invention. In various embodiments, the present invention provides for a host cell comprising a nucleic acid encoding a chimeric protein of the present invention.

Pharmaceutically Acceptable Salts and Excipients

The chimeric proteins described herein can possess a sufficiently basic functional group, which can react with an inorganic or organic acid, or a carboxyl group, which can react with an inorganic or organic base, to form a pharmaceutically acceptable salt. A pharmaceutically acceptable acid addition salt is formed from a

pharmaceutically acceptable acid, as is well known in the art. Such salts include the pharmaceutically acceptable salts listed in, for example, *Journal of Pharmaceutical Science*, 66, 2-19 (1977) and *The Handbook of Pharmaceutical Salts; Properties, Selection, and Use*. P. H. Stahl and C. G. Wermuth (eds.), Verlag, Zurich (Switzerland) 2002, which are hereby incorporated by reference in their entirety.

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

The term "pharmaceutically acceptable salt" also refers to a salt of the compositions of the present invention having an acidic functional group, such as a carboxylic acid functional group, and a base. Suitable bases include, but are not limited to, hydroxides of alkali metals such as sodium, potassium, and lithium; hydroxides of alkaline earth metal such as calcium and magnesium; hydroxides of other metals, such as aluminum and zinc; ammonia, and organic amines, such as unsubstituted or hydroxy-substituted mono-, di-, or tri-alkylamines, dicyclohexylamine; tributyl amine; pyridine; N-methyl, N-ethylamine; diethylamine; triethylamine; mono-, bis-, or tris-(2-OH-lower alkylamines), such as mono-; bis-, or tris-(2-hydroxyethyl)amine, 2-hydroxy-tert-butylamine, or tris-(hydroxymethyl)methylamine, N,N-di-lower alkyl-N-(hydroxyl-lower alkyl)-amines, such as N,N-dimethyl-N-(2-hydroxyethyl)amine or tri-(2-hydroxyethyl)amine; N-methyl-D-glucamine; and amino acids such as arginine, lysine, and the like.

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

Pharmaceutical Compositions and Formulations

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

In various embodiments, pharmaceutical excipients can be liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the

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

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

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

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

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

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

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

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

For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). The carrier should be stable under the

conditions of manufacture and storage, and should be preserved against microorganisms. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol), and suitable mixtures thereof.

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

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

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

In another embodiment, a controlled-release system can be placed in proximity of the target area to be treated, thus requiring only a fraction of the systemic dose (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.

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

Administration and Dosage

It will be appreciated that the actual dose of the chimeric protein to be administered according to the present invention will vary according to the particular dosage form, and the mode of administration. Many factors that may modify the action of the chimeric protein (*e.g.*, body weight, gender, diet, time of administration, route of

administration, rate of excretion, condition of the subject, drug combinations, genetic disposition and reaction sensitivities) can be taken into account by those skilled in the art. Administration can be carried out continuously or in one or more discrete doses within the maximum tolerated dose. Optimal administration rates for a given set of conditions can be ascertained by those skilled in the art using conventional dosage administration tests.

In some embodiments, a suitable dosage of the chimeric protein is in a range of about 0.01 mg/kg to about 10 g/kg of body weight of the subject, about 0.01 mg/kg to about 1 g/kg of body weight of the subject, about 0.01 mg/kg to about 100 mg/kg of body weight of the subject, 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, about 100 mg/kg body weight, about 1 g/kg of body weight, about 10 g/kg of body weight, inclusive of all values and ranges therebetween.

Individual doses of the chimeric protein can be administered in unit dosage forms (*e.g.*, tablets or capsules) containing, for example, from about 0.01 mg to about 100 g, from about 0.01 mg to about 75 g, from about 0.01 mg to about 50 g, from about 0.01 mg to about 25 g, about 0.01 mg to about 10 g, about 0.01 mg to about 7.5 g, about 0.01 mg to about 5 g, about 0.01 mg to about 2.5 g, about 0.01 mg to about 1 g, about 0.01 mg to about 100 mg, from about 0.1 mg to about 100 mg, from about 0.1 mg to about 90 mg, from about 0.1 mg to about 80 mg, from about 0.1 mg to about 70 mg, from about 0.1 mg to about 60 mg, from about 0.1 mg to about 50 mg, from about 0.1 mg to about 40 mg active ingredient, from about 0.1 mg to about 30 mg, from about 0.1 mg to about 20 mg, from about 0.1 mg to about 10 mg, from about 0.1 mg to about 5 mg, from about 0.1 mg to about 3 mg, from about 0.1 mg to about 1 mg per unit dosage form, or from about 5 mg to about 80 mg per unit dosage form. For example, a unit dosage form can be about 0.01 mg, about 0.02 mg, about 0.03 mg, about 0.04 mg, about 0.05 mg, about 0.06 mg, about 0.07 mg, about 0.08 mg, about 0.09 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, about 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1 mg, about 2 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 200 mg, about 500 mg, about 1 g, about 2.5 g, about 5 g, about 10 g, about 25 g, about 50 g, about 75 g, about 100 g, inclusive of all values and ranges therebetween.

In one embodiment, the chimeric protein is administered at an amount of from about 0.01 mg to about 100 g daily, from about 0.01 mg to about 75 g daily, from about 0.01 mg to about 50 g daily, from about 0.01 mg to about 25 g daily, from about 0.01 mg to about 10 g daily, from about 0.01 mg to about 7.5 g daily, from about

0.01 mg to about 5 g daily, from about 0.01 mg to about 2.5 g daily, from about 0.01 mg to about 1 g daily, from about 0.01 mg to about 100 mg daily, from about 0.1 mg to about 100 mg daily, from about 0.1 mg to about 95 mg daily, from about 0.1 mg to about 90 mg daily, from about 0.1 mg to about 85 mg daily, from about 0.1 mg to about 80 mg daily, from about 0.1 mg to about 75 mg daily, from about 0.1 mg to about 70 mg daily, from about 0.1 mg to about 65 mg daily, from about 0.1 mg to about 60 mg daily, from about 0.1 mg to about 55 mg daily, from about 0.1 mg to about 50 mg daily, from about 0.1 mg to about 45 mg daily, from about 0.1 mg to about 40 mg daily, from about 0.1 mg to about 35 mg daily, from about 0.1 mg to about 30 mg daily, from about 0.1 mg to about 25 mg daily, from about 0.1 mg to about 20 mg daily, from about 0.1 mg to about 15 mg daily, from about 0.1 mg to about 10 mg daily, from about 0.1 mg to about 5 mg daily, from about 0.1 mg to about 3 mg daily, from about 0.1 mg to about 1 mg daily, or from about 5 mg to about 80 mg daily. In various embodiments, the chimeric protein is administered at a daily dose of about 0.01 mg, about 0.02 mg, about 0.03 mg, about 0.04 mg, about 0.05 mg, about 0.06 mg, about 0.07 mg, about 0.08 mg, about 0.09 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, about 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1 mg, about 2 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 200 mg, about 500 mg, about 1 g, about 2.5 g, about 5 g, about 7.5 g, about 10 g, about 25 g, about 50 g, about 75 g, about 100 g, inclusive of all values and ranges therebetween.

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

Combination Therapy and Additional Therapeutic Agents

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

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

(*e.g.*, a first formulation including the additional therapeutic agent and a second formulation including the chimeric protein).

Co-administration does not require the therapeutic agents to be administered simultaneously, if the timing of their administration is such that the pharmacological activities of the additional therapeutic agent and the chimeric protein overlap in time, thereby exerting a combined therapeutic effect. For example, the additional therapeutic agent and the chimeric protein can be administered sequentially. The term "sequentially" as used herein means that the additional therapeutic agent and the chimeric protein are administered with a time separation of more than about 60 minutes. For example, the time between the sequential administration of the additional therapeutic agent and the chimeric protein can be more than about 60 minutes, more than about 2 hours, more than about 5 hours, more than about 10 hours, more than about 1 day, more than about 2 days, more than about 3 days, more than about 1 week apart, more than about 2 weeks apart, or more than about one month apart. The optimal administration times will depend on the rates of metabolism, excretion, and/or the pharmacodynamic activity of the additional therapeutic agent and the chimeric protein being administered. Either the additional therapeutic agent or the chimeric protein cell may be administered first.

