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(12) **United States Patent**
Bedi et al.(10) **Patent No.: US 12,295,968 B2**(45) **Date of Patent: *May 13, 2025**(54) **COMPOSITIONS AND METHODS FOR
TARGETED IMMUNOMODULATORY
ANTIBODIES AND FUSION PROTEINS**(71) Applicant: **The Johns Hopkins University,**
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Baltimore, MD (US)(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.This patent is subject to a terminal dis-
claimer.(21) Appl. No.: **18/883,719**(22) Filed: **Sep. 12, 2024**(65) **Prior Publication Data**

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C07K 19/00 (2013.01); **A61K 2039/505**
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See application file for complete search history.

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Alignment of SEQ ID No. 10 of D3 with SEQ ID No. 87 of B1.
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Primary Examiner — Bridget E Bunner(74) *Attorney, Agent, or Firm* — DLA Piper LLP (US)(57) **ABSTRACT**The present invention is based on the seminal discovery that
targeted immunomodulatory antibodies and fusion proteins
can counter act or reverse immune tolerance of cancer cells.
Cancer cells are able to escape elimination by chemothera-
peutic agents or tumor-targeted antibodies via specific
immunosuppressive mechanisms in the tumor microenvi-
ronment and such ability of cancer cells is recognized as
immune tolerance. Such immunosuppressive mechanisms
include immunosuppressive cytokines (for example, Trans-
forming growth factor beta (TGF-β)) and regulatory T cells
and/or immunosuppressive myeloid dendritic cells (DCs).
By counteracting tumor-induced immune tolerance, the
present invention provides effective compositions and meth-
ods for cancer treatment, optional in combination with
another existing cancer treatment. The present invention
provides strategies to counteract tumor-induced immune
tolerance and enhance the antitumor efficacy of chemo-
therapy by activating and leveraging T cell-mediated adap-
tive antitumor immunity against resistant or disseminated
cancer cells.**9 Claims, 75 Drawing Sheets****Specification includes a Sequence Listing.**

Related U.S. Application Data

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- (60) Provisional application No. 61/435,671, filed on Jan. 24, 2011, provisional application No. 61/311,255, filed on Mar. 5, 2010.

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1. Amino acid sequences of transforming growth factor beta receptor type II (TGF- β -RII) or TGF- β -RIIB or a fragment thereof:

(i) Transforming growth factor beta receptor type II (TGF- β -RII) (SEQ ID NO: 79):

MGRGLLRGLW PLHIVLWTRI *ASTIPPHVQK* *SVNNDMIVTD* *NNGAVKFPQL* *CKFCDVRFST*
CDNQKSCMSN *CSITSICEKP* *QEVCAVWRK* *NDENITLETV* *CHDPKLPYHD* *FILEDAASPK*
CIMKEKKKPG *ETFFMCSCSS* *DECNDNIIFS* *EEYNTPNDL* *LLVIFQVTGI* *SLLPPLGVAI*
SVIIIFYCYR VNRQQLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELKQ YWLITAFHAK GNMQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQVVG
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC WDHDPEARLT AQCVARFSE
LEHLDRLSGR SCSEEKIPED GSLNTTK

[Italic – Extracellular domain of Transforming growth factor beta Receptor II (TGF β RII)]
[Underlined = TGF β RII Extracellular domain (ECD) region that binds TGF- β]

(ii) Transforming growth factor beta receptor type IIB (TGF- β -RIIB) (SEQ ID NO: 80):

MGRGLLRGLW PLHIVLWTRI *ASTIPPHVQK* *SDVEMEAQKD* *EIICPSCNRT* *AHPLRHINND*
MIVTDNNGAV *KFPQLCKFCD* *VRFSTCDNQK* *SCMSNCSITS* *ICEKPQEVCAV* *AVWRKNDENI*
TLETVCHDPK *LPYHDFIED* *AASPKCIMKE* *KKKPGETFFM* *CSCSSDECND* *NIIFSEEYNT*
SNPDL *LLVIF* *QVTGISLLPP* *LGVAISVII* *FCYRVNRQQ* *KLSTWETGK* *TRKLMEFSEH*
CAIILEDERS DISSTCANNI NHNTPELLPIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA VKI FP
YEEYASWKTE KDIFSDINLK HENILQFLTA EERKTELKQ YWLITAFHAK GNMQEYLTRH
VISWEDLRKL GSSLARGIAH LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL
SLRLDPTLSV DDLANSQVVG TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR
CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC
WDHDPEARLT AQCVARFSE LEHLDRLSGR SCSEEKIPED GSLNTTK

[Italic – Extracellular domain of Transforming growth factor beta Receptor IIB (TGF β RIIB)]
[Underlined = TGF β RIIB Extracellular domain (ECD) region that binds TGF- β]

2. Truncated mutants of Transforming growth factor beta Receptor II (TGF- β -RII) or TGF- β -RIIB comprising the Extracellular domain (ECD) region that binds TGF- β

(i) TGF- β R-II (Δ C terminus): TGF β RII lacking the last 38 amino acids from the C-terminus (SEQ ID NO: 81):

MGRGLLRGLW PLHIVLWTRI *ASTIPPHVQK* *SVNNDMIVTD* *NNGAVKFPQL* *CKFCDVRFST*
CDNQKSCMSN *CSITSICEKP* *QEVCAVWRK* *NDENITLETV* *CHDPKLPYHD* *FILEDAASPK*
CIMKEKKKPG *ETFFMCSCSS* *DECNDNIIFS* *EEYNTPNDL* *LLVIFQVTGI* *SLLPPLGVAI*
SVIIIFYCYR VNRQQLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELKQ YWLITAFHAK GNMQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQVVG
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC WDHDPEARLT

FIG. 1A

TGF- β R-IIB (Δ C terminus): TGF β RII lacking the last 38 aa from the C-terminus (SEQ ID NO: 82):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK **SDVEMEAQKD EIICPSCNRT AHPLRHINND**
MIVTDNNGAV KFPQLCKFCD VRFSTCDNOK SCMSNCSITS ICEKPQEVCV AVWRKNDENI
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT
SNPDLLLIVIF QVTGISLLPP LGVAISVIII FYCYRVNRQQ KLSSTWETGK TRKLMEFSEH
CAIILEDERS DISSTCANNI NHNTELLPIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA VKI FP
YEEYASWKTE KDIFSDINLK HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH
VISWEDLRKL GSSLARGIAH LHSHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL
SLRLDPTLSV DDLANSQVVG TARYMAPEVL ESRMNLNVE SFKQTDVYSM ALVLWEMTSR
CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLLEC
WDHDPPEARL

(ii) TGF- β R-II (Δ cyt): TGF β RII lacking the kinase domain & juxtamembrane region (SEQ ID NO: 83):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNOKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD LLVIFQVTGI SLLPPLGVAI
SVIIIFYCYR VNRQQLSS

TGF- β R-IIB (Δ cyt): TGF β RII lacking the kinase domain & juxtamembrane region (SEQ ID NO: 84):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK **SDVEMEAQKD EIICPSCNRT AHPLRHINND**
MIVTDNNGAV KFPQLCKFCD VRFSTCDNOK SCMSNCSITS ICEKPQEVCV AVWRKNDENI
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT
SNPDLLLIVIF QVTGISLLPP LGVAISVIII FYCYRVNRQQ KLSS

(iii) TGF- β R-II containing the N-terminus region including the extracellular domain (SEQ ID NO: 85):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNOKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD LLVIFQ

TGF- β R-IIB containing the N-terminus region including the extracellular domain (SEQ ID NO: 86):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK **SDVEMEAQKD EIICPSCNRT AHPLRHINND**
MIVTDNNGAV KFPQLCKFCD VRFSTCDNOK SCMSNCSITS ICEKPQEVCV AVWRKNDENI
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT
SNPDLLLIVIF Q

(iv) TGF- β R-II containing the extracellular domain that binds TGF- β (SEQ ID NO: 87):

TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNOKSCMSN CSITSICEKP
QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS
DECNDNIIFS EYNTSNPD

TGF- β R-IIB containing the extracellular domain that binds TGF- β (SEQ ID NO: 88):

TIPPHVQK **SDVEMEAQKD EIICPSCNRT AHPLRHINND** MIVTDNNGAV KFPQLCKFCD
VRFSTCDNOK SCMSNCSITS ICEKPQEVCV AVWRKNDENI TLETVCHDPK LPYHDFILED
AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT SNPD

(v) TGF- β R-II containing the region of the extracellular domain that binds TGF- β (SEQ ID NO: 89):

QQL CKFCDVRFST CDNOKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD
FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

FIG. 1B

3. Kinase-deficient mutants, deletion mutants, or point mutants of Transforming growth factor beta Receptor II (TGF β -RII) or TGF β -RIIB or a fragment thereof which binds TGF- β

(i) Transforming growth factor beta Receptor II containing point mutations:

Amino acid sequence of TGF- β R-II (K277R) contains a point mutation in its ATP-binding site and is inactive as a kinase (SEQ ID NO: 90):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPDL LLVIFQVTGI SLLPPLGVAI
SVIIIFYCYR VNRQQLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVRIFF YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQQVG
TARYMAPEVL ESRMNLNVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
LEHLDRLSGR SCSEKIPED GSLNTTK

(ii) Transforming growth factor beta Receptor II containing deletions in the amino acid sequence (deletion mutants): Transforming growth factor beta Receptor II (Δ i)

TGF- β R-II (Δ i2) contains a deletion of amino acids 498 to 508 and is inactive as a kinase (SEQ ID NO: 91):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPDL LLVIFQVTGI SLLPPLGVAI
SVIIIFYCYR VNRQQLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQQVG
TARYMAPEVL ESRMNLNVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
HPCVESMKDA SGIQMV CETL TECWDHDPEA RLTAQCVAER FSELEHLDRL SGRSCSEKI
PEDGSLNTTK

4. Amino acid sequences of transforming growth factor beta receptor type III (TGF- β -RIII) or a fragment thereof.
5. Hybrid or fusion proteins containing amino acid sequences of transforming growth factor beta receptor type II (TGF- β -RII) and TGF- β -RIII.
6. Hybrid or fusion proteins containing amino acid sequences of transforming growth factor beta receptor type IIB (TGF- β -RIIB) and TGF- β -RIII.

Linker sequence used in fusion proteins (SEQ ID NO: 104):

GGGGSGGGSGGGGS

FIG. 1C

Fusion proteins comprising Anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).

Anti-HER2/neu heavy chain + TGF β -RII ECD fusion amino acid sequence

(SEQ ID NO: 1):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKG
RFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTSVNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT
QTYICNVNNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST**
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

Anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSSGSR
SGTDFTLTISSLPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-HER2 antibody variable region

Plain: Anti-HER2 antibody constant region

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 2

Fusion proteins comprising Anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).

Anti-EGFR1 heavy chain + TGF β -RII ECD fusion amino acid sequence

(SEQ ID NO: 2):

QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPTTSR
LSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGTLVTVSAASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

Anti-EGFR1 light chain amino acid sequence (SEQ ID NO: 71):

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGSG
SGTDFTLISINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-EGFR antibody variable region

Plain: Anti-EGFR antibody constant region

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 3

Fusion proteins comprising Anti-CD20 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).

Anti-CD20 heavy chain+ TGF β -RII ECD fusion amino acid sequence
(SEQ ID NO: 3):

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

Anti-CD20 light chain amino acid sequence (SEQ ID NO: 72):

QIVLSQSPAILASAPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVFVRFSGSGS
GTSYSLTISRVEAEDAATYYCQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Underlined: Anti-CD20 antibody variable region

Plain: Anti-CD20 antibody constant region

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 4

Fusion proteins comprising anti-VEGF antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).

Anti-VEGF heavy chain+ TGF β -RII ECD fusion amino acid sequence
(SEQ ID NO: 4):

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFKR
RFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYGSSHWYFDVWGQGLTVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSS
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSV
NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

Anti-VEGF Light chain sequence (SEQ ID NO: 73):

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSRFSGSG
SGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYERHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-VEGF antibody variable region

Plain: Anti-VEGF antibody constant region

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 5

Fusion proteins comprising anti-human CTLA-4 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).

Anti-CTLA-4 heavy chain + TGF β -RII Extracellular domain fusion amino acid sequence
(SEQ ID NO: 5):

QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYTMHWVRQA PGKGLEWVTF ISYDGNNKYY
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGQ GTLVTVSSAS
TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL
YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPELLGGPS
VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ
GNVFSCSVMH EALHNHYTQK SLSLSPGK **GGGGSGGGGSGGGGS** **TIPPHVQK SVNNDMIVTD**
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

Anti-CTLA-4 light chain (SEQ ID NO: 74):

EIVLTQSPGT LSLSPGERAT LSCRASQSVG SSYLAWYQQK PGQAPRLLIY GAFSRATGIP
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPWTFG QGTKVEIKRT VAAPSVFIFP
PSDEQLKSGT ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYLSSTL
TLISKADYEKH KVYACEVTHQ GLSSPVTKSF NRGEC

Underlined: Anti-CTLA-4 antibody heavy chain variable region

Plain: Anti-CTLA-4 antibody heavy chain constant region

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 6

Fusion proteins comprising IL-2, Fc, and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).IL-2 + Fc + TGF β -RII Extracellular domain (SEQ ID NO: 6):

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T GGGGSGGGGSGGGGS
THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**EE**MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS **TIPPHVQK SVNNDMIVTD**
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCAVWRK NDENITLETV
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFSEEYNTSNPD

TGF β -RII Extracellular domain + Fc + IL-2 (SEQ ID NO: 7):

TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP
QEVCAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS
DECNDNIIFS EEYNTSNPD GGGGSGGGGSGGGGS
THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**EE**MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T

Bold: TGF β RII ectodomain*Italic: Linker (Optional; other linker sequences)*

Plain: Fc

Underlined: IL-2 fragment

(Note: Can replace linker GGGGSGGGGSGGGGS SEQ ID NO: 104 with

EPKSCDK SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: **E** with D and **M** with L in Fc)

FIG. 7

Fusion proteins comprising anti-CD25 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).