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

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

In some embodiments, the present invention pertains to chemotherapeutic agents as additional therapeutic agents. For example, without limitation, such combination of the present chimeric proteins and chemotherapeutic agent find use in the treatment of cancers, as described elsewhere herein. Examples of chemotherapeutic agents include, but are not limited to, alkylating agents such as thiotepa and CYTOXAN cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (*e.g.*, bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; cally statin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (*e.g.*, cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB 1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the

enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammall and calicheamicin omegall (*see*, *e.g.*, Agnew, Chem. Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN doxorubicin (including morpholino- doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxy doxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as minoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (*e.g.*, T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, TAXOL paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, 111.), and TAXOTERE doxetaxel (Rhone-Poulenc Rorer, Antony, France); chloranbucil; GEMZAR gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE. vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11) (including the treatment regimen of irinotecan with 5-FU and leucovorin); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; combretastatin; leucovorin (LV); oxaliplatin, including the oxaliplatin treatment regimen (FOLFOX); lapatinib (Tykerb); inhibitors of PKC-α, Raf, H-Ras, EGFR (*e.g.*, erlotinib (Tarceva)) and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above. In addition, the methods of treatment can further include the use of radiation. In addition, the methods of treatment can further include the use of photodynamic therapy.

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

In some embodiments, inclusive, without limitation, of autoimmune applications, the additional therapeutic 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). Additional anti-inflammatory agents are described, for example, in U.S. Patent No. 4,537,776, the entire contents of which is incorporated by reference herein.

In some embodiments, the present invention relates to combination therapy with one or more immune-modulating agents, for example, without limitation, agents that modulate immune checkpoint. In various embodiments, the immune-modulating agent targets one or more of PD-1, PD-L1, and PD-L2. In various embodiments, the immune-modulating agent is PD-1 inhibitor. In various embodiments, the immune-modulating agent is an antibody specific for one or more of PD-1, PD-L1, and PD-L2 as described herein. For instance, in some embodiments, the immune-modulating agent is an antibody such as, by way of non-limitation, nivolumab, (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), pidilizumab (CT-011, CURE TECH), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), MPDL328OA (ROCHE). In some embodiments, the immune-modulating agent targets one or more of CD137 or CD137L. In various embodiments, the immune-modulating agent is an antibody specific for one or more of CD137 or CD137L. For instance, in some embodiments, the immune-modulating agent is an antibody such as, by way of non-limitation, urelumab (also known as BMS-663513 and anti-4-1BB antibody). In some embodiments, the present chimeric protein is combined with urelumab (optionally with one or more of nivolumab, lirilumab, and urelumab) for the treatment of solid tumors and/or B-cell non-Hodgkins lymphoma and/or head and neck cancer and/or multiple myeloma. In some embodiments, the immune-modulating agent is an agent that targets one or more of CTLA-4, AP2M1, CD80, CD86, SHP-2, and PPP2R5A. In various embodiments, the immune-modulating agent is an antibody specific for one or more of CTLA-4, AP2M1, CD80, CD86, SHP-2, and PPP2R5A. For instance, in some embodiments, the immune-modulating agent is an antibody such as, by way of non-limitation, ipilimumab (MDX-010, MDX-101, Yervoy, BMS) and/or tremelimumab (Pfizer). In some embodiments, the present chimeric protein is combined with ipilimumab (optionally with bavituximab) for the treatment of one or more of melanoma, prostate cancer, and lung cancer. In various embodiments, the immune-modulating agent targets CD20. In various embodiments, the immune-modulating agent is an antibody specific CD20. For instance, in some embodiments, the immune-modulating agent is an antibody such as, by way of non-limitation, Ofatumumab (GENMAB), obinutuzumab (GAZYVA), AME-133v (APPLIED MOLECULAR EVOLUTION), Ocrelizumab (GENENTECH), TRU-015 (TRUBION/EMERGENT), veltuzumab (IMMU-106).

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

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

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

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

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

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

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

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

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

Methods of Treatment

Methods and compositions described herein have application to treating various diseases and disorders, including, but not limited to cancer, infections, immune disorders, autoimmune diseases, and many other diseases and disorders.

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

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

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

Illustrative cancers that may be treated include, but are not limited to, basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.*, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (*e.g.* that associated with brain tumors), and Meigs' syndrome.

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

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

In various embodiments, the present compositions are used to treat or prevent one or more autoimmune diseases or conditions, such as multiple sclerosis, diabetes mellitus, lupus, celiac disease, Crohn's disease, ulcerative colitis, Guillain-Barre syndrome, scleroderms, Goodpasture's syndrome, Wegener's granulomatosis, autoimmune epilepsy, Rasmussen's encephalitis, Primary biliary sclerosis, Sclerosing cholangitis, Autoimmune hepatitis, Addison's disease, Hashimoto's thyroiditis, Fibromyalgia, Menier's syndrome; transplantation rejection (*e.g.*, prevention of allograft rejection) pernicious anemia, rheumatoid arthritis, systemic lupus erythematosus,

dermatomyositis, Sjogren's syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, Reiter's syndrome, Grave's disease, and other autoimmune diseases.

Kits

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

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

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

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

Definitions

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

Further, the term “about” when used in connection with a referenced numeric indication means the referenced numeric indication plus or minus up to 10% of that referenced numeric indication. For example, the language “about 50” covers the range of 45 to 55.

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

As used herein, something is “decreased” if a read-out of activity and/or effect is reduced by a significant amount, such as by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, or more, up to and including at least about 100%, in the presence of an agent or stimulus relative to the absence of such modulation. As will be understood by one of ordinary skill in the art, in some embodiments, activity is decreased and some downstream read-outs will decrease but others can increase.

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

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

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

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

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

Effective amounts, toxicity, and therapeutic efficacy can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to about 50% of the population) and the ED50 (the dose therapeutically effective in about 50% of the population). The dosage can

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

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

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

EXAMPLES

In the following examples, unless noted, mutations to IFN are relative to human IFN-α2 - SEQ ID NO: 179.

The Q124R mutant is representative of an attenuated human IFN alpha 2 mutant that can be assayed in vivo in a murine model. Specifically, Q124R is a human IFN mutation that is suitable for use in the mouse (*i.e.* it is a human mutant IFN that functions in mouse). *See Nat. Comm.* 2014;5:3016. doi: 10.1038/ncomms4016, the entire contents of which are hereby incorporated by reference.

Anti-human PD-1 VHH used in these Examples is SEQ ID NO: 23.

Anti-human PD-L1 VHH used in these Examples is SEQ ID NO: 158.

Example 1: Characterization of anti-mouse PD-L1/Attenuated Human IFN Chimera

FIG. 1 shows the anti-tumor activities of an anti-PD-L1-human IFN Q124R chimera construct as compared to anti-PD-L1 VHH and PBS. In the experiment, C57BL/6 mice were inoculated subcutaneously (50 µl) with $6x10^5$ B16mCD20cl1 melanoma tumor cells (a mouse melanoma cell line). Perilesional treatment with 30 µg of various constructs (100 µl) was started when tumors reached a size of ± 10 mm^2 as measured by caliper. **FIG. 1** shows that anti-PD-L1 had minimal effect in the B16 tumor model while the anti-PD-L1-human IFN Q124R chimera showed anti-tumor activity and reduced tumor size.

The anti-PD-L1-human IFN Q124R chimera was also shown to be safe (**FIG. 2**). In each figure, panels A-G show: change in body weight of the mice in the tumor studies described above (panel A), white blood cell counts ("wbc") and lymphocytes count ("ly") (panel B), neutrophil count ("ne") and monocyte count ("mo") (panel C); red blood cell count ("rbc") and, for FIG. 4, hemoglobin ("hb") (panel D); hemocrit ("hct"), mean corpuscular volume ("mcv"), mean corpuscular hemoglobin ("mch"), mean corpuscular hemoglobin concentration ("mchc") (panel E); pitted red blood cells ("pit") (panel F); and mean platelet volume ("mpv") (panel G). Importantly, the anti-PD-L1-human IFN Q124R chimera showed safety parameters that were far superior than what was expected from wild type interferons.

Example 2: Characterization of Anti-Human PD1 and PD-L1 VHHs

PD-1 (programmed cell death protein 1) functions as an immune checkpoint and plays an important role in the down regulation of the immune system by preventing activation of T-cells by binding to its ligands PD-L1 (programmed death-ligand 1) and PD-L2. A plate-binding assay was set up to evaluate the effect of PD-1 and PD-L1 VHHs on the interaction between these two proteins.

Specifically, maxisorp plates were coated overnight with anti-FLAG Ab (Sigma; 2 µg/ml in PBS), washed with washing buffer (PBS + 0,05% Tween-20) and blocked with PBS + 5% Tween-20 (2 hours at room temperature). Conditioned medium containing FLAG-tagged hPD-L1 extracellular domain was incubated for two hours at room temperature. Excess unbound protein was washed away with washing buffer. hPD1-Fc (500 ng/ml; R&D systems; cat: 1086-PD) in the presence or absence of VHHs was allowed to bind for 2 hours at room temperature. Bound hPD1-Fc was quantified with an horseradish peroxidase (HRP)-coupled anti-human secondary AB and the TMB HRP substrate (KPL).

In this experiment, hPD-1-Fc was allowed to bind to immobilized hPD-L1 in the presence of a serial dilution (1000 ng/ml; 1 over 10) of VHHs specific for hPD-1, hDP-L1, mPD-L1 or an irrelevant target. Data clearly illustrate that both hPD-1 and hPD-L1 VHHs blocked the interaction PD-1/PD-L1, while no effect for mPD-L1 or irrelevant VHHs was observed. See **FIG. 3**.

Example 3: Anti-Human PD-L1 targeting of IFN Chimeras

Efficiency of human PD-L1 (programmed death-ligand 1)/attenuated IFN chimera ("AcTaferon") targeting was examined by quantification of STAT1 phosphorylation in the human PD-L1 positive MDA-MB-321 cell-line by FACS analysis.

Specifically, MDA-MB-321 cells were stimulated with anti-human PD-L1/attenuated IFN chimeras as indicated for 15 minutes at 37°C in DMEM medium supplemented with 10% FBS. After stimulation, cells were fixed by adding 1 volume Fix Buffer I (BD Biosciences) for 10 minutes at 37°C, and permeabilized by resuspension in 2 volumes Perm III Buffer I (BD Biosciences) for 30 minutes on ice. Samples were stained with an anti-STAT1 pY701 Ab (BD Biosciences) for 20 minutes at 4°C and analyzed with a FACSCalibur (BD Biosciences) and the CellQuest Pro Version 4.0.2 software (BD Biosciences).

In this experiment, MDA-MB-321 cells were stimulated with a serial dilution (100 ng/ml; 1 over 5) of anti-human PD-L1/attenuated IFN chimeras for 15 minutes at 37°C. After fixation and permeabilization, cells were stained for phospho STAT1 and analyzed in FACS. Data clearly illustrate that PD-L1 targeting of anti-human PD-L1/attenuated IFN chimeras strongly increased STAT1 phosphorylation of anti-human PD-L1/attenuated R149A IFN chimeras, but not anti-human PD-L1/attenuated R33A/E120R IFN chimeras. Untargeted (BcII10 VHH) chimera was unable to signal even at 100 ng/ml. See **FIG. 4**.

Example 4: Signaling Induction in Human Dendritic Cells with Anti-human PD-L1 VHH Chimeras

A dendritic cell pSTAT signaling assay was undertaken. Chimeras studied were anti-human PD-L1 VHH/human IFN R149A and anti-human PD-L1 VHH/ human IFN R33A/E120R. Two doses of the agents were studied: 100 ng/ml and 500 ng/ml.

Briefly, human PBMCs were isolated from blood obtained from healthy donors. Approximately 120 ml of blood was collected from each donor using heparin coated tubes (12 tubes). The blood was kept at room temperature and processed immediately Briefly, blood was diluted 1:1 with DPBS and 25 ml was gently layered onto 15 ml of Lympholyte H. After centrifugation, the mononuclear cell rings were collected and cells were washed three times with DPBS (PBS Dulbecco's Phosphate Buffered Saline, Wisent, catalog #311-425-LL) and counted. Dendritic cells were enriched from the PBMC population using "DC- enrichment kit" containing a combination of lineage specific monoclonal antibodies in PBS and a suspension of magnetic particles (STEMCELL Technologies Catalogue number 19251), according to manufacturer's instructions.

Dendritic cells (DC) were stimulated for 15 minutes in the presence or absence of test items and controls (PBS) and the level of phosphorylated-STAT1 (pSTAT1, specifically pY701-STAT1) was determined in isolated DC cell populations (Lin-(CD14/CD16/CD20/CD56/CD3)/HLA-DR+) by flow cytometry. Post stimulation, cells were fixed (BD Cytofix fixation buffer, BD Bioscience, catalog #554655), then permeabilized with Perm buffer II (BD PhosFlow Perm Buffer, BD Bioscience, catalog #558052). Cells were then stained for phosphoSTAT1 and for DC surface markers (Lin-/HLA-DR+) (see table below). Both intra-cellular and surface staining were performed at the same time. Flow cytometry and data acquisition was performed after cell washing with DPBS.

Table showing list of antibodies for flow cytometry staining

Marker/Product Name	Fluorochrome	Clone	Purpose	Supplier-Catalog Number
pSTAT1	AlexaFluor647	4a	phospho-STAT1	BD-562070
Anti-human CD3	PE	UCHT1	T cells marker Lineage depletion	BD-561809
Anti-human CD14	PE	M5E2	Monocytes markers Lineage depletion	BD-555398
anti-human CD16	PE	B73.1	NK, Neutrophils, Monocytes marker Lineage depletion	BD-561313
anti-human CD19	PE	HIB19	B cells marker Lineage depletion	BD-555413
anti-human CD56	PE	B159	NK cells marker Lineage depletion	BD-555516
Anti-human HLA-DR	FITC	TU36	MHC II marker DC discrimination	BD-555560
Anti-human CD11c	BV421	B-Ly6	DC discrimination	BD-562561
LIVE/DEAD Fixable Aqua Dead Cell Stain	Aqua	N/Ap	Viability dye	ThermoFisher-L34957
Normal mouse IgG	N/Ap	N/Ap	Fc receptor blocker Blocking agent	ThermoFisher-10400C

FIG. 5 shows the data, expressed as a fold change of the percentage of $pSTAT^+$ dendritic cells.

This study clearly shows that a human PD-L1 antigen-targeting construct comprising an IFN signaling agent whose activity is recoverable upon cell targeting (IFN R149A) promotes IFN signaling in human dendritic cells (as determined by pSTAT1 induction). In contrast, no IFN signaling activation is observed with a PD-L1-targeting construct that incorporates an IFN signaling agent whose activity is not recoverable (IFN R33A/E120R). Thus, as observed for comparable IFN fusion constructs targeting mouse PD-L1, targeting IFN to human dendritic cells using a targeting moiety directed at human PD-L1 antigen results in triggering of a pronounced IFN signal transduction.

EQUIVALENTS

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific embodiments described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

INCORPORATION BY REFERENCE

All patents and publications referenced herein are hereby incorporated by reference in their entireties.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

As used herein, all headings are simply for organization and are not intended to limit the disclosure in any manner. The content of any individual section may be equally applicable to all sections.

CLAIMS

What is claimed is:

1. A chimeric protein comprising:

(a) one targeting moiety, said targeting moiety comprising a recognition domain which specifically binds to programmed cell death protein 1 ligand 1 (PD-L1); and

(b) a modified signaling agent, said modified signaling agent having one or more mutations that confer improved safety as compared to a wild type signaling agent; and

wherein the targeting moiety and the modified signaling agent are optionally connected with one or more linkers.

2. The chimeric protein of claim 1, wherein the recognition domain comprises a full-length antibody, a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a shark heavy-chain-only antibody (VNAR), a microprotein, a darpin, an anticalin, an adnectin, an aptamer, a Fv, a Fab, a Fab', a $F(ab')_2$, a peptide mimetic molecule, a natural ligand for a receptor, or a synthetic molecule.