Anti-CD25 (Daclizumab) heavy chain and TGF β -RII Extracellular domain

(SEQ ID NO: 8):

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY
NQKFKDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LVTVSSASTK
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKENWYVDG VEVHNAKTKP REEQYNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN
VFSCSVMEHA LHNHYTQKSL SLSPGK **GGGGSGGGGSGGGGS** **TIPPHVQK SVNNDMIVTD**
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDNITLETV
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

Anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75):

DIQMTQSPST LSASVGDRVT ITCSASSSIS YMHWYQQKPG KAPKLLIYTT SNLASGVPAR
FSGSGSGTEF TLTISSLQPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS
DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLT
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

Plain: Anti-CD25 antibody

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 8A

Anti-CD25 (Basiliximab) heavy chain and TGF β -RII Extracellular domain

(SEQ ID NO: 9):

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPG QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDYGY YFDFWQGGTT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
FSCSVMEAL HNHYTQKSLS LSPGK GGGGSGGGGSGGGGS **TIPPHVQK SVNNDMIVTD**
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

Anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76):

QIVSTQSPAI MSASPGEKVT MTCSASSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR
FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE
QLKSGTASVV CLLNMFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK
ADYEKHKVYA CEVTHQGLSS PVTKSFNRGE

Plain: Anti-CD25 antibody

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 8B

Fusion proteins comprising anti-CD4 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).

Anti-CD4 heavy chain and TGF β -RII Extracellular domain (SEQ ID NO: 10):

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFSCLKLRSVTAADTAVYYCASNILKYLHWLLYWGQGVLTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGSGGGSGGGGS **TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRF**
STCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMK
EKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

Anti-CD4 light chain (SEQ ID NO: 77):

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQOKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Plain: Anti-CD4 antibody (variable region – underlined)

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 9

Fusion proteins comprising PD-1 Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ectodomain).PD-1 ectodomain + Fc + TGF β RII ectodomain (SEQ ID NO: 11):PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV *GGGSGGGSGGGGS*

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK

TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS

REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG

NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVW****RKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYN****TSNPD**TGF β RII ectodomain + Fc + PD-1 ectodomain (SEQ ID NO: 12):**TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVW****RKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYN****TSNPD** *GGGSGGGSGGGGS*

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK

TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS

REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG

NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLVUnderlined: PD-1 ectodomain*Italic: Linkers*

Plain: IgG1 Fc

Bold: TGF β RII ectodomain(Note: The first linker sequence *GGGSGGGSGGGGS* is optional and may be replaced with *EPKSCDK* SEQ ID NO: 105 or deleted)

FIG. 10

Fusion proteins comprising Transforming growth factor-beta receptor II (TGF β -RII) ectodomain, Fc, and Receptor activator of nuclear factor- κ B (RANK) Ectodomain:

TGF β RII ectodomain -- Fc - RANK ectodomain (SEQ. ID. NO: 13):

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEV
CVAVWRKNDENITL**ETVCHDPKLPYHDFILED****AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD**
GGGGSGGGSGGGSGGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS
*DGSFFLYSKLTVDKSRWQQGNVFS**CSVMHEALHNHYTQKSLSLSPGK* *GGGGSGGGSGGGSG* QI
APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

RANK ectodomain -- Fc - TGF β RII ectodomain (SEQ. ID. NO: 14):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
GGGGSGGGSGGGSGGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS
DGSFFLYSKLTVDKSRWQQGNVFS
*CSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGSG***TIPPHVQKS**
VNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEV
CVAVWRKNDENITL
ETVCHDPKLPYHDFILED**AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD**

Bold: TGF β RII ectodomain

Plain: IgG1 Fc

Italics: Linker

Underlined: RANK ectodomain

(optional linker 1 may be deleted or replaced with another linker such as IEGRDMD (SEQ. ID.

NO:106) or EPKSCDK (SEQ.ID. NO: 105))

FIG. 11

(i) Full-length PD-1 or fragment thereof (SEQ ID NO: 92):

1 MQIPQAPWPFV VWAFLQLGWR PGWFLDSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS
61 ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT
121 YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPPSP RPAGQFQTLV VGVVGGLLGS
181 LVLLVWVLAV ICSRAARGTI GARRTGQPLK EDPSAVPVFS VDYGELDFQW REKTPEPPVP
241 CVPEQTEYAT IVFPSGMGTS SPARRGSADG PRSAQPLRPE DGHCSWPL

PD-1 ectodomain

Ligand-binding domain

(ii) PD-1 extracellular domain (ectodomain) or fragment thereof
(SEQ ID NO: 93):

PGWFLDSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA
AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA
ELRVTERRAE VPTAHPSPPSP RPAGQFQTLV

(iii) PD-1 extracellular domain (ectodomain) ligand-binding region
(SEQ ID NO: 94):

DSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA
AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA
ELRVTERRAE VPTAHPSPPSP RPAGQFQ

(iv). Mutant of PD-1 or a fragment thereof which binds Programmed Death-1 ligand
[PD-L1 (B7-H1) or PD-L2 (B7-DC)]

FIG. 12

Fusion proteins comprising Anti-HER2/neu antibody and PD-1 Ectodomain.

Anti-HER2/neu heavy chain + PD-1 ectodomain fusion amino acid sequence

(SEQ ID NO: 15):

EVQLVESGGGLVQPGGSLRLSCAASG**FN**IKD**TY**IHWVRQAPGKGLEWVARIYPTNGYTRYADSVKG
RFTISADTSKNTAYLQMNSLRAEDTAVYYCSR**WG**GDGFYAMDYWGQGT**LV**TVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVT**SV**WNSGALTSGVHTFPAVLQSSGLYSLSSVTV**PSS**SLGT
QTYICNVNHKPSNTKVDKKVEPKSCDK**TH**TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN**ST**YRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNV**F**SCSV**M**HEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **PGWFLDSPDR PWNPPFTSPA LLVVTEGDNA TFTCSFSNTS**
ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT
YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

Anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFS**SGSR**
SGTDFTLTISSSLQPEDFATYYCQQHYTTPPTFGQGT**KVEIKRTVAAPS**VFI**FPPSDEQLKSGTASV**
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK**D**STYSLSS**TLT**LSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-HER2/neu Variable region

Plain: Anti-HER2/neu Constant region

*Italic: Linker***Bold: PD-1 ectodomain**

FIG. 13

Fusion proteins comprising Anti-EGFR1 antibody and PD-1 Ectodomain.

Anti-EGFR heavy chain + PD-1 ectodomain fusion amino acid sequence

(SEQ ID NO: 16):

QVQLKQSGPGLVQPSSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGGNTDYNTPF
TSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT
LVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
SSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCP
PCPAPELLGGPSVFLFPPKPKDTLMISRTPEV
TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **PGWFLDSPDR** **PWNPPTFSPA** **LLVVTEGDNA** **TFTCSFSNTS**
ESFVLNWYRM **SPSNQTDKLA** **AFPEDRSQPG** **QDCRFRTQL** **PNGRDFHMSV** **VRARRNDSGT**
YLCGAISLAP **KAQIKESLRA** **ELRV**TERRAE **VPTAHPSPSP** **RPAGQFQTLV**

Anti-EGFR light chain amino acid sequence (SEQ ID NO: 71):

DILLTQSPVILSVSPGERVSF
SCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSG
SGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLEL
KRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-EGFR Variable region

Plain: Anti-EGFR Constant region

*Italic: Linker***Bold: PD-1 ectodomain**

FIG. 14

Fusion proteins comprising Anti-CD20 antibody and PD-1 Ectodomain.

Anti-CD20 heavy chain + PD-1 ectodomain fusion amino acid sequence

(SEQ ID NO: 17):

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS
ESFVLNWMYR SPSNQTDKLA AFPEDRSQPG QDCRFRTQL PNGRDFHMSV VRARRNDSGT
YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

Anti-CD20 light chain amino acid sequence (SEQ ID NO: 72):

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVFVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Underlined: Anti-CD20 Variable region

Plain: Anti-CD20 Constant region

*Italic: Linker***Bold: PD-1 ectodomain**

FIG. 15

Fusion proteins comprising Anti-VEGF antibody and PD-1 Ectodomain.

Anti-VEGF heavy chain + PD-1 ectodomain fusion amino acid sequence

(SEQ ID NO: 18):

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFKR
RFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLLTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS
ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRTQL PNGRDFHMSV VRARRNDSGT
YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

Anti-VEGF Light chain sequence (SEQ ID NO: 73):

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSRFS
SGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-VEGF Variable region

Plain: Anti-VEGF Constant region

*Italic: Linker***Bold: PD-1 ectodomain**

FIG. 16

Fusion proteins comprising anti-human CTLA-4 antibody and PD-1 Ectodomain.

Anti-(human CTLA-4) (human γ 1-chain)-PD-1 ectodomain fusion protein, disulfide with human κ -chain, dimer

Anti-CTLA-4 heavy chain + PD-1 ectodomain fusion amino acid sequence

(SEQ ID NO: 19):

QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYTMHWVRQA PGKGLEWVTF ISYDGNKYY
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYFCARTG WLGPFDYWGQ GTLTVTVSSAS
TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL
YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPELLGGPS
VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPISRDELT
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ
GNVFSCSVMH EALHNHYTQKSLSLSPGK **GGGSGGGSGGGGS** **PGWFLDSPDR**
PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG
QDCRFRTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA ELRVTERRAE
VPTAHPSPSP RPAGQFQTLV

Anti-CTLA-4 light chain (SEQ ID NO: 74):

EIVLTQSPGT LSLSPGERAT LSCRASQSVG SSYLAWYQQK PGQAPRLLIY GAFSRATGIP
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPWTFG QGTKVEIKRT VAAPSVFIFP
PSDEQLKSGT ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYLSSTL
TLISKADYEH KVIYACEVTHQ GLSSPVTKSF NRGEK

Underlined: Anti-CTLA-4 Variable region

Plain: Anti-CTLA-4 Constant region

Italic: Linker

Bold: PD-1 ectodomain

FIG. 17

Fusion proteins comprising anti-CD25 antibody and PD-1 Ectodomain.

Anti-CD25 (Daclizumab) heavy chain and PD-1 ectodomain (SEQ ID NO: 20):

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY
NQKFKDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LVTVSSASTK
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGS

PGWFLDSPDRPWNPPPTFSPALLVVTEGDNATFTCSFSNT**SESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRAR****RNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPPRPAGQFQTLV**

Anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75):

DIQMTQSPST LSASVGDRVIT ITCSASSSIS YMHWYQQKPG KAPKLLIYTT SNLASGVPAR
FSGSGSGTEF TLTISSLQPD DEATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS
DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTLL
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

Plain: Anti-CD25 antibody

*Italic: Linker***Bold: PD-1 ectodomain**

FIG. 18A

Anti-CD25 (Basiliximab) heavy chain and PD-1 ectodomain (SEQ ID NO: 21):

QLQQSGTVLA RPGASVKMSC KASGYSETRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YDFEWGQGT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYITLP PSRDELTKNQ
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
FSCSVMEAL HNHYTQKSLS LSPGK GGGGSGGGGSGGGGS
PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNT
SESFVLNWRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRAR
RNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV

Anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76):

QIVSTQSPAI MSASPGEKVT MTCSASSSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR
FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE
QLKSGTASVV CLLNFPYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK
ADYEKHKVYA CEVTHQGLSS PVTKSFNRGE

Plain: Anti-CD25 antibody

Italic: Linker

Bold: PD-1 ectodomain

FIG. 18B

Fusion proteins comprising IL-2, Fc, and PD-1 ectodomain.

IL-2 + Fc + PD-1 ectodomain (SEQ ID NO: 22):

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T *GGGGSGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHREALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV

PD-1 ectodomain + Fc + IL-2 (SEQ ID NO: 23):

PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV *GGGGSGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHREALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T

Underlined: IL-2 fragment*Italic: Linker*

Plain: Fc

Bold: human PD-1 ectodomain(Note: Can replace optional linker 1 *GGGGSGGGSGGGGS* SEQ ID NO: 104 with
EPKSCDK SEQ ID NO: 105)(Note: Can replace underlined aa in Fc: E with D and M with L)

FIG. 19

Fusion proteins comprising anti-CD4 antibody and PD-1 Extracellular domain

Anti-CD4 heavy chain and PD-1 Extracellular domain (SEQ ID NO: 24):

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFSCLKRSVTAADTAVYYCASNILKYLHWLLYWGQGVLTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS
PGWFLDSPDRPWNPTTFSPALLVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV

Anti-CD4 light chain (SEQ ID NO: 77):

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGI PARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRITVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Plain: Anti-CD4 antibody (Variable region -- underlined)

*Italic: Linker***Bold: PD-1 ectodomain**

FIG. 20

Fusion proteins comprising PD-1 Ectodomain, Fc, and Receptor activator of nuclear factor- κ B (RANK) Extracellular domain (ectodomain):

RANK ectodomain – Fc - PD-1 ectodomain (SEQ. ID. NO: 25):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RCTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVERHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
GGGGSGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHREALHNHYTQKSLSLSPGK GGGGSGGGSGGGGS
PGWFLDSPDRPWNPTTFSPALLVTEGDNATFTCSFSNTSESFVLNWMSPSNQTDKLAAPEDR
SQPGQDCFRVLTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV

PD-1 ectodomain – Fc - RANK ectodomain (SEQ. ID. NO: 26):

PGWFLDSPDRPWNPTTFSPALLVTEGDNATFTCSFSNTSESFVLNWMSPSNQTDKLAAPEDR
SQPGQDCFRVLTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLVGGGGSGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHREALHNHYTQKSLSLSPGK GGGGSGGGSGGGGS QI APPCTSEKHY EHLGRCCNKC
EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR
RCTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR
PWTNCTFLGK RVERHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Bold: PD-1 ectodomain*Italics: Linker* (optional: may delete linker 1 or replace with IEGRDMD (SEQ. ID. NO: 106) orEPKSCDK (SEQ. ID. NO: 105))

Plain: IgG1 Fc

Underlined: RANK ectodomain

FIG. 21

Moieties that bind Receptor activator of nuclear factor- κ B (RANK) ligand(i) Full-length RANK (TNFRSF11A) or fragment thereof (SEQ. ID. NO: 95):

1 MAPRARRRRP LFALLLLCAL LARLQVALQI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC
 61 TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW
 121 SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
 181 RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPGLIILLLF ASVALVAII FGV CYRKKGK
 241 ALTANLWHWI NEACGRLSGD KESSGDSCVS THTANFGQOG ACEGVLLLT EEKTFPEDMC
 301 YPDQGGVCQG TCVGGGPY AQ GEDARMLSLV SKTEIEEDSF RQMPTEDEYM DRPSQPTDQL
 361 LFLTEPGSKS TPPFSEPLEV GENDSLSQCF TGTQSTVGSE SCNCTEPLCR TDWTPMSEN
 421 YLQKEVDSGH CPHWAASPSP NWADVCTGCR NPPGEDCEPL VGSPKRGFLP QCAYGMGLPP
 481 EEEASRTEAR DQPEDGADGR LPSSARAGAG SGSSPGGQSP ASGNVTGNSN STFISSGQVM
 541 NFKGDIIVVY VSQTSQEGAA AAAEPMGRPV QEETLARRDS FAGNGPRFPD PCGGPEGLRE
 601 PEKASRFVQE QGGAKA

Bold: RANK extracellular domain (RANK ligand-binding domain)(ii) RANK extracellular domain (ectodomain) or fragment thereof (SEQ. ID. NO: 96):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA
 VVAGNSTTPR RACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR
 PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

(iii) Peptide sequences in RANKL binding domains of RANK or peptides containing key RANKL-binding residues of RANK (SEQ. ID. NO: 97):

1 MAPRARRRRP LFALLLLCAL LARLQVALQI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC
 61 TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW
 121 SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
 181 RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Bold: RANK extracellular domain (RANK ligand-binding domain)

RANK peptide sequences (Underlined) and residues (Italics) involved in binding RANKL.