3. The chimeric protein of claim 1 or 2, wherein the recognition domain comprises a single-domain antibody (VHH).

4. The chimeric protein of any one of claims 1-3, wherein the recognition domain comprises a V_{HH}, humanized V_{HH}, or camelized V_{HH}.

5. The chimeric protein of any one of claims 1-4, wherein the recognition domain functionally modulates PD-L1.

6. The chimeric protein of any one of claims 1-4, wherein the recognition domain binds but does not functionally modulate PD-L1.

7. The chimeric protein of claim 1, wherein the modified signaling agent comprises one or more mutations conferring reduced affinity or activity for a receptor relative to a wild type signaling agent.

8. The chimeric protein of claim 1, wherein the modified signaling agent comprises one or more mutations conferring substantially reduced or ablated affinity or activity for a receptor relative to a wild type signaling agent.

9. The chimeric protein of claim 1, wherein the modified signaling agent comprises both (a) one or more mutations conferring substantially reduced or ablated affinity for a receptor relative to a wild type signaling agent and (b) one or more mutations conferring reduced affinity or activity for a receptor relative to a wild type signaling agent; and wherein the receptors are different.

10. The chimeric protein of claim 7, wherein the one or more mutations allow for attenuation of activity.

11. The chimeric protein of claim 10, wherein agonistic or antagonistic activity is attenuated.

12. The chimeric protein of claim 10 or 11, wherein the modified signaling agent comprises one or more mutations which convert its activity from agonistic to antagonistic.

13. The chimeric protein of claim 7, wherein the mutation confers reduced affinity or activity that is restorable by attachment to one or more targeting moiety.

14. The chimeric protein of claim 8, wherein the mutation confers substantially reduced or ablated affinity or activity that is not substantially restorable by attachment to one or more targeting moiety.

15. The chimeric protein of any one of claims 1-14, wherein the modified signaling agent is modified human IFN-α2.

16. The chimeric protein of claim 15, wherein the modified human IFN-α2 comprises one or more mutations at positions R120, M148, R149, and L153.

17. The chimeric protein of claim 16, wherein the modified human IFN-α2 comprises one or more mutations selected from R120E, R149A, and L153A.

18. The chimeric protein of claim 17, wherein the modified human IFN-α2 comprises a R120E mutation and either a R149A or a L153A mutation.

19. The chimeric protein of any one of claims 1-14, wherein the modified signaling agent is modified human TNF-α.

20. The chimeric protein of claim 19, wherein the modified human TNF-α comprises one or more mutations at positions R32, N34, Q67, H73, L75, T77, S86, Y87, V91 , I97, T105, P106, A109, P113, Y115, E127, N137, D143, and A145.

21. The chimeric protein of claim 20, wherein the modified human TNF-α comprises one or more mutations selected from Y87Q, Y87L, Y87A, and Y87F.

22. The chimeric protein of claim 20, wherein the modified human TNF-α comprises one or more mutations selected from I97A, I97Q, and I97S.

23. The chimeric protein of claim 20, wherein the modified human TNF-α comprises one or more mutations selected from Y115G and Y115A.

24. The chimeric protein of any one of claims 1-14, wherein the modified signaling agent is modified human IL-1β.

25. The chimeric protein of claim 24, wherein the modified human IL-1β comprises one or more mutations selected from A117G/P118G, R120G, R120X, L122A, T125G/L126G, R127G, Q130X, Q131G, K132A, S137G/Q138Y, L145G, H146X, L145A/L147A, Q148X, Q148G/Q150G, Q150G/D151A, M152G, F162A, F162A/Q164E, F166A, Q164E/E167K, N169G/D170G, I172A, V174A, K208E, K209A, K209X, K209A/K210A, K219X, E221K, E221X, E221S/N224A, N224S/K225S, E244K and N245Q, and wherein X is any amino acid.

26. The chimeric protein of claim 25, wherein the modified human IL-1β comprises one or more mutations selected from Q131G and Q148G.

27. The chimeric protein of claim 25, wherein the modified human IL-1β comprises one or more mutations selected from Q148G and K208E.

28. The chimeric protein of claim 25, wherein the modified human IL-1β comprises one or more mutations selected from R120G and Q131G.

29. The chimeric protein of claim 25, wherein the modified human IL-1β comprises one or more mutations selected from R120G and H146A.

30. The chimeric protein of claim 25, wherein the modified human IL-1β comprises one or more mutations selected from R120G and K208E.

31. The chimeric protein of claim 25, wherein the modified human IL-1β comprises one or more mutations selected from R120G, F162A, and Q164E.

32. A chimeric protein comprising:

(a) one targeting moiety, said targeting moiety comprises a recognition domain which specifically binds to programmed cell death protein 1 (PD-1); and

(b) a modified signaling agent, said modified signaling agent having one or more mutations that confer improved safety as compared to a wild type signaling agent; and

wherein the targeting moiety and the modified signaling agent are optionally connected with one or more linkers.

33. The chimeric protein of claim 32, wherein the recognition domain comprises a full-length antibody, a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a shark heavy-chain-only antibody (VNAR), a microprotein (*e.g.* cysteine knot protein, knottin), a darpin, an anticalin, an adnectin, an aptamer, a Fv, a Fab, a Fab', a $F(ab')_2$, a peptide mimetic molecule, a natural ligand for a receptor, or a synthetic molecule.

34. The chimeric protein of claim 32 or 33, wherein the recognition domain comprises a single-domain antibody (VHH).

35. The chimeric protein of any one of claims 32-34, wherein the recognition domain comprises a V_{HH}, humanized V_{HH}, or camelized V_{HH}.

36. The chimeric protein of any one of claims 32-35, wherein the recognition domain functionally modulates PD-1.

37. The chimeric protein of any one of claims 32-35, wherein the recognition domain binds but does not functionally modulate PD-1.

38. The chimeric protein of claim 32, wherein the modified signaling agent comprises one or more mutations conferring reduced affinity or activity for a receptor relative to a wild type signaling agent.

39. The chimeric protein of claim 32, wherein the modified signaling agent comprises one or more mutations conferring substantially reduced or ablated affinity or activity for a receptor relative to a wild type signaling agent.

40. The chimeric protein of claim 32, wherein the modified signaling agent comprises both (a) one or more mutations conferring substantially reduced or ablated affinity for a receptor relative to a wild type signaling agent and (b) one or more mutations conferring reduced affinity or activity for a receptor relative to a wild type signaling agent; and wherein the receptors are different.

41. The chimeric protein of claim 38, wherein the one or more mutations allow for attenuation of activity.

42. The chimeric protein of claim 41, wherein agonistic or antagonistic activity is attenuated.

43. The chimeric protein of claim 41 or 42, wherein the modified signaling agent comprises one or more mutations which convert its activity from agonistic to antagonistic.

44. The chimeric protein of claim 38, wherein the mutation confers reduced affinity or activity that is restorable by attachment to one or more targeting moiety.

45. The chimeric protein of claim 39, wherein the mutation confers substantially reduced or ablated affinity or activity that is not substantially restorable by attachment to one or more targeting moiety.

46. The chimeric protein of any one of claims 32-45, wherein the modified signaling agent is modified human IFN-α2.

47. The chimeric protein of claim 46, wherein the modified human IFN-α2 comprises one or more mutations at positions R120, M148, R149, and L153.

48. The chimeric protein of claim 47, wherein the modified human IFN-α2 comprises one or more mutations selected from R120E, M148A, R149A, and L153A.

49. The chimeric protein of claim 48, wherein the modified human IFN-α2 comprises a R120E mutation and either a R149A or a L153A mutation.

50. The chimeric protein of any one of claims 32-45, wherein the modified signaling agent is modified human TNF-α.

51. The chimeric protein of claim 50, wherein the modified human TNF-α comprises one or more mutations at positions R32, N34, Q67, H73, L75, T77, S86, Y87, V91 , I97, T105, P106, A109, P113, Y115, E127, N137, D143, and A145.