(iv) Mutant of RANK or a fragment thereof which binds Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL)(v) Full-length Osteoprotegerin (OPG, TNFRSF11B) or fragment thereof (SEQ. ID. NO: 98):

1 MNKLLOCALV FLDISIKWTF QETFPKYLH YDEETSHQLL CDKCPPGYL KQHCTAKWKT
 61 VCAPCPDHYY TDSWHTSDEC LYCSPVCKEL QYVKQECNRT HNRVCECKEG RYLEIEFCLK
 121 HRSCPPGFGV VQAGTPERN T VCKRCPDGFF SNETSSKAPC RKHTNCSVFG LLLTQKGNAT
 181 HDNICSGNSE STQKCGIDVF LCEEAFRFA VPTKFTPNWL SVLVNLPGT KVNAESVERI
 241 KRQHSSQEQT FQLKLWKHQ NKAQDIVKKI IQDIDLCE NS VQRHIGHANL TFEQLRSLME
 301 SLP GKKGVAE DIEKTIKACK PSDQILKLLS LWRIKNGDQD TLKGLMHALK HSKTYHFPKT
 361 VTQSLKKTIR FLHSFTMYKL YQKLFLEMIG NQVQSVKISC L

Bold: RANK ligand-binding domain

FIG. 22

Fusion proteins comprising Anti-HER2/neu antibody and Receptor activator of nuclear factor- κ B (RANK) Extracellular domain (ECD).

Anti-HER2/neu heavy chain+ RANK ECD fusion amino acid sequence (SEQ. ID. NO: 27):

EVQLVESGGGLVQPGGSLRLSCAASGFMNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISA
DTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVFSSSLGTQTYICNVNHKPSNTKVDK
KVEPKSCDKTHTCTPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMH
EALHNYHTQKSLSLSPGK **GGGGSGGGSGGGGS** **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW
SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Anti-HER2/neu light chain amino acid sequence (SEQ. ID. NO: 70):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFRSGSRSGTDFT
LTISLQPEDFATYYCQHHYTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC

Underlined: Anti-HER2 antibody variable region

Plain: Anti-HER2 antibody constant region

Italics: Linker

Bold: RANK ectodomain

FIG. 23

Fusion proteins comprising Anti-EGFR1 antibody and Receptor activator of nuclear factor- κ B (RANK) Extracellular domain (ECD).

Anti-EGFR1 heavy chain + RANK ECD fusion amino acid sequence (SEQ. ID. NO: 28):

QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTFPFTRLSINKD
NSKSQVFFKMNLSQSNDAIYYCARALTYDYEFAYWGQGTLVTVSAASTKGPSVFPLAPSSKSTSGGTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVKDKR
VEPKSCDKTHTCTPPCPAPELLGGPSVFLFPPPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCFVSVMHE
ALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW
SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Anti-EGFR1 light chain amino acid sequence (SEQ. ID. NO: 71):

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGSGSGTDFT
LSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Underlined: Anti-EGFR antibody variable region

Plain: Anti-EGFR antibody constant region

Italics: Linker

Bold: RANK ectodomain

FIG. 24

Fusion proteins comprising Anti-CD20 antibody and Receptor activator of nuclear factor- κ B (RANK) Extracellular domain (ECD).

Anti-CD20 heavy chain+ RANK ECD fusion amino acid sequence (SEQ. ID. NO: 29):

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKGKATLTA
DKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD
KKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW
SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Anti-CD20 light chain amino acid sequence (SEQ. ID. NO: 72):

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFOQKPGSSPKPWYATSNLASGVPVRFSGSGSGTSYSL
TISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEEKHKVYACEVTHQGLSPVTKSFNRGEC

Underlined: Anti-CD20 antibody variable region

Plain: Anti-CD20 antibody constant region

Italics: Linker

Bold: RANK ectodomain

FIG. 25

Fusion proteins comprising anti-VEGF antibody and Receptor activator of nuclear factor- κ B (RANK) Extracellular domain (ECD).

Anti-VEGF heavy chain+ RANK ECD fusion amino acid sequence (SEQ. ID. NO: 30):

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVROAPGKGLEWVGWINTYTGEPTYAADF
KRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWFYFDVWGQGLTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
EVTCTVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP**
CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW **SQDCECCRRN**
TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS
DAVCSSSLPA RKPPNEPHVY LPG

Anti-VEGF Light chain sequence (SEQ. ID. NO: 73):

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSRFGSGSG
TDFTLTISLQPEDFATYYCOQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYEEKHKVYACEVTHQG
LSSPVTKSFNRGEC

Underlined: Anti-VEGF antibody variable region

Plain: Anti-VEGF antibody constant region

Italics: Linker

Bold: RANK ectodomain

FIG. 26

Fusion proteins comprising anti-human CTLA-4 antibody and Receptor activator of nuclear factor- κ B (RANK) Extracellular domain (ECD).

Anti-CTLA-4 heavy chain + RANK ECD fusion sequence (SEQ. ID. NO: 31):

QVQLVESGGG VVQPGRSRLR SCAASGFTFS SYTMHWVRQA PGKGLEWVTF ISYDGNNKYY
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGQ GTLVTVSSAS
TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL
YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAFELLGGPS
VFLFPPKPKD TLMISRTPEV TCVVDVDSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTPPVLD SDGSFFLYSK LTVDKSRWQQ
GNVFSCSVMH EALHNHYTQK SLSLSPGK **GGGSGGGSGGGGS** **QI APPCTSEKHY EHLGRCCNKC**
EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR
RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR
PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Anti-CTLA-4 light chain (SEQ. ID. NO: 74):

EIVLTQSPGT LSLSPGERAT LSCRASQSVG SSYLAWYQQK PGQAPRLLIY GAFSRATGIP
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPWTFG QGTKVEIKRT VAAPSVFIFP
PSDEQLKSGT ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYSLSSSTL
TLSKADYEKH KVIACEVTHQ GLSSPVTKSF NRGEC

Underlined: Anti-CTLA-4 antibody variable region

Plain: Anti-CTLA-4 antibody constant region

Italics: Linker

Bold: RANK ectodomain

FIG. 27

Fusion proteins comprising anti-CD25 antibody and Receptor activator of nuclear factor- κ B (RANK) Extracellular domain (ECD).

Anti-CD25 and RANK ectodomain - Heavy chain (SEQ. ID. NO: 32):

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY
NQKFQDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LTVVSSASTK
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSNAKLPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNQGPENNY KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC**
EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR
RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR
PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Anti-CD25 - Light chain (SEQ. ID. NO. 75):

DIQMTQSPST LSASVGDRVT ITCSASSSIS YMHWYQQKPG KAPKLLIYTT SNLASGVPAR
FSGSGSGTEF TLTISSLQPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS
DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLT
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

Plain: Anti-CD25 antibody

*Italics: Linker***Bold: RANK ectodomain**

FIG. 28A

Fusion proteins comprising anti-CD25 antibody and Receptor activator of nuclear factor- κ B (RANK) Extracellular domain (ECD).

Anti-CD25 and RANK ectodomain - Heavy chain (SEQ. ID. NO: 33):

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPG QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDYGY YDFWQGQTT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTL PPSRDELTKNQ
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPVLDSDG SFFLYSKLTV DKSRWQQGNV
FSCSVMHEAL HNHYTQKSLS LSPGK *GGGSGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC**
EPGKMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR
RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR
PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Anti-CD25 – Light chain (SEQ. ID. NO: 76):

QIVSTQSPAI MSASPGEKVT MTCSASSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR
FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFI FPPSDE
QLKSGTASVV CLLNNFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK
ADYEKHKVYA CEVTHQGLSS PVTKSFNRGEC

Plain: Anti-CD25 antibody

*Italics: Linker***Bold: RANK ectodomain**

FIG. 28B

Fusion proteins comprising IL-2, Fc, and Receptor activator of nuclear factor- κ B (RANK) Extracellular domain (ECD).

IL2 – Fc – RANK ECD (SEQ. ID. NO: 34):

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEEELKPLEEVLNL
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGGSG
 GGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
 PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
 VSLTCLVKGFPYSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHN
 HYTQKSLSLSPGK GGGGSGGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW
SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

RANK ECD – Fc – IL-2 (SEQ. ID. NO: 35):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
GGGGSGGGGSGGGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFPYSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKN
PKLTRMLTFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMC
EYADETATIVEFLNRWITFCQSIISTLT

Bold: RANK ectodomain

Italics: Linker (Optional; any linker sequence)

Plain: Fc

Underlined: IL2 fragment that binds CD25 (IL-2R α)

(Can replace linker 1 with EPKSCDK (SEQ. ID. NO: 105) or IEGRDMD (SEQ. ID. NO: 106))

(Can replace underlined aa in Fc: E with D and M with L)

FIG. 29

Fusion protein comprising anti-CD4 antibody and Receptor activator of nuclear factor- κ B (RANK) Extracellular domain (ECD)

Anti-CD4 heavy chain and RANK Extracellular domain (SEQ ID NO: 36):

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFSLKLRSVTAADTAVYYCASNILKYLHWLLYWGQGVLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTSVWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP**
CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR R**CACTAGYHW SQDCECCRRN**
TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS
DAVCSSSLPA RKPPNEPHVY LPG

Anti-CD4 light chain (SEQ ID NO: 77):

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYICQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Underlined: Anti-CD4 antibody variable region*Italic: Linker***Bold: PD-1 ectodomain**

FIG. 30

- (i) Full-length human PD-1 ligand 1 (B7-H1; PDCD1L1; PDL1; CD274) protein or a fragment thereof (SEQ ID NO: 99):

MRIFAVFIFM TYWHLNNAFT VTPKDLVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME
DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG
ADYKRITVKV NAPYNKINQR ILVVDPTSE HELTCQAEY PKAEVIWTSS DHQVLSGKTT
TTNSKREEKL FNVSTILRIN TTTNEIFYCT FRRLDPEENH TAEIVIPPELP LAHPPNERTH
LVILGAILLC LGVALTFIER LRKGRMDVK KCGIQDTNSK KQSDTHLEET

- (ii) PD-L1 extracellular binding domain (ectodomain) or fragment thereof
(SEQ ID NO: 100):

AFT VTPKDLVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG
EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV
NAPYNKINQR ILVVDPTSE HELTCQAEY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL
FNVTSTILRIN TTTNEIFYCT FRRLDPEENH TAEIVI

- (iii) Human PD-1 ligand 2 [PD-L2 (B7-DC)] or a fragment thereof.
- (iv). Mutant of Programmed Death-1 ligand [PD-L1 (B7-H1) or PD-L2 (B7-DC)] or a fragment thereof which binds Programmed Death-1 (PD-1).

Sequence used in fusion proteins (SEQ ID NO: 101):

RIFAVFIFM TYWHLNNAFT VTPKDLVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME
DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG
ADYKRITVKV NAPYNKINQR ILVVDPTSE HELTCQAEY PKAEVIWTSS DHQVLSGKTT
TTNSKREEKL FNVSTILRIN TTTNEIFYCT FRRLDPEENH TAEIVIPPELP LAHPPNERTH
LVILGAILLC LGVALTFIER LRKGRMDVK KCGIQDTNSK KQSDTHLEET

Bold: PD-L1 extracellular domain (ectodomain)

Underlined Bold: Ligand Binding domain

FIG. 31

Fusion proteins comprising anti-tumor necrosis factor (TNF α) antibody and PD-1 ligand 1 or PD-1 ligand 2

Anti-TNF α heavy chain + PD-L1 (SEQ ID NO: 37):

EVQLVESGGGLVQPGRSRLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSV
EG
RFTISRDNKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGT
LVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
TQTYICNVNHKPSNTKVD**KKAE**PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

GGGGSGGGSGGGGS

RIFAVFIFMTYWHLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQF
VHGEEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDPVTSEHELTCQAEQYPAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

Anti-TNF α light chain (SEQ ID NO: 78):

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPGKAPKLLIYAAS
TLSQSGVPSRFS
SGSG
SGTDFTLTIS
SLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: anti-TNF α antibody variable region

Plain: anti-TNF α antibody constant region

Italic: Linker

Bold: human PD-1 ligand 1 (PD-L1)

(Note: Fc region **KKAE** SEQ ID NO: 107 can be replaced with **KRVE** SEQ ID NO: 108
or **KKVE** SEQ ID NO: 109)

FIG. 32

Fusion proteins comprising TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand: (TNFR2 ECD + IgG C γ 1 + PD-L1)

TNFR2 ECD + IgG C γ 1 + PD-L1 (SEQ ID NO: 38):

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPMGPSPPAEGSTGD *EPKSCDK*
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVSMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
RIFAVFIFMTYWHLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNI IQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGK
RMMDVKKCGIQDTNSKKQSDTHLEET

Fusion proteins comprising PD-1 ligand, Fc, and TNFR2 Extracellular ligand binding domain: (PD-L1 + IgG C γ 1 + TNFR2 ECD)

PD-L1 + IgG C γ 1 + TNFR2 ECD (SEQ ID NO: 39):

MRIFAVFIFMTYWHLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNI IQF
FVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
PYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFR
LRKGRMMDVKKCGIQDTNSKKQSDTHLEET *GGGGSGGGSGGGGS* *EPKSCDK*
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVSMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPMGPSPPAEGSTGD

Underlined: TNFR2 ligand-binding domain

Plain: human IgG1 heavy chain constant region (IgG C γ 1 domain = H, CH2, CH3)

Italic: Linker (Linker 1 is optional and may be replaced with another linker such as EPKSCDK (SEQ. ID. NO: 105) or GGGSGGGSGGGGS (SEQ. ID. NO: 104))

Bold: human PD-1 ligand 1 or extracellular binding domain of PD-L1

FIG. 33

Fusion proteins comprising anti-CD20 antibody and PD-1 ligand 1 (PD-L1).