52. The chimeric protein of claim 51, wherein the modified human TNF-α comprises one or more mutations selected from Y87Q, Y87L, Y87A, and Y87F.

53. The chimeric protein of claim 51, wherein the modified human TNF-α comprises one or more mutations selected from I97A, I97Q, and I97S.

54. The chimeric protein of claim 51, wherein the modified human TNF-α comprises one or more mutations selected from Y115G and Y115A.

55. The chimeric protein of any one of claims 32-45, wherein the modified signaling agent is modified human IL-1β.

56. The chimeric protein of claim 55, wherein the modified human IL-1β comprises one or more mutations selected from A117G/P118G, R120G, R120X, L122A, T125G/L126G, R127G, Q130X, Q131G, K132A, S137G/Q138Y, L145G, H146X, L145A/L147A, Q148X, Q148G/Q150G, Q150G/D151A, M152G, F162A, F162A/Q164E, F166A, Q164E/E167K, N169G/D170G, I172A, V174A, K208E, K209A, K209X, K209A/K210A, K219X, E221K, E221X, E221S/N224A, N224S/K225S, E244K and N245Q, and wherein X is any amino acid.

57. The chimeric protein of claim 56, wherein the modified human IL-1β comprises one or more mutations selected from Q131G and Q148G.

58. The chimeric protein of claim 56, wherein the modified human IL-1β comprises one or more mutations selected from Q148G and K208E.

59. The chimeric protein of claim 56, wherein the modified human IL-1β comprises one or more mutations selected from R120G and Q131G.

60. The chimeric protein of claim 56, wherein the modified human IL-1β comprises one or more mutations selected from R120G and H146A.

61. The chimeric protein of claim 56, wherein the modified human IL-1β comprises one or more mutations selected from R120G and K208E.

62. The chimeric protein of claim 56, wherein the modified human IL-1β comprises one or more mutations selected from R120G, F162A, and Q164E.

63. A chimeric protein comprising:

(a) one targeting moiety, said targeting moiety comprises a recognition domain which specifically binds to PD-1 ligand 2 (PD-L2); and

(b) a modified signaling agent, said modified signaling agent having one or more mutations that confer improved safety as compared to a wild type signaling agent; and

wherein the targeting moiety and the modified signaling agent are optionally connected with one or more linkers.

64. The chimeric protein of claim 63, wherein the recognition domain comprises a full-length antibody, a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a shark heavy-chain-only antibody (VNAR), a microprotein (*e.g.* cysteine knot protein, knottin), a darpin, an anticalin, an adnectin, an aptamer, a Fv, a Fab, a Fab', a $F(ab')_2$, a peptide mimetic molecule, a natural ligand for a receptor, or a synthetic molecule.

65. The chimeric protein of claim 63 or 64, wherein the recognition domain comprises a single-domain antibody (VHH).

66. The chimeric protein of any one of claims 63-65, wherein the recognition domain comprises a V_{HH}, humanized V_{HH}, or camelized V_{HH}.

67. The chimeric protein of any one of claims 63-66, wherein the recognition domain functionally modulates PD-L2.

68. The chimeric protein of any one claims 63-66, wherein the recognition domain binds but does not functionally modulate PD- L2.

69. The chimeric protein of claim 63, wherein the modified signaling agent comprises one or more mutations conferring reduced affinity or activity for a receptor relative to a wild type signaling agent.

70. The chimeric protein of claim 63, wherein the modified signaling agent comprises one or more mutations conferring substantially reduced or ablated affinity or activity for a receptor relative to a wild type signaling agent.

71. The chimeric protein of claim 63, wherein the modified signaling agent comprises both (a) one or more mutations conferring substantially reduced or ablated affinity for a receptor relative to a wild type signaling agent and (b) one or more mutations conferring reduced affinity or activity for a receptor relative to a wild type signaling agent; and wherein the receptors are different.

72. The chimeric protein of claim 69, wherein the one or more mutations allow for attenuation of activity.

73. The chimeric protein of claim 72, wherein agonistic or antagonistic activity is attenuated.

74. The chimeric protein of claim 72 or 73, wherein the modified signaling agent comprises one or more mutations which convert its activity from agonistic to antagonistic.

75. The chimeric protein of claim 69, wherein the mutation confers reduced affinity or activity that is restorable by attachment to one or more targeting moiety.

76. The chimeric protein of claim 70, wherein the mutation confers substantially reduced or ablated affinity or activity that is not substantially restorable by attachment to one or more targeting moiety.

77. The chimeric protein of any one of claims 63-76, wherein the modified signaling agent is modified human TNF-α.

78. The chimeric protein of claim 77, wherein the modified human TNF-α comprises one or more mutations at positions R32, N34, Q67, H73, L75, T77, S86, Y87, V91 , I97, T105, P106, A109, P113, Y115, E127, N137, D143, and A145.

79. The chimeric protein of claim 78, wherein the modified human TNF-α comprises one or more mutations selected from Y87Q, Y87L, Y87A, and Y87F.

80. The chimeric protein of claim 78, wherein the modified human TNF-α comprises one or more mutations selected from I97A, I97Q, and I97S.

81. The chimeric protein of claim 78, wherein the modified human TNF-α comprises one or more mutations selected from Y115G and Y115A.

82. The chimeric protein of any one of claims 63-76, wherein the modified signaling agent is modified human IL-1β.

83. The chimeric protein of claim 82, wherein the modified human IL-1β comprises one or more mutations selected from A117G/P118G, R120G, R120X, L122A, T125G/L126G, R127G, Q130X, Q131G, K132A, S137G/Q138Y, L145G, H146X, L145A/L147A, Q148X, Q148G/Q150G, Q150G/D151A, M152G, F162A, F162A/Q164E, F166A, Q164E/E167K, N169G/D170G, I172A, V174A, K208E, K209A, K209X, K209A/K210A, K219X, E221K, E221X, E221S/N224A, N224S/K225S, E244K and N245Q, and wherein X is any amino acid.

84. The chimeric protein of claim 83, wherein the modified human IL-1β comprises one or more mutations selected from Q131G and Q148G.

85. The chimeric protein of claim 83, wherein the modified human IL-1β comprises one or more mutations selected from Q148G and K208E.

86. The chimeric protein of claim 83, wherein the modified human IL-1β comprises one or more mutations selected from R120G and Q131G.

87. The chimeric protein of claim 83, wherein the modified human IL-1β comprises one or more mutations selected from R120G and H146A.

88. The chimeric protein of claim 83, wherein the modified human IL-1β comprises one or more mutations selected from R120G and K208E.

89. The chimeric protein of claim 83, wherein the modified human IL-1β comprises one or more mutations selected from R120G, F162A, and Q164E.

90. The chimeric protein of any one of the above claims, wherein the chimeric protein is suitable for use in a patient having one or more of: cancer, infections, immune disorders, and autoimmune diseases.

91. A recombinant nucleic acid composition encoding one or chimeric proteins of any one of the above claims.

92. A host cell comprising a nucleic acid of claim 91.

93. A method for treating cancer, comprising administering an effective amount of the chimeric protein of any of the above claims to a patient in need thereof.

94. The method of claim 93, wherein the cancer is selected form one or more of basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the

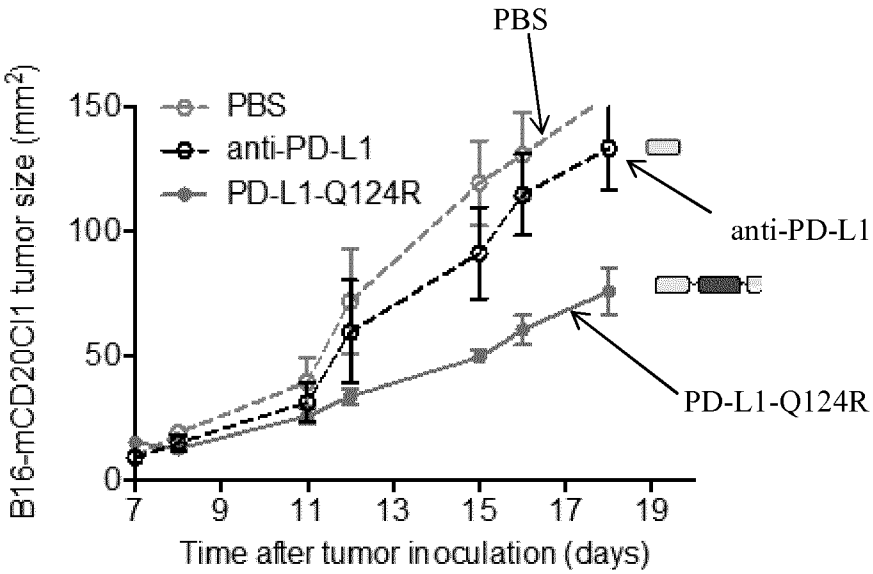
peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.*, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (*e.g.* that associated with brain tumors), and Meigs' syndrome.

95. A method for treating an autoimmune disease or disorder, comprising administering an effective amount of the chimeric protein of any of the above claims to a patient in need thereof.

96. The method of claim 95, wherein the autoimmune disease or disorder, is selected from one or more of Crohn's Disease, systemic lupus erythematosis, rheumatoid arthritis or juvenile rheumatoid arthritis, ulcerative colitis immune disorders such as eosinophilic fasciitis, hypoimmunoglobulinemia, or thymoma/thymic carcinoma, graft versus host disease, preleukemia, Nonhematologic syndrome (*e.g.* Down's, Dubowwitz, Seckel), Felty syndrome, hemolytic uremic syndrome, myelodysplasic syndrome, nocturnal paroxysmal hemoglobinuria, osteomyelofibrosis, pancytopenia, pure red-cell aplasia, Schoenlein-Henoch purpura, malaria, protein starvation, menorrhagia, systemic 97. A chimeric protein of any of the above claims for use in the treatment of one or more of: cancer, infections, immune disorders, and/or autoimmune diseases, as described herein.

97. Use of a chimeric protein of any of the above claims for the manufacture of a medicament for treating one or more of: cancer, infections, immune disorders, and/or autoimmune diseases, as described herein.

FIG. 1



1 exp, 6 mice

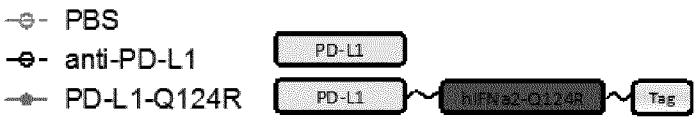
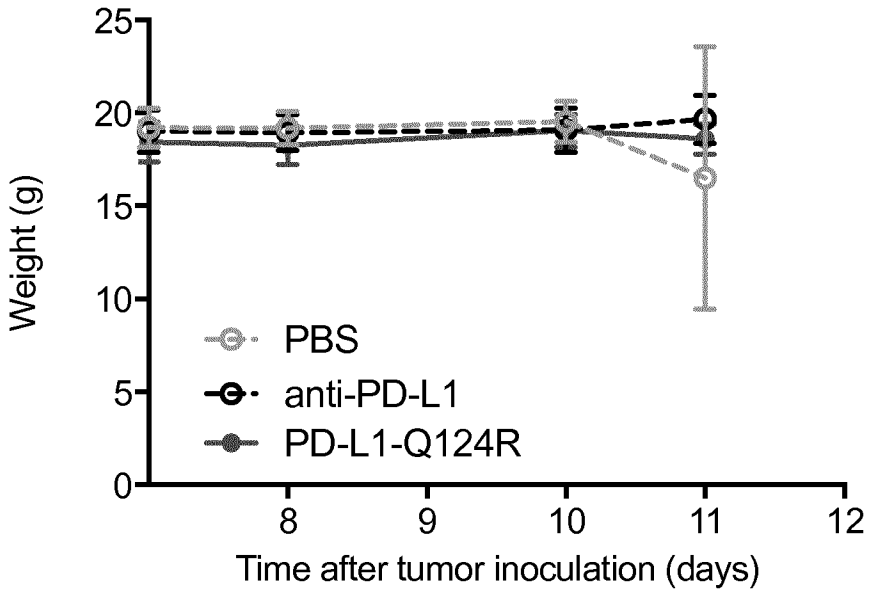
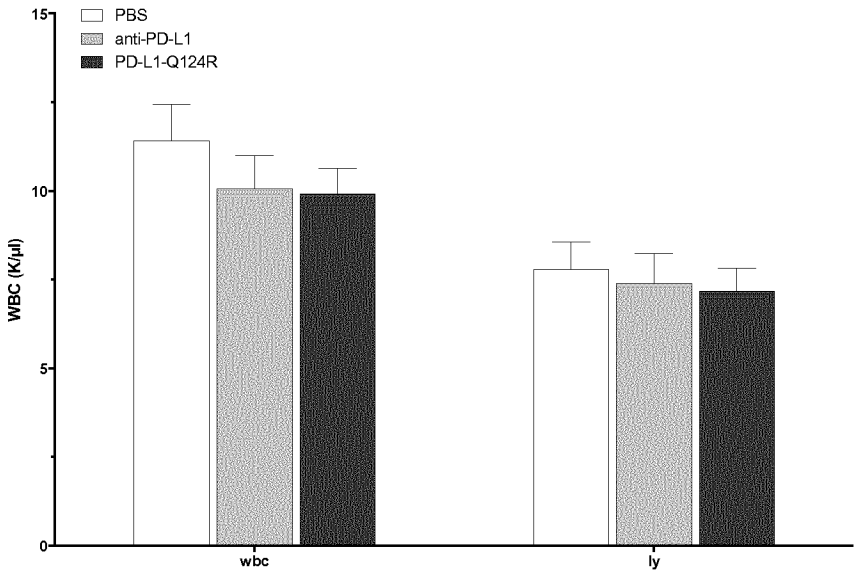


FIG. 2
A.


25
20
15
10
5
0
Weight (g)
PBS
anti-PD-L1
PD-L1-Q124R
8
9
10
11
12
Time after tumor inoculation (days)

B.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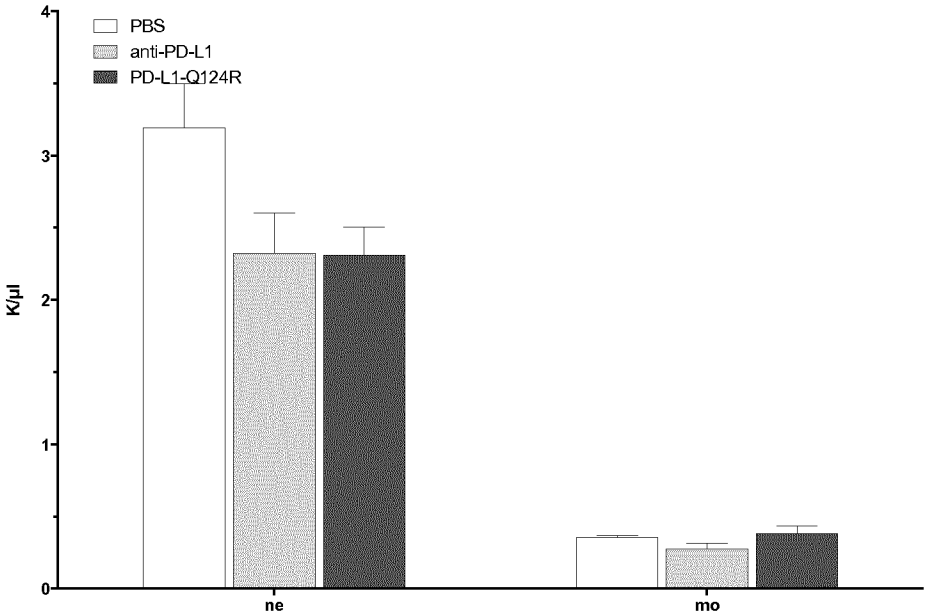
10
5
0
WBC (K/µl)
PBS
anti-PD-L1
PD-L1-Q124R
wbc
ly