Anti-CD20 heavy chain + PD-L1 sequence (SEQ ID NO: 40):

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

GGGSGGGSGGGGS

RIFAVFIFMTYWHLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDPVITSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

Anti-CD20 light chain sequence (SEQ ID NO: 72):

QIVLSQSPAILSASPGKVTMTCRASSSVSYIHWFOQKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQTWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Underlined: anti-CD20 antibody variable region

Plain: anti-CD20 antibody constant region

*Italic: Linker***Bold: human PD-1 ligand 1 or extracellular binding domain of PD-L1**

FIG. 34

Fusion proteins comprising anti-CD25 antibody and PD-1 ligand 1 (PD-L1).

Anti-CD25 (Daclizumab) heavy chain and PD-L1 (SEQ ID NO: 41):

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY
NQKFQDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LVTVSSASTK
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMEHA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGS

RIFAVFIFMTYWHLNNAFTVTPKDLYVVEYGSNMTIECK

FPVEKQLDLAALIVYWEMEDKNI IQFVHGEEDLKVQHSSYRQARLLKDQLSLGNA

ALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHEL

TCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLEFNVSTLRLNTTTNEIFYC

TFRRLDPEENHTAELVIPELPLAHPPNERHLVILGAILLCLGVALTFIFRLRKGR

MMDVKKCGIQDTNSKKQSDTHLEET

Anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75):

DIQMTQSPST LSASVGDRVT ITCSASSSIS YMHWYQQKPG KAPKLLIYTT SNLASGVPAR
FSGSGSGTEF TLTISSLQPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS
DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

Plain: anti-CD25 antibody

Italic: Linker (optional and may be replaced)**Human PD-1 ligand 1 or extracellular binding domain of PD-L1 (underlined)**

FIG. 35A

Anti-CD25 (Basiliximab) heavy chain and PD-1 ectodomain (SEQ ID NO: 42)

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDYGY YFDFWGQGT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLF PSRDELTKNQ
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPVLDSDG SFFLYSKLTV DKSRWQQGNV
FSCSVMEAL HNHYTQKSLS LSPGK GGGGSGGGGSGGGGS

RIFAVFIFMTYWHLNAFTVTVPKDLYVVEYGSNMTIECK

FPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNA

ALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDVPTSEHEL

TCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYC

TFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR

MMDVKKCGIQDTNSKKQSDTHLEET

Anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76):

QIVSTQSPAI MSASPGEKVT MTCSASSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR
FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE
QLKSGTASVV CLLNFPYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK
ADYEKHKVYA CEVTHQGLSS PVTKSFNRRGE

Plain: anti-CD25 antibody

Italic: Linker

Bold: human PD-1 ligand 1 or extracellular binding domain of PD-L1 (underlined)

FIG. 35B

Fusion proteins comprising IL-2, Fc, and PD-1 ligand 1 (PD-L1)

Fusion protein: hPD-1 ligand 1 + Fc + IL-2 (SEQ ID NO: 43):

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQFVHGEE
DLKVOHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKV
NAPYNKINQRILVVDPVTSEHELTCQAEYGPKAQEVITWSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTT
TNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFR
 LRKGRMDVKKCGIQDTNSKKQSDTHLEET GGGGSGGGGSGGGGS
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
 CLVKGFPYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQ
 KSLSLSPGK GGGGSGGGGSGGGGS
APTSSSTKKTQLQLEHLLLDLQMI LGINNYKNPKLTRMLTFKFYMPKKATELKHLCLEEEELKPLEEVLNL
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

Fusion protein: IL-2 + Fc + PD-1 ligand 1 (SEQ ID NO: 44):

APTSSSTKKTQLQLEHLLLDLQMI LGINNYKNPKLTRMLTFKFYMPKKATELKHLCLEEEELKPLEEVLNL
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
 GGGGSGGGGSGGGGS
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
 CLVKGFPYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQ
 KSLSLSPGK GGGGSGGGGSGGGGS
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQFVHGEE
LKVOHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD
PVTSEHELTCQAEYGPKAQEVITWSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGRMDVKKCGIQDTNSKKQSDTHLEE
 T

Underlined: IL-2 fragment*Italic: Linker (optional)*

Plain: Fc

Bold: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)

(Note: Can replace linker 1 GGGGSGGGGSGGGGS SEQ ID NO: 104 with

EPKSCDK SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: E with D and M with L)

FIG. 36

Fusion protein comprising anti-CD4 antibody and PD-1 ligand 1 (PD-L1)

Anti-CD4 heavy chain and PD-1 ligand 1 (SEQ ID NO: 45)

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFSCLKRSVTAADTAVYYCASNILKYLHWLLYWGQGVLTSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TPPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNI IQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDPVTSEHELTCQAEQYPAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

Anti-CD4 light chain (SEQ ID NO: 77)

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Underlined: Anti-CD4 antibody variable region

Plain: Anti-CD4 antibody constant region

*Italic: Linker***Bold: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)**

FIG. 37

Fusion proteins comprising the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C γ 1), and a sequence from PD-1 ligand (PD-L1)

Oncostatin M signal peptide + CTLA-4 ECD + IgG C γ 1 + PD-L1 (SEQ ID NO: 46):

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQA
DSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGI
GNGTQIYVIDPEPCPDSD QEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQDLAALIVWEMEDKNIIQ
VHGEEDLKVOHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
YNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLR
KGRMMDVKKCGIQDTNSKKQSDTHLEET

Fusion proteins comprising the extracellular domain of PD-1 ligand (PD-L1), Immunoglobulin Fc (IgG C γ 1), and a sequence from the extracellular domain of CTLA-4.

PD-L1 + IgG C γ 1 + CTLA-4 ECD (SEQ ID NO: 47):

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQDLAALIVWEMEDKNIIQ
FVHGEEDLKVOHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
PYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFR
LKGRMMDVKKCGIQDTNSKKQSDTHLEET GGGGSGGGSGGGGS
QEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK GGGGSGGGSGGGGS
AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSI
CTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDS

Underlined Italic: *Oncostatin M Signal Peptide (-25 to -1)*

Underlined: CTLA-4 extracellular domain (1-125)

Plain: human IgG1 heavy chain constant region (IgG C γ 1 domain = H, CH2, CH3)

Italic: *Linker (optional)*

Underlined: human PD-1 ligand 1 (PD-1L) or extracellular binding domain of PD-1L

(Note: Optional C to S conversion in IgG sequence (bold underlined))

(Note: The linker QEPKSCDK SEQ ID NO: 110 can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence)

FIG. 38

Fusion proteins comprising a sequence of transforming growth factor- β (TGF- β), Immunoglobulin Fc (IgG C β 1), and a sequence of PD-1 ligand (PD-L1)

TGF β -1 + Fc + PD-L1 (SEQ ID NO: 48):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
 QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS *GGGSGGGSGGGGS*
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNI IQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRIILVVDVPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKG
RMDVKKCGIQDTNSKKQSDTHLEET

Fusion protein comprising a sequence of PD-1 ligand (PD-L1), Immunoglobulin Fc (IgG C γ 1), and a sequence of Transforming growth factor beta (TGF- β)

PD-L1 + Fc + TGF β -1 (SEQ ID NO: 49):

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNI IQF
FVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
PYNKINQRIILVVDVPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKG
RMDVKKCGIQDTNSKKQSDTHLEET *GGGSGGGSGGGGS*
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*
 ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
 QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Underlined: TGF β -1

Italic: Linker (optional)

Plain: Fc

Bold: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)

(Note: Can replace linker *GGGSGGGSGGGGS* SEQ ID NO: 104 with

EPKSCDK SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: E with D and M with L)

FIG. 39

- (i) Transforming growth factor-beta (TGF- β 1, TGF- β 2, or TGF- β 3 or a fragment thereof:

TGF- β 1 full sequence (SEQ ID NO: 102):

1 MPPSGLRLLP LLLPLLRLLV LTPGRPAAGL STCKTIDMEL VKRKRIEAIR GQILSKLRLS
61 SPPSQGEVPP VPLPEAVLAL YNSTRDRVAG ESAEPEPEPE ADYYAKEVTR VLMVENTNKI
121 YEKVKKSPHS IYMLFNTSEL REAVPEPVLL SRAELRLLRL KLKAEQHVEL YQKYSNDSWR
181 YLSNRLLAPS DTPEWLSFDV TGVVRQWLSH GGEVEGFRLS AHCSCDSKDN TLQVDINGFS
241 SSRRGDLATI HGMNRPFLLL MATPLERAQH LHSSRQRRAL DTNYCFSSTE KNCCVRQLYI
301 DFRKDLGWKW IHEPKGYHAN FCLGPCPYIW SLDTQYSKVL ALYNQHNPQA SAAPCCVPQA
361 LEPLPIVYYV GRKPKVEQLS NMIVRSCKCS

Underlined: Mature (active) TGF- β 1 sequence (Ala 279 – Ser 390; 112 aa).

- (ii) Mature (active) TGF- β 1 sequence (Ala 279 – Ser 390; 112 aa)
(SEQ ID NO: 103):

AL DTNYCFSSTE KNCCVRQLYI DFRKDLGWKW IHEPKGYHAN FCLGPCPYIW
SLDTQYSKVL ALYNQHNPQA SAAPCCVPQA LEPLPIVYYV GRKPKVEQLS NMIVRSCKCS

FIG. 40

Fusion proteins comprising an antibody that binds TNF- α , and a sequence of transforming growth factor- β (TGF- β).

Anti-TNF α heavy chain + TGF- β 1 (SEQ ID NO: 50):

EVQLVESGGGLVQPGKSLRLSCAASGFTEDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSVEG
RFTISRDNKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGTLLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVD**KKAE**PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTTPVLDSDGSFFLYSKLTVDKSRWQQGNVSCFVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Anti-TNF α light chain (SEQ ID NO: 78):

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPKGAPKLLIYAASLTQSGVPSRFSGSG
SGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: anti-TNF α antibody variable region

Plain: anti-TNF α antibody constant region

Italic: Linker

Bold: Mature (active) human TGF- β 1

(Note: **KKAE** SEQ ID NO: 107 can be replaced with **KRVE** SEQ ID NO: 108 or **KKVE** SEQ ID NO: 109)

FIG. 41

Fusion proteins comprising TNFR2 Extracellular ligand binding domain, Fc, and a sequence from transforming growth factor- β (TGF- β)

TNFR2 ECD + IgG C γ 1 + TGF- β 1 (SEQ ID NO: 51):

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPMGSPSPAEGSTGD *EPKSCDK*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**EE**MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Fusion proteins comprising a sequence from transforming growth factor- β (TGF- β), Fc, and TNFR2 Extracellular ligand binding domain:

TGF- β 1 + IgG C γ 1 + TNFR2 ECD (SEQ ID NO: 52):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS *GGGSGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**EE**MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPMGSPSPAEGSTGD

Underlined: TNFR2 ligand-binding domain

Plain: human IgG1 heavy chain constant region (IgG C γ 1 domain = H, CH2, CH3)

Italic: Linker

Bold: Mature (active) human TGF- β 1

(Note: Can replace linker 1 with *GGGSGGGSGGGGS* SEQ ID NO: 104 or *EPKSCDK* SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: **E** with D and **M** with L)

FIG. 42

Fusion proteins comprising anti-CD20 antibody and a sequence from transforming growth factor- β (TGF- β)

Anti-CD20 heavy chain + mature TGF β 1 sequence (SEQ ID NO: 53):

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

GGGGSGGGSGGGGS

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Anti-CD20 light chain sequence (SEQ ID NO: 72):

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVFVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Underlined: anti-CD20 antibody variable region

Plain: anti-CD20 antibody constant region

Italic: Linker

Bold: Mature (active) human TGF- β 1

FIG. 43

Fusion proteins comprising anti-CD25 antibody and a sequence from transforming growth factor- β (TGF- β).

Anti-CD25 (Daclizumab) heavy chain and TGF- β 1 (SEQ ID NO: 54):

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY
NQKFKDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LVTVSSASTK
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSNAKLPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNQGPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGS **ALDTNYCFSSTEKNCCVRQLY**
IDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNP GASAAPCCVPQALEPLP
IVYYVGRKPKVEQLSNMIVRSCKCS

Anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75):

DIQMTQSPST LSASVGDRVT ITCSASSSIS YMHWYQQKPG KAPKLLIYTT SNLASGVPAR
FSGSGSGTEF TLTISSLQPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS
DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLT
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

Plain: anti-CD25 antibody

Italic: Linker

Bold: Mature (active) human TGF- β 1

FIG. 44A

Anti-CD25 (Basiliximab) heavy chain and TGF- β 1 (SEQ ID NO: 55):

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YFDFWGQGTT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLF PSRDELTKNQ
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRRWQQGNV
FSCSVMEAL HNHYTQKSLS LSPGK GGGGSGGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHAN
FCLGPCPYIWSLDTQYSKVLALYNQHNPASAAAPCCVPQALEPLPIVYYYVGRKPKVEQ
LSNMIVRSCKCS

Anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76):

QIVSTQSPAI MSASPGEKVT MTCSASSSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR
FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE
QLKSGTASVV CLLNMFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK
ADYEKHKVYA CEVTHQGLSS PVTKSFNRGE

Plain: anti-CD25 antibody

Italic: Linker

Bold: Mature (active) human TGF- β 1

FIG. 44B

Fusion proteins comprising IL-2, Fc, and a sequence from transforming growth factor- β (TGF- β).

TGF- β 1 + Fc + IL-2 (SEQ ID NO: 56):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS *GGGGSGGGSGGGGS*
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 R**EE****M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQG
 NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T

IL-2 + Fc + TGF- β 1 (SEQ ID NO: 57):

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T *GGGGSGGGSGGGGS*
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 R**EE****M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQG
 NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Underlined: IL-2 fragment

Italic: Linker (optional)

Plain: Fc

Bold: Mature (active) human TGF- β 1

(Note: Can replace linker 1 *GGGGSGGGSGGGGS* SEQ ID NO: 104 with

EPKSCDK SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: E with D and M with L)

FIG. 45

Fusion protein comprising anti-CD4 antibody and a sequence from transforming growth factor- β (TGF- β).

Anti-CD4 heavy chain and TGF- β 1 (SEQ ID NO: 58)

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFSCLKRSVTAADTAVYYCASNILKYLHWLLYWGQGVLTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

GGGGSGGGSGGGGS

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Anti-CD4 light chain (SEQ ID NO: 77)

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Underlined: Anti-CD4 antibody variable region

Plain: Anti-CD4 antibody constant region

Italic: Linker

Bold: Mature (active) human TGF- β 1

FIG. 46

Fusion proteins comprising the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C γ 1), and a sequence from a sequence from transforming growth factor- β (TGF- β)

Oncostatin M signal peptide + CTLA-4 ECD + IgG C γ 1 + TGF- β 1 (SEQ ID NO: 59):

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIA SFVCEYASPGKATEVRVTVLRQA
DSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLGI
GNGTQIYVIDPEPCPDSDQEPKSCDK THT**C**PP**C**PAPELLGGPSVFLFPPKPKDTLM
 ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
 YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
 GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK
 GGGGSGGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Fusion proteins comprising a sequence from transforming growth factor- β (TGF- β), Immunoglobulin Fc (IgG C γ 1), and a sequence from the extracellular domain of CTLA-4

TGF- β 1 + IgG C γ 1 + CTLA-4 ECD (SEQ ID NO: 60):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS *EPKSCDK*
 THT**C**PP**C**PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
AMHVAQPAVVLASSRGIA SFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSI
CTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLGIGNGTQIYVIDPEPCPDSD

Underlined Italic: *Oncostatin M Signal Peptide (-25 to -1)*

Underlined: CTLA-4 extracellular domain (1-125)

Plain: human IgG1 heavy chain constant region (IgG C γ 1 domain = H, CH2, CH3)

Italic: Linker (optional)

Bold: Mature (active) human TGF- β 1

(Note: Optional C to S conversion in IgG sequence (bold underlined))

(Note: The linker QEPKSCDK SEQ ID NO: 110 can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence)

FIG. 47

Fusion proteins comprising an antibody that binds TNF- α , and a RANKL-binding sequence of receptor activator of nuclear factor-kB (RANK)

Anti-TNF α heavy chain + RANK ectodomain (SEQ ID NO: 61):

EVQLVESGGGLVQPGKSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSV
EG
RFTISRDNKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGT
LVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSSLG
TQTYICNVNHKPSNTKVD**KKAE**PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP
CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR R**CACTAGYHW SQDCECCRRN**
TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS
DAVCSSSLPA RKPPNEPHVY LPG

Anti-TNF α light chain (SEQ ID NO: 78):

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPPGKAPKLLIYAASTLQSGVPSRFSSGSG
SGTDFTLTISSLPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: anti-TNF α antibody variable region

Plain: anti-TNF α antibody constant region

Italic: Linker

Bold: RANK ectodomain

(Note: **KKAE** SEQ ID NO: 107 can be replaced with **KRVE** SEQ ID NO: 108 or **KKVE** SEQ ID NO: 109)

FIG. 48

Fusion proteins comprising TNFR2 Extracellular ligand binding domain, Fc, and a RANKL-binding sequence of receptor activator of nuclear factor- κ B (RANK)
TNFR2 ECD + IgG C γ 1 + RANK ectodomain (SEQ ID NO: 62):

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKEGCRLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSENNTSSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPLMGPSPPAEGSTGD *EPKSCDK*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**EEM**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS* **QI APPCTSEKHY**
EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA
VVAGNSTTPR RACTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS
DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Fusion proteins comprising a RANKL-binding sequence of receptor activator of nuclear factor- κ B (RANK), Fc, and TNFR2 Extracellular ligand binding domain:
RANK ectodomain + IgG C γ 1 + TNFR2 ECD (SEQ ID NO: 63):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW
NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRN TECAPGLGAQ
HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA
RKPPNEPHVY LPG
GGGSGGGSGGGGS THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSR**EEM**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKEGCRLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSENNTSSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPLMGPSPPAEGSTGD

Underlined: TNFR2 ligand-binding domain

Plain: human IgG1 heavy chain constant region (IgG C γ 1 domain = H, CH2, CH3)

Italic: Linker

Bold: RANK ectodomain

(Note: Can replace linker 1 with *GGGSGGGSGGGGS* SEQ ID NO: 104 or *EPKSCDK* SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: **E** with D and **M** with L)

FIG. 49

Fusion proteins comprising the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C γ 1), and a RANKL-binding sequence of receptor activator of nuclear factor- κ B (RANK)

Oncostatin M signal peptide + CTLA-4 ECD + IgG C γ 1 + RANK ectodomain (SEQ ID NO: 64):

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLAASSRGIA SFVCEYASPGKATEVRVTVLRQA
 DSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLGI
 GNGTQIYVIDPEPCPDS QEPKSCDK THTCPPCPAPELLGGPSVFLFPPKPKDTLM
 ISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
 YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
 GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK
 GGGGSGGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP**
CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRRN
TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS
DAVCSSSLPA RKPPNEPHVY LPG

Fusion proteins comprising a RANKL-binding sequence of receptor activator of nuclear factor- κ B (RANK), Immunoglobulin Fc (IgG C γ 1), and a sequence from the extracellular domain of CTLA-4

RANK ectodomain + IgG C γ 1 + CTLA-4 ECD (SEQ ID NO: 65):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW
NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRRN TECAPGLGAQ
HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA
RKPPNEPHVY LPG *EPKSCDK*
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCVMSHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
 AMHVAQPAVVLAASSRGIA SFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSI
 CTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLGI GNGTQIYVIDPEPCPDS

Underlined Italic: Oncostatin M Signal Peptide (-25 to -1)

Underlined: CTLA-4 extracellular domain (1-125)

Plain: human IgG1 heavy chain constant region (IgG C γ 1 domain = H, CH2, CH3)

Italic: Linker (optional)

Bold: RANK ectodomain

(Note: Optional C to S conversion in IgG sequence (bold underlined))

(Note: The linker *QEPKSCDK* SEQ ID NO: 110 can be replaced with *EPKSCDK* SEQ ID NO: 105 or another linker sequence)

FIG. 50

Fusion proteins comprising a RANKL-binding sequence of receptor activator of nuclear factor- κ B (RANK), Fc, and a sequence from transforming growth factor- β (TGF- β).

TGF- β 1 + Fc + RANK ectodomain (SEQ ID NO: 66):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPASAAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS GGGGSGGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS **QI APPCTSEKHY**
EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA
VVAGNSTTPR RACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS
DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

RANK ectodomain + Fc + TGF- β 1 (SEQ ID NO: 67):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW
NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRRN TECAPGLGAQ
HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA
RKPPNEPHVY LPG GGGGSGGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPASAAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Underlined: Mature (active) human TGF- β 1

Italic: Linker (optional)

Plain: Fc

Bold: RANK ectodomain

(Note: Can replace linker 1 GGGGSGGGGSGGGGS SEQ ID NO: 104 with

EPKSCDK SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: E with D and M with L)

FIG. 51

Fusion proteins comprising a RANKL-binding sequence of receptor activator of nuclear factor- κ B (RANK), Fc, and a sequence from PD-1 ligand 1

PD-1L1 + Fc + RANK ectodomain (SEQ ID NO: 68):

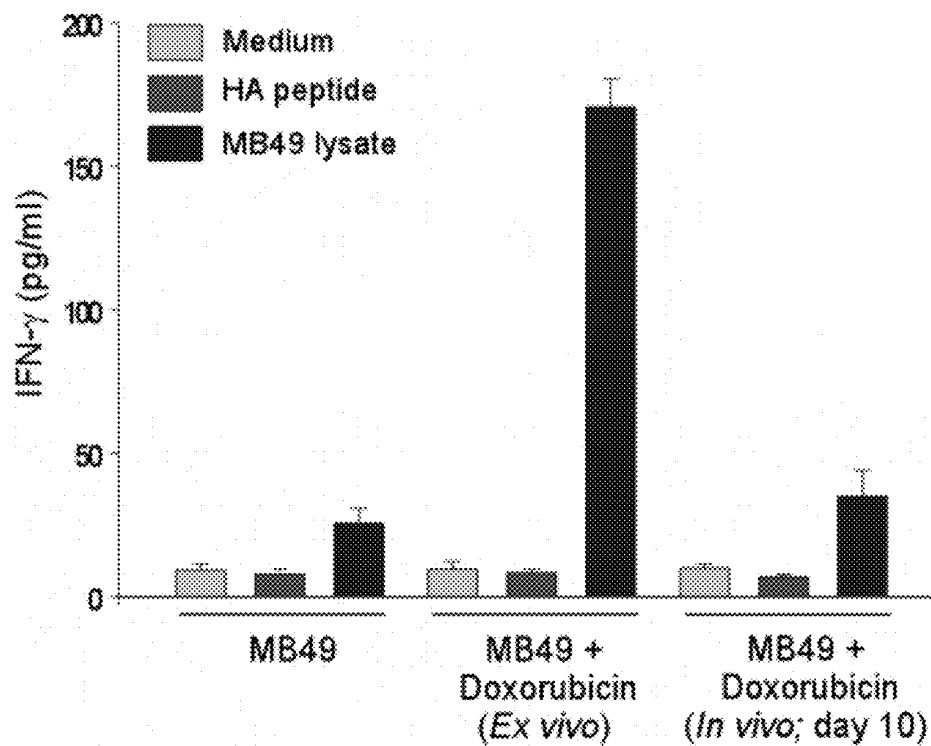
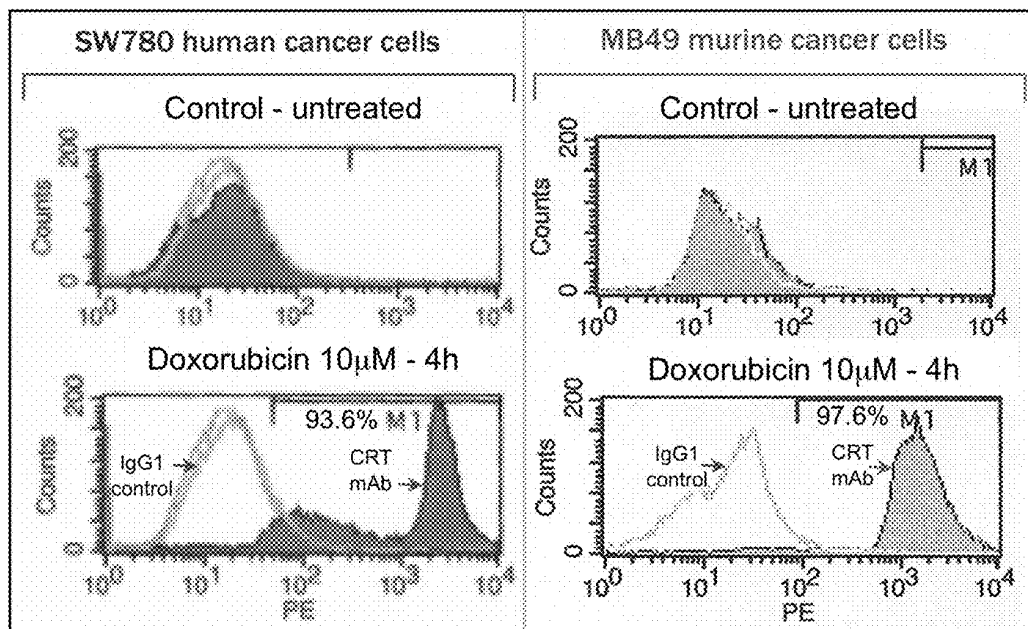
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQFVHGEED
LKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD
PVTSEHELTCQAEQYPAEVIWTSSDHQVLSGKTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMDVKKCGIQDTNSKKQSDTHLEE
T GGGGSGGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQ
KSLSLSPGK GGGGSGGGGSGGGGS QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTSDSVCLP
CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRN
TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS
DAVCSSSLPA RKPPNEPHVY LPG

RANK ectodomain + Fc + PD-1L1 (SEQ ID NO: 69):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTSDSVCLP CGPDEYLDW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
GGGGSGGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQ
KSLSLSPGK GGGGSGGGGSGGGGS
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQFVHGEED
LKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD
PVTSEHELTCQAEQYPAEVIWTSSDHQVLSGKTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMDVKKCGIQDTNSKKQSDTHLEE
T

Bold: PD-1L1 (May use PD-1L1 extracellular domain – underlined)