FIG. 2 (CONT.)
C.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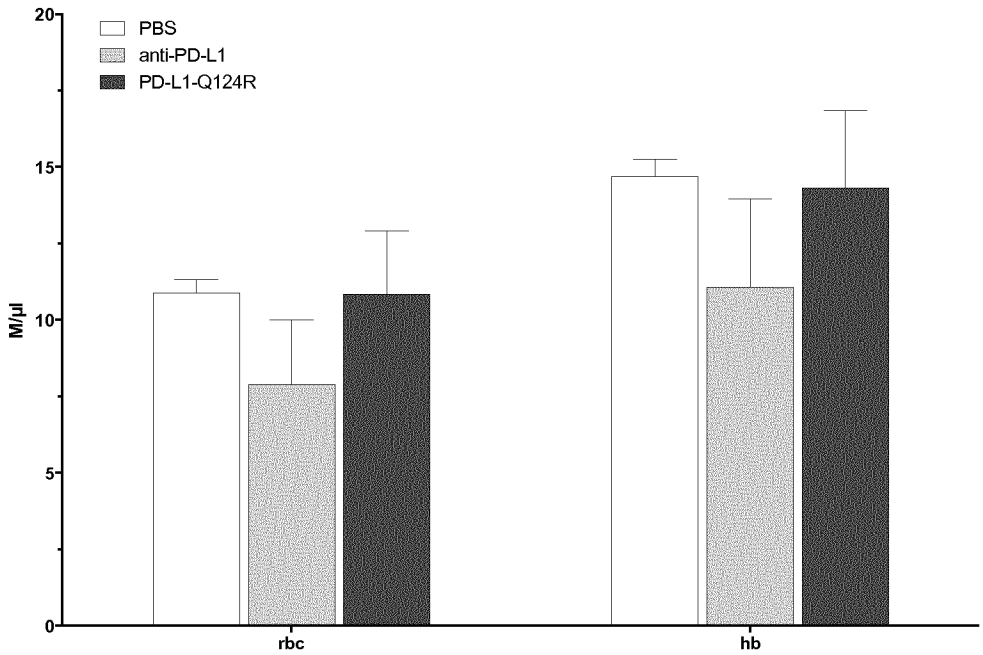
PD-L1-Q124R
K/µl
4
3
2
1
0
ne
mo

D.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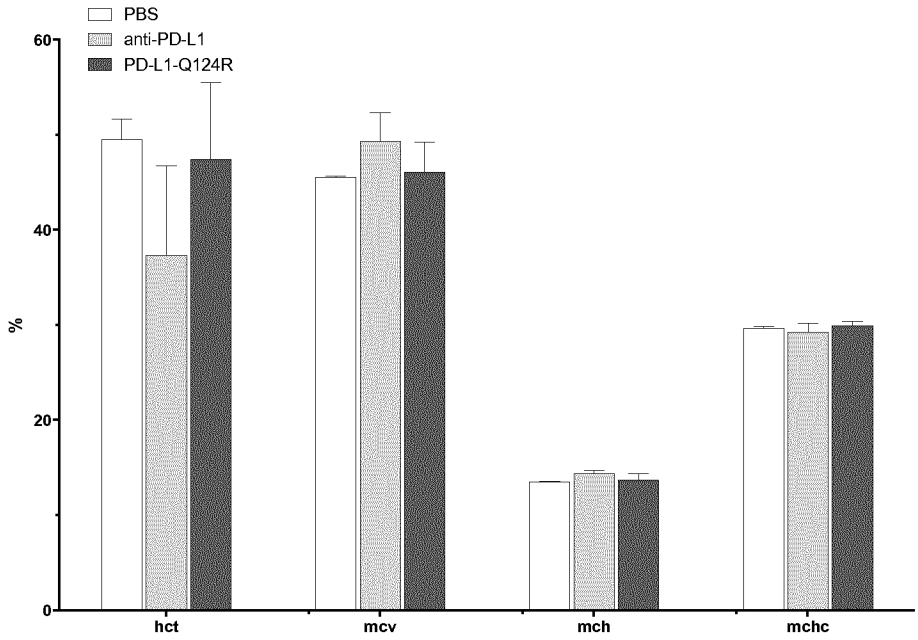
PD-L1-Q124R
M/µl
20
15
10
5
0
rbc
hb

FIG. 2 (CONT.)

E.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40
20
0
%
hct
mcv
mch
mchc

F.

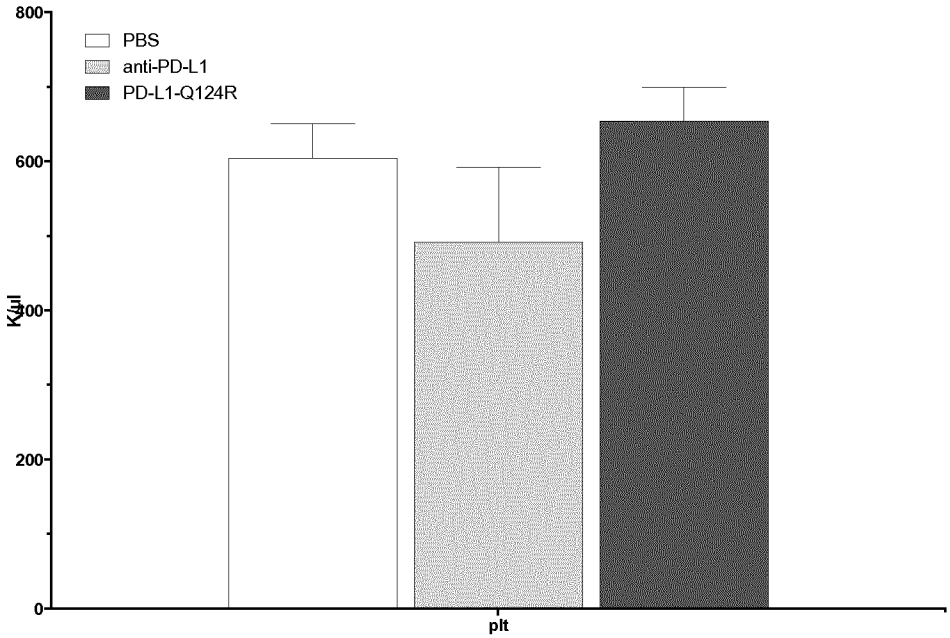

PBS
anti-PD-L1
PD-L1-Q124R
800
600
400
200
0
K/µl
plt

FIG. 2 (CONT.)
G.