FIG. 52



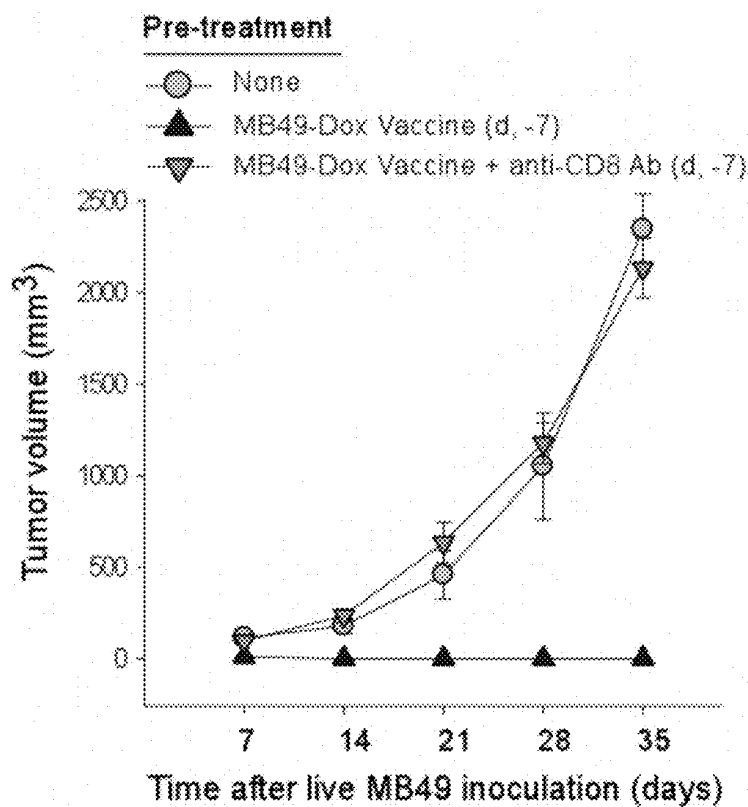


FIG. 53C

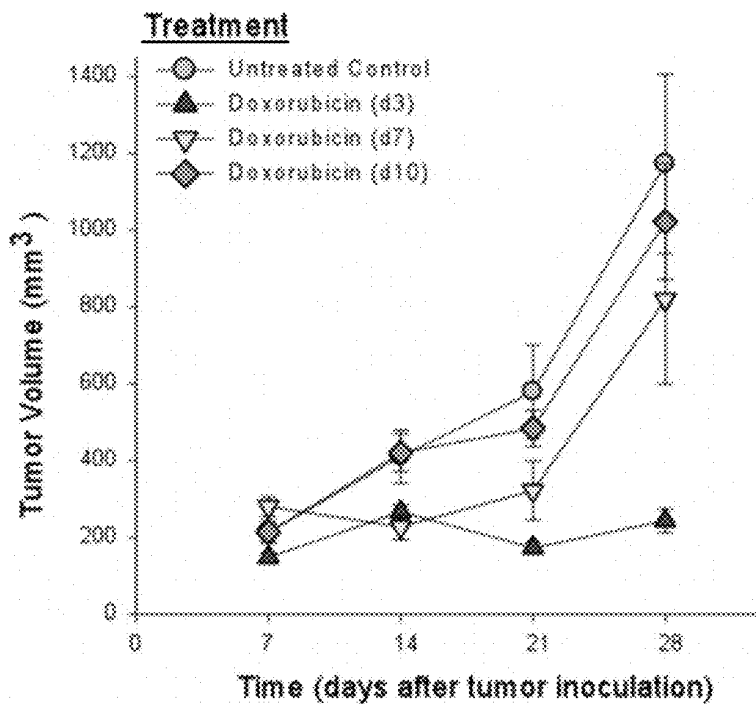


FIG. 53D

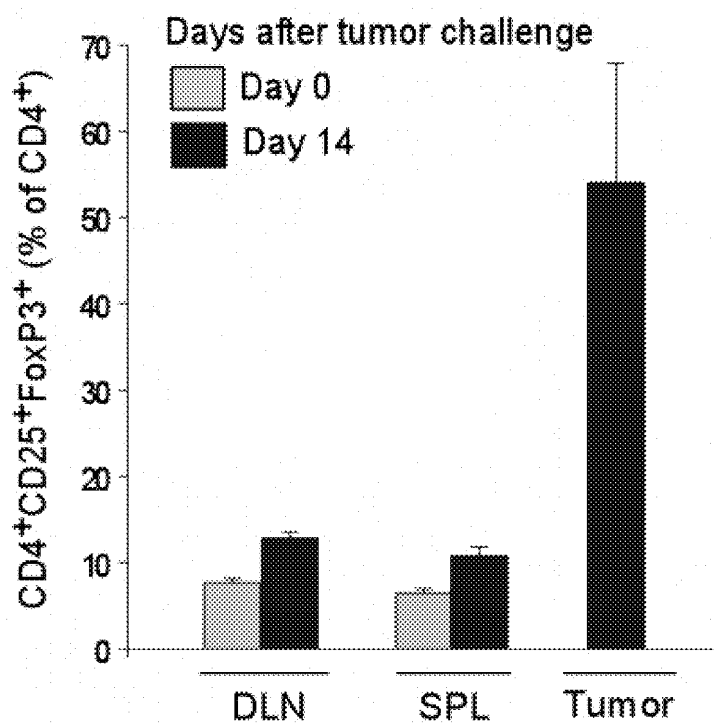


FIG. 53E

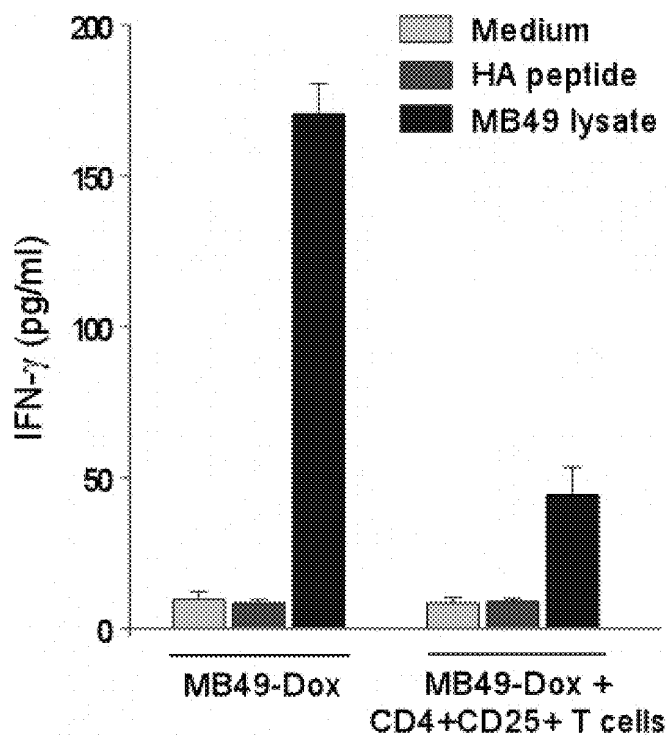


FIG. 53F

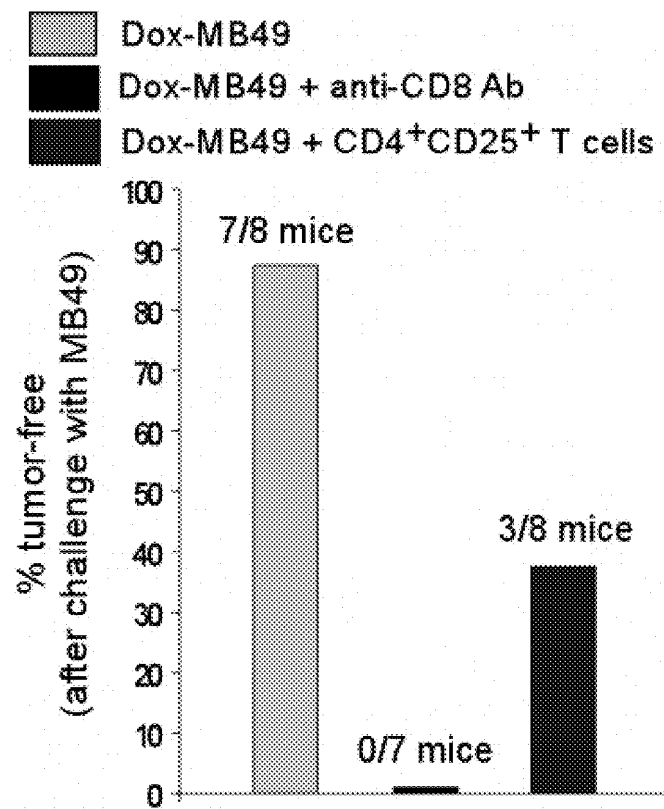


FIG. 53G

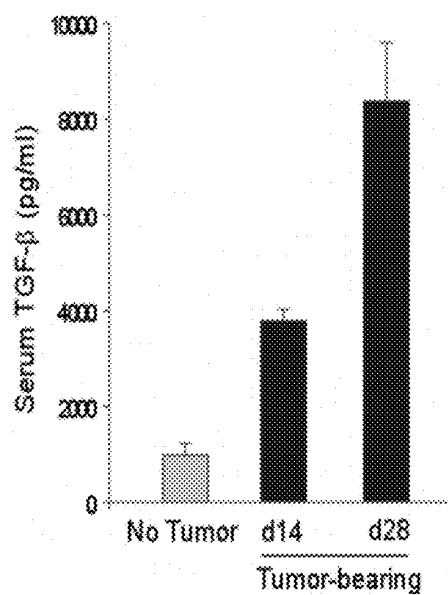


FIG. 54A

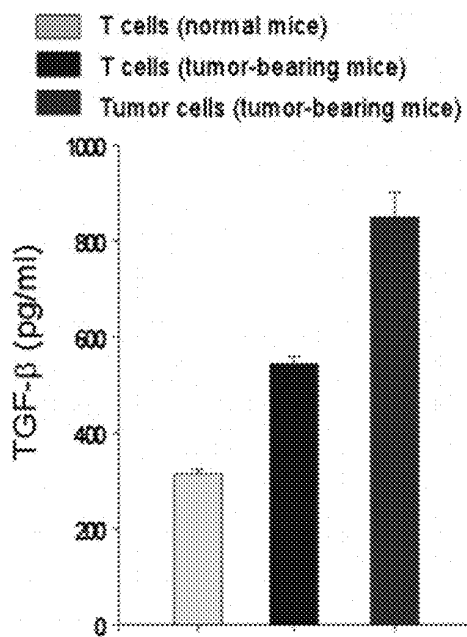


FIG. 54B

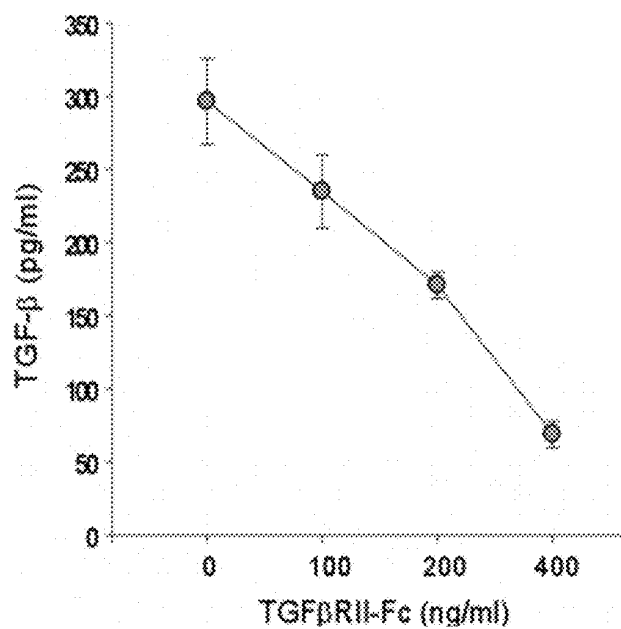


FIG. 54C

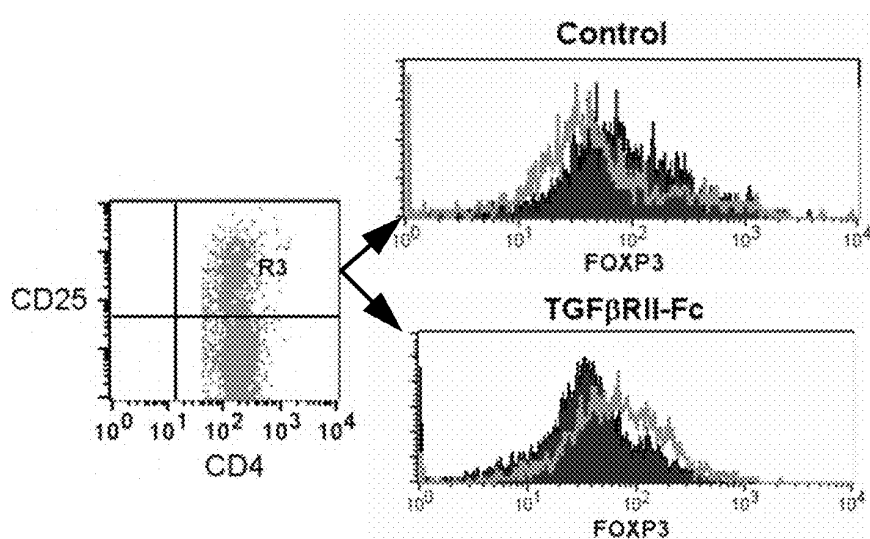


FIG. 54D

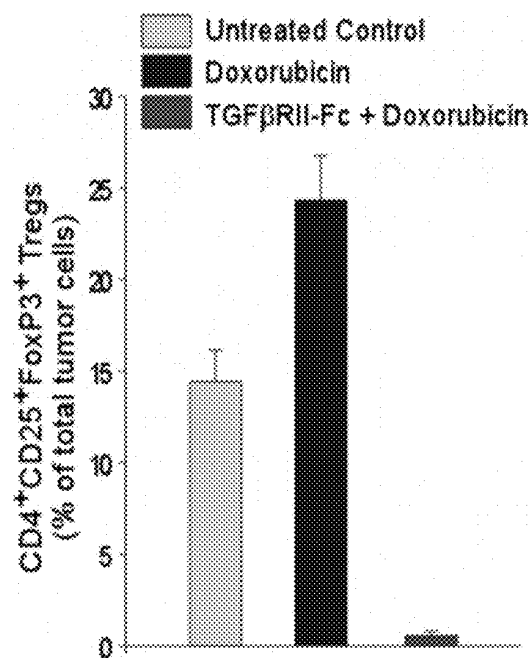


FIG. 54E

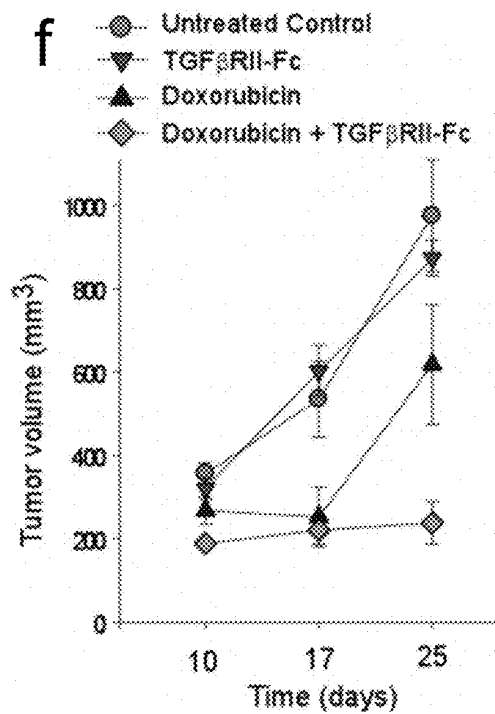


FIG. 54F

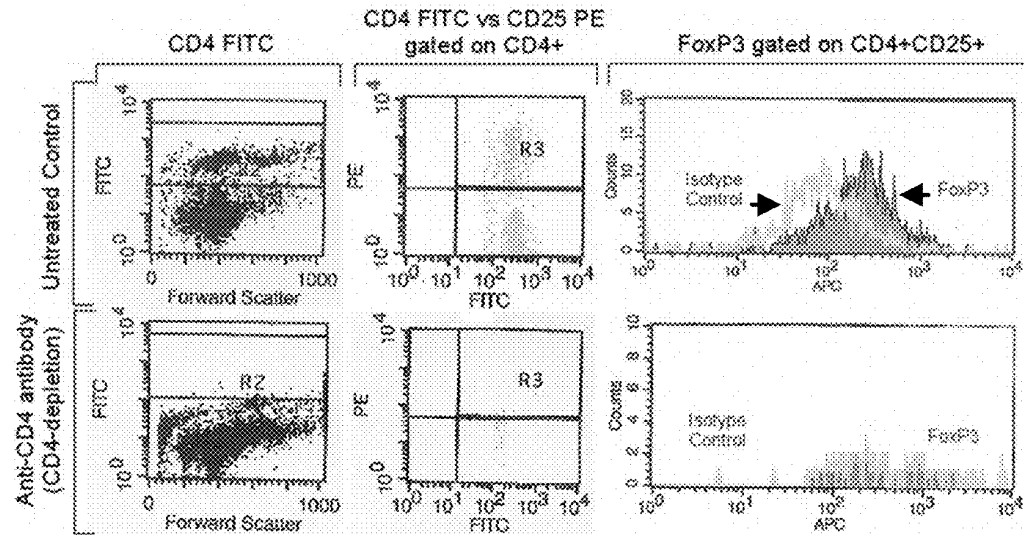


FIG. 55A

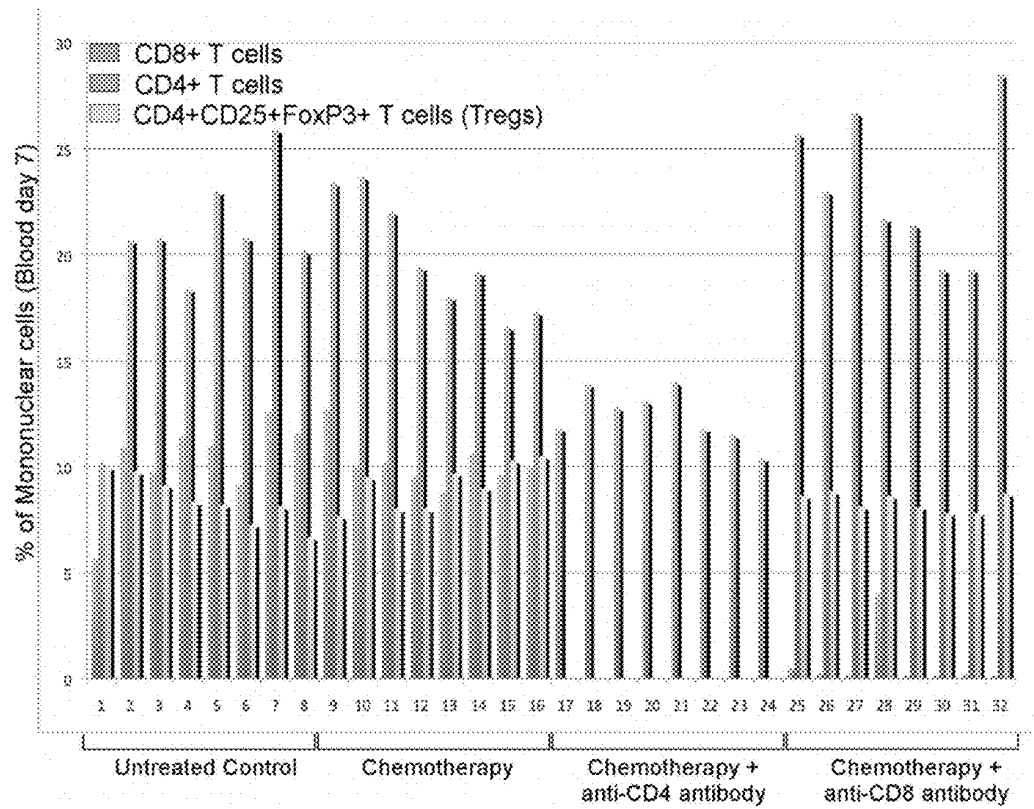


FIG. 55B

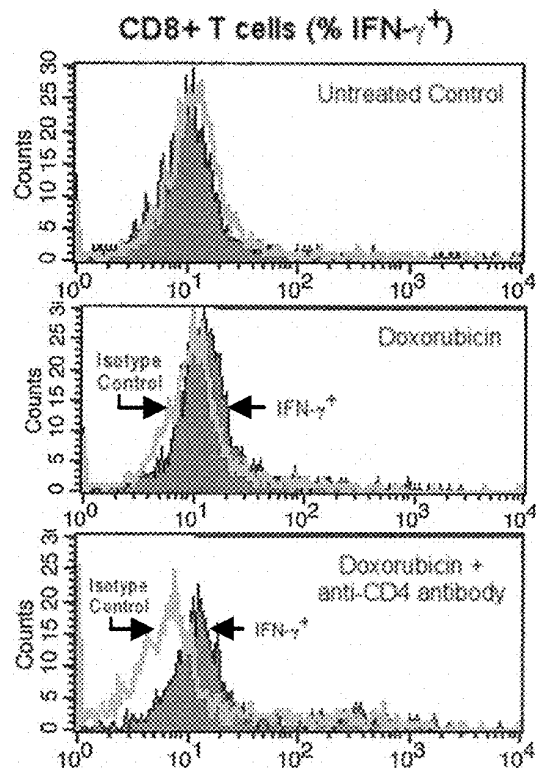


FIG. 55C

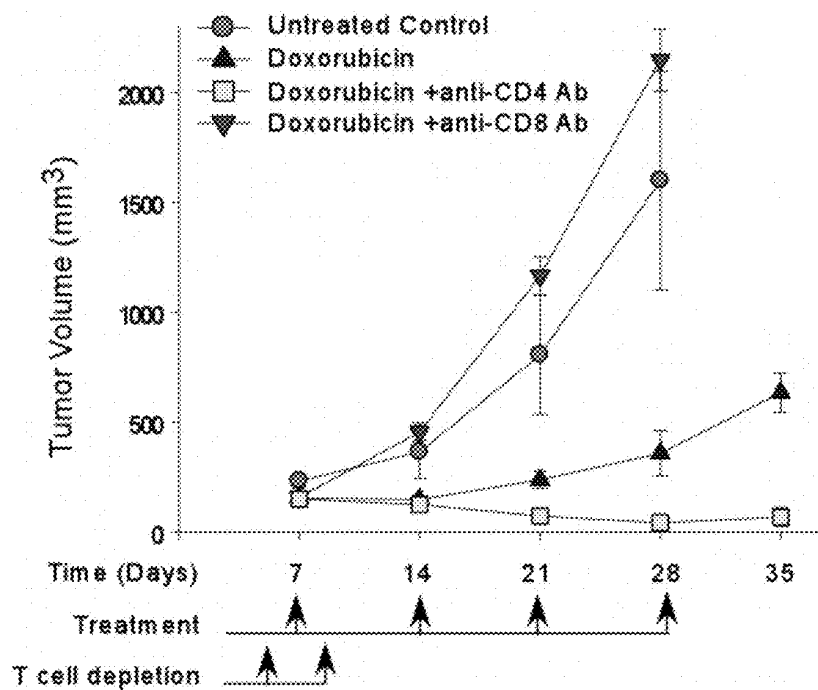


FIG. 55D

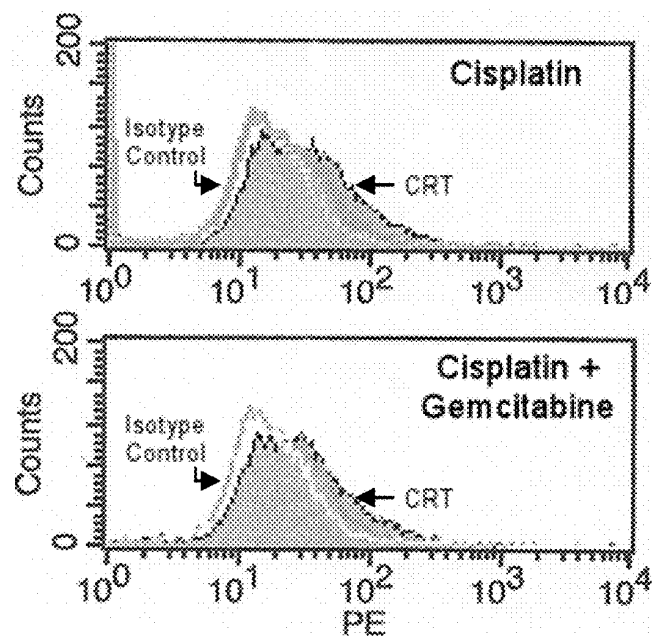


FIG. 56A

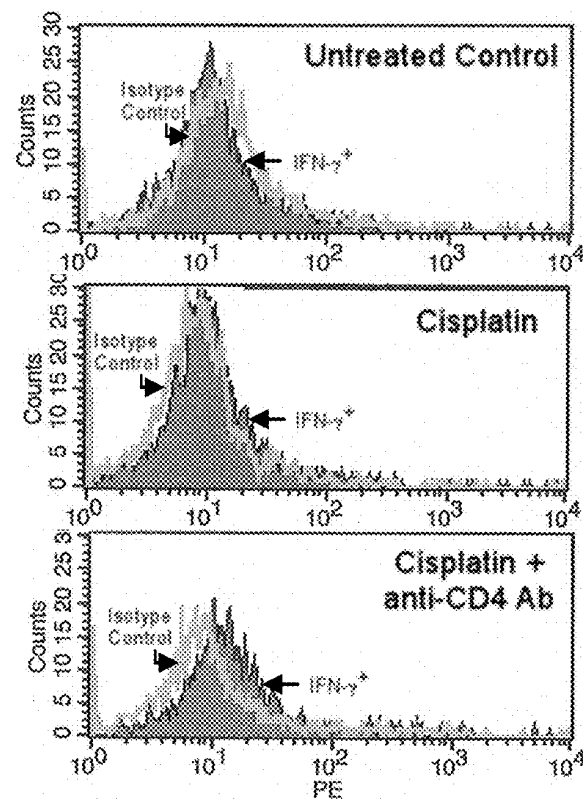


FIG. 56B

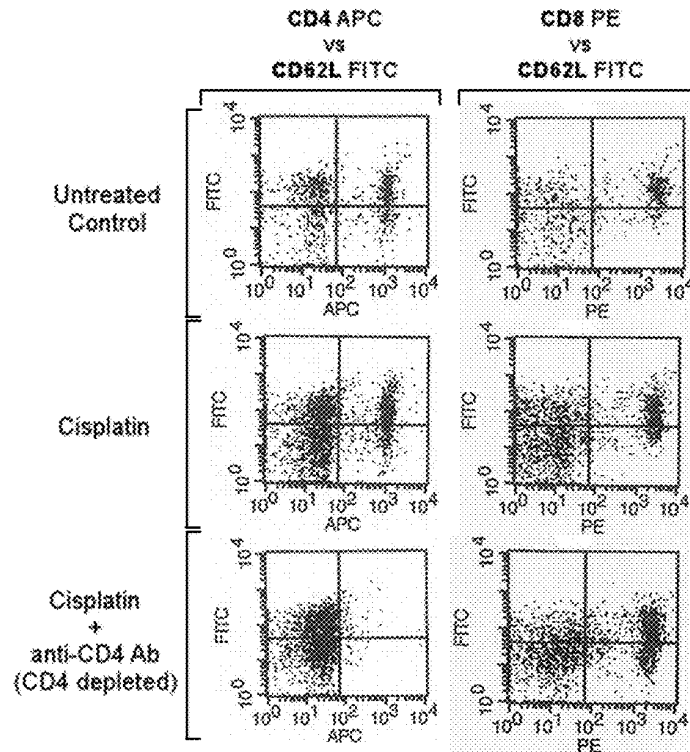


FIG. 56C

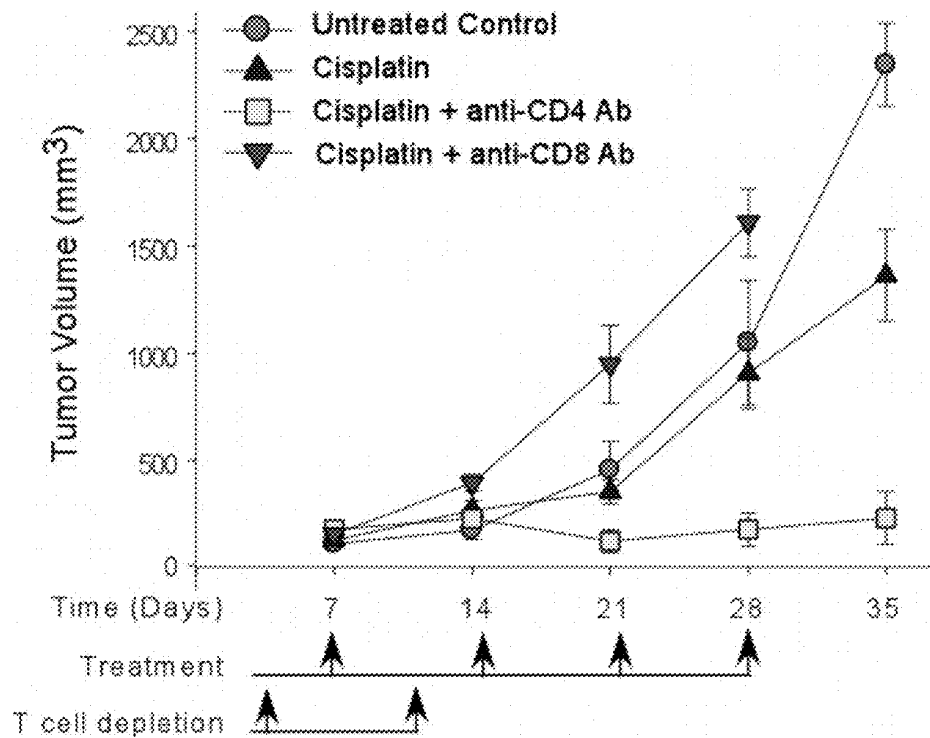