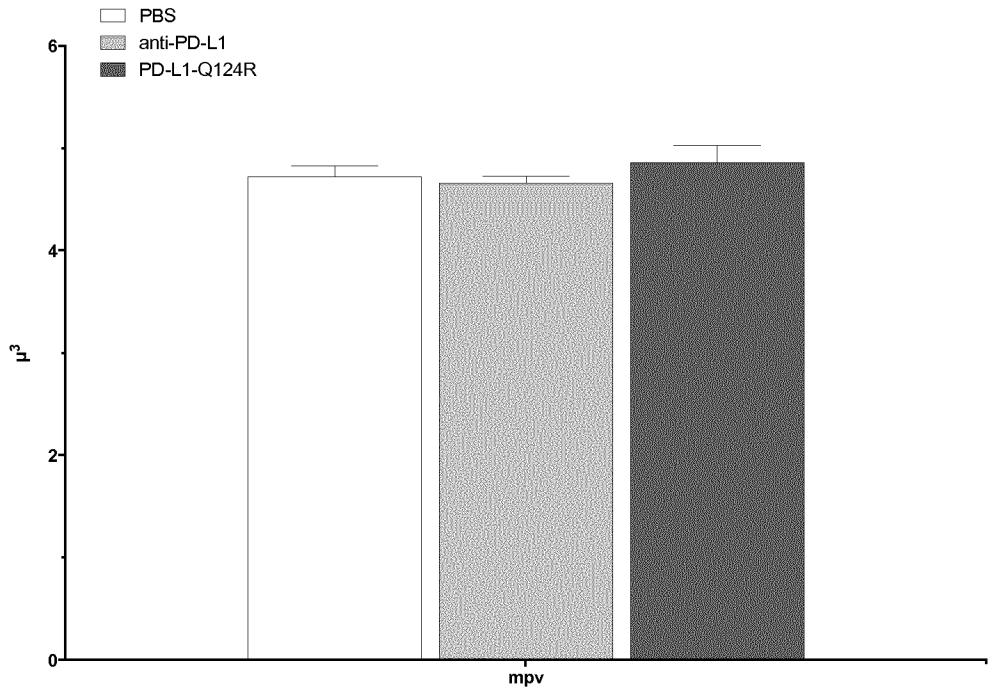


FIG. 3

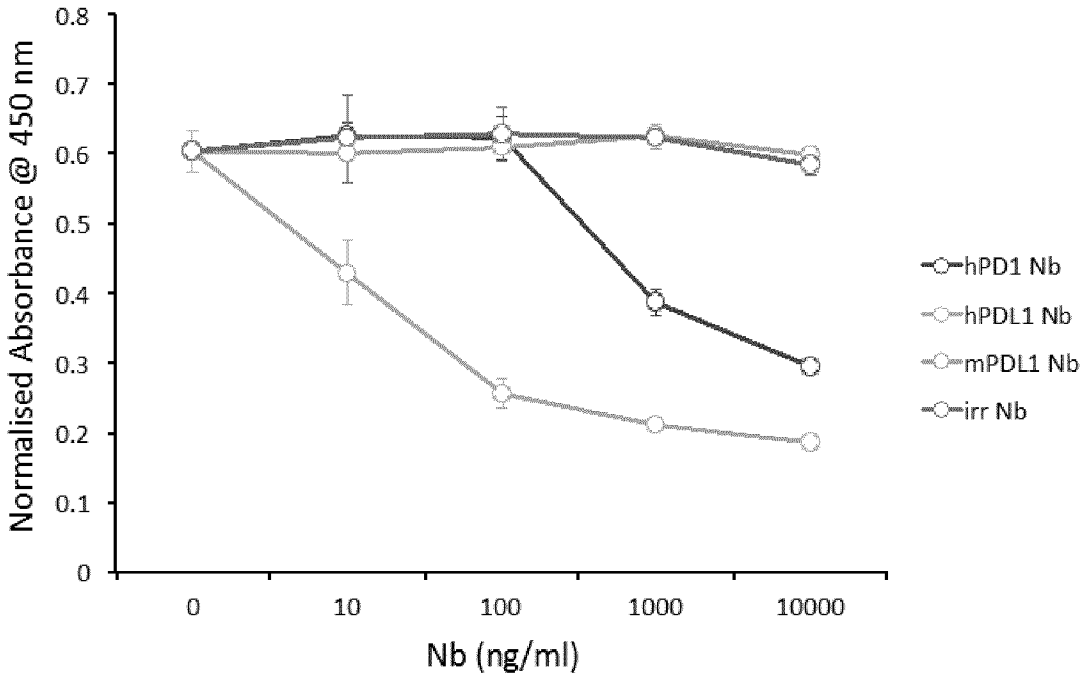


FIG. 4

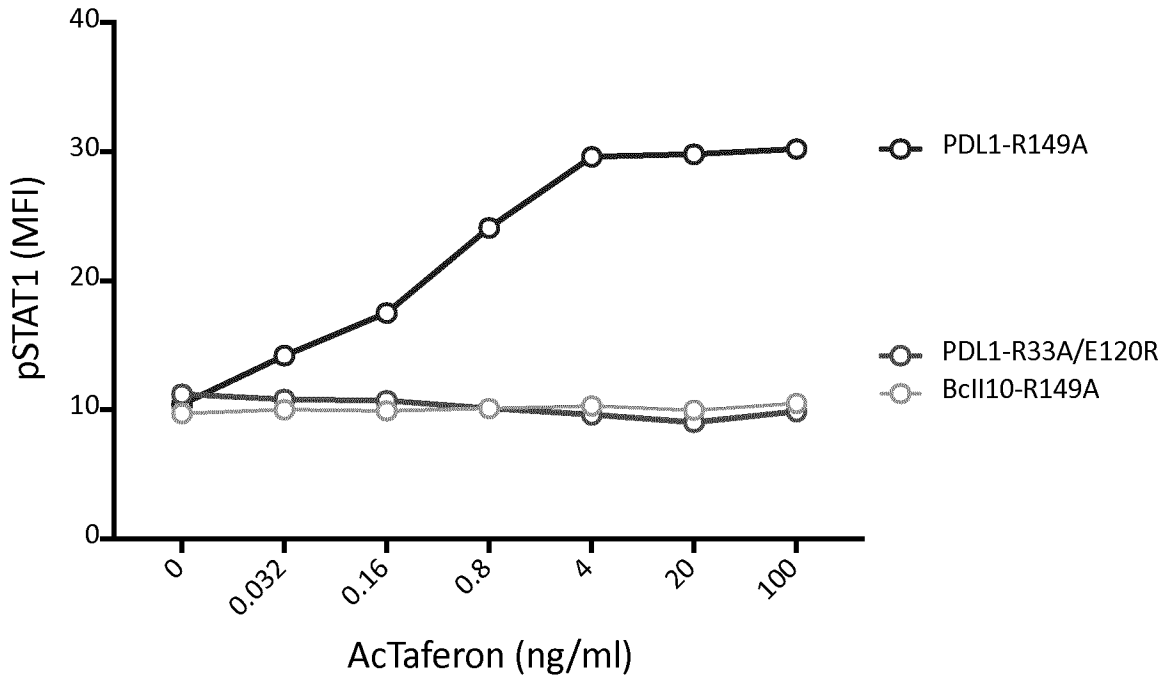
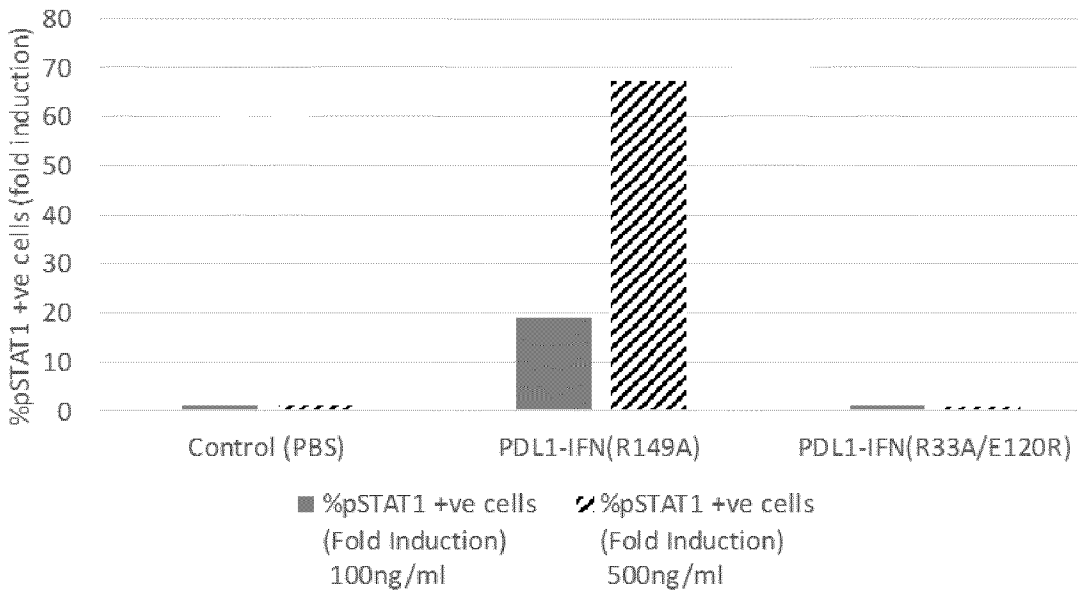

40
30
20
10
0
pSTAT1 (MFI)
PDL1-R149A
PDL1-R33A/E120R
BcII10-R149A
0
0.032
0.16
0.8
4
20
100
AcTaferon (ng/ml)

FIG. 5


80
70
60
50
40
30
20
10
0
%pSTAT1 +ve cells (fold induction)
Control (PBS)
PDL1-IFN(R149A)
PDL1-IFN(R33A/E120R)
%pSTAT1 +ve cells (Fold Induction) 100ng/ml
%pSTAT1 +ve cells (Fold Induction) 500ng/ml