FIG. 56D

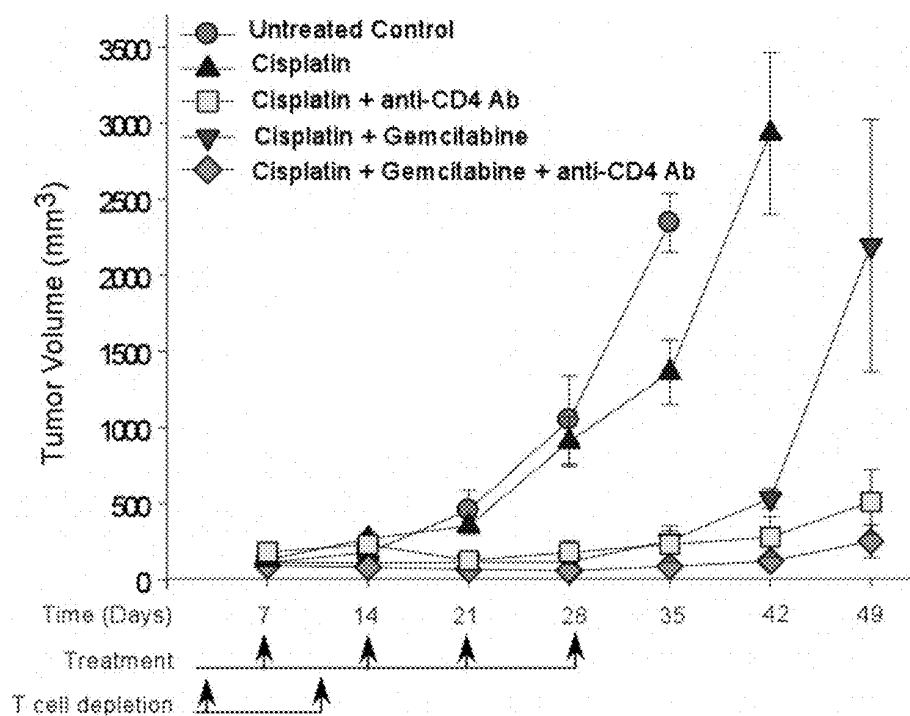


FIG. 56E

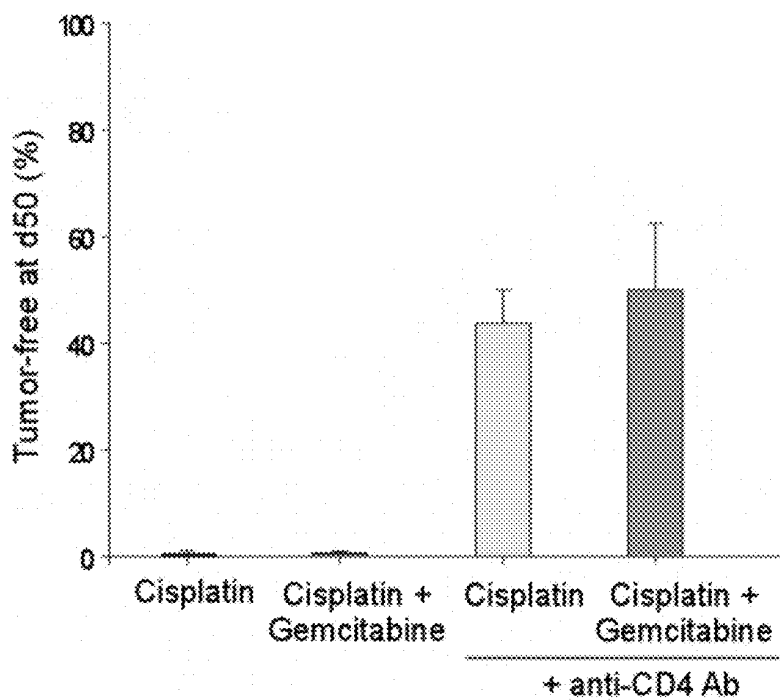


FIG. 56F

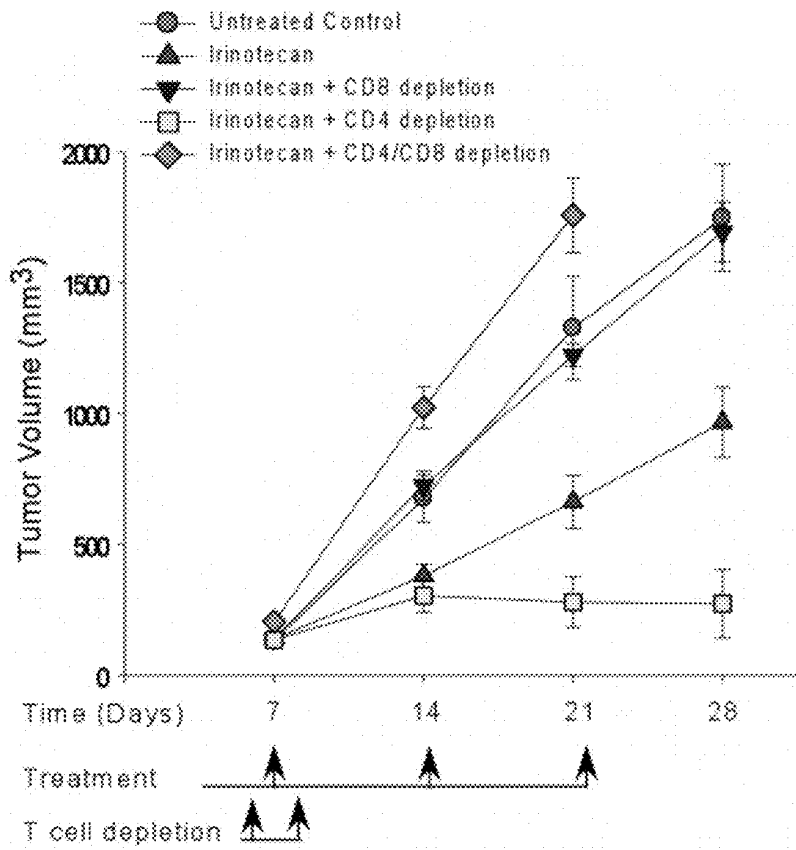


FIG. 57A

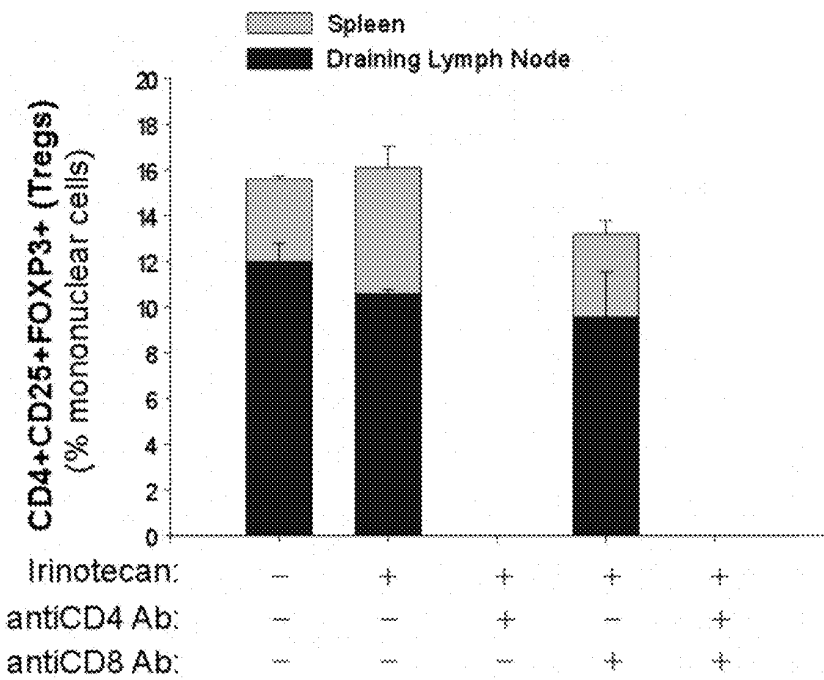


FIG. 57B

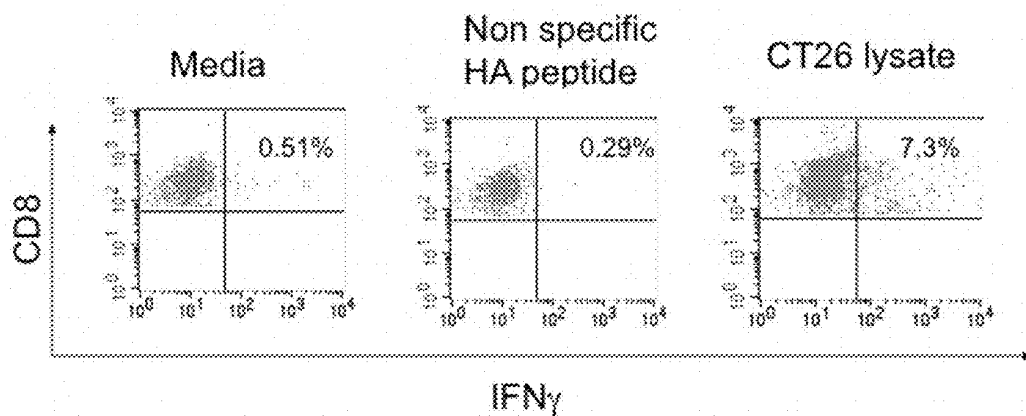


FIG. 57C

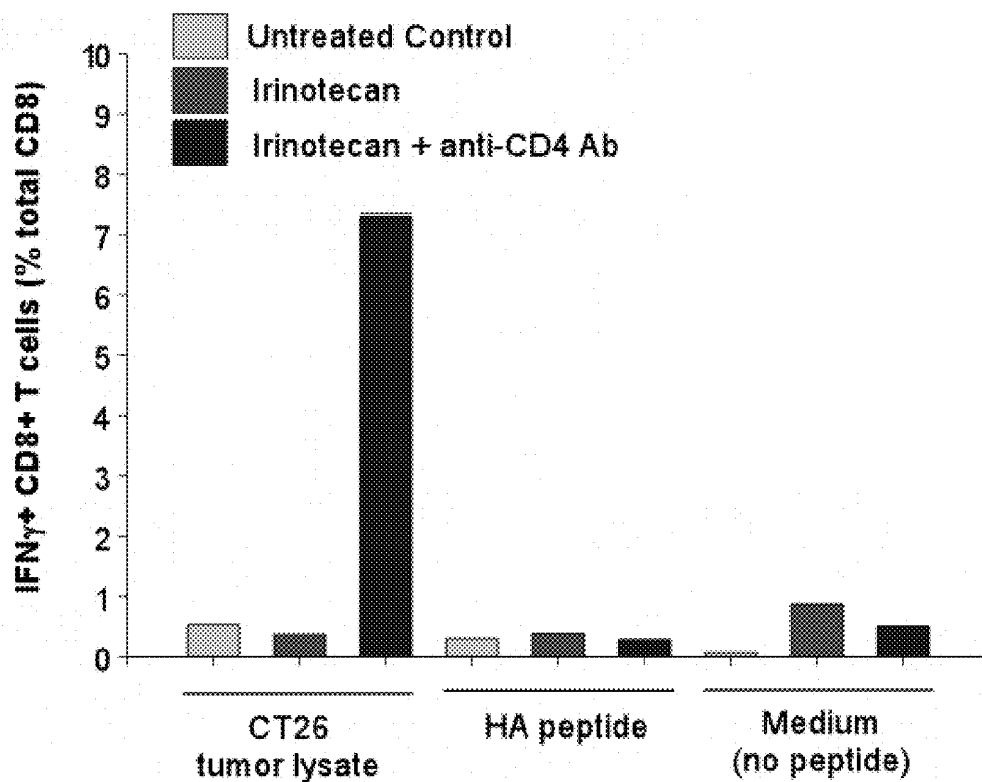


FIG. 57D

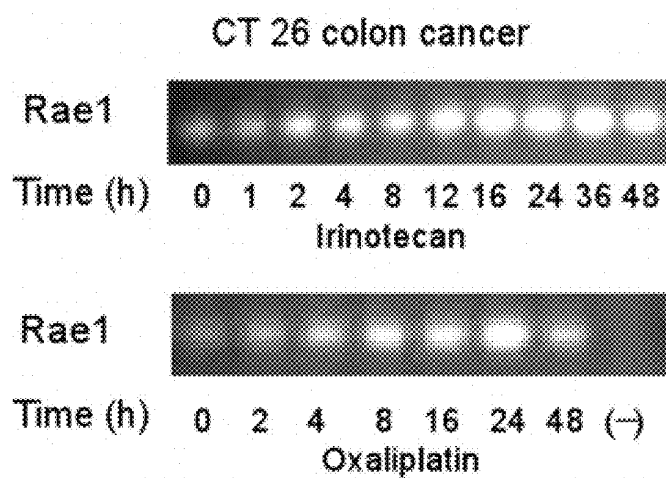


FIG. 57E

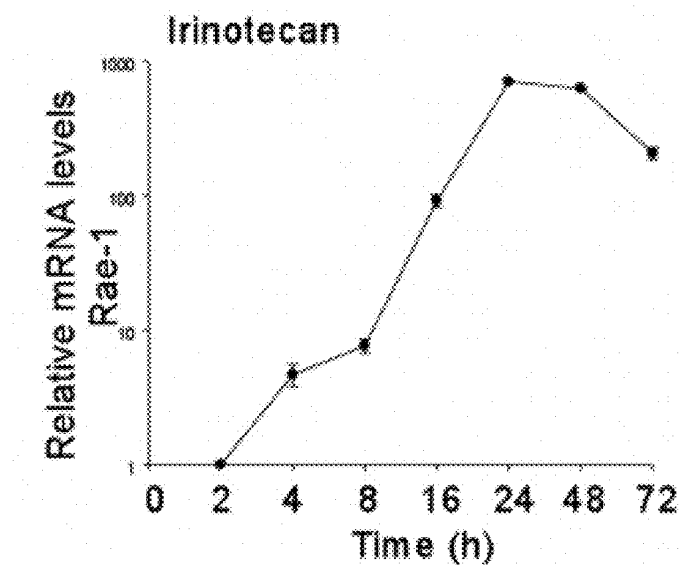


FIG. 57F

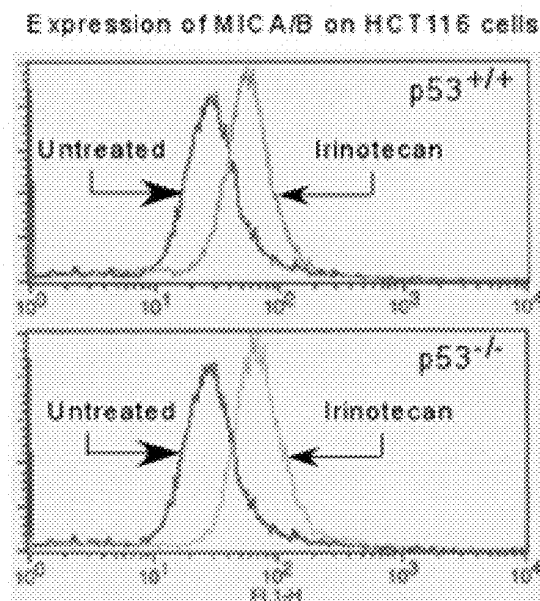


FIG. 57G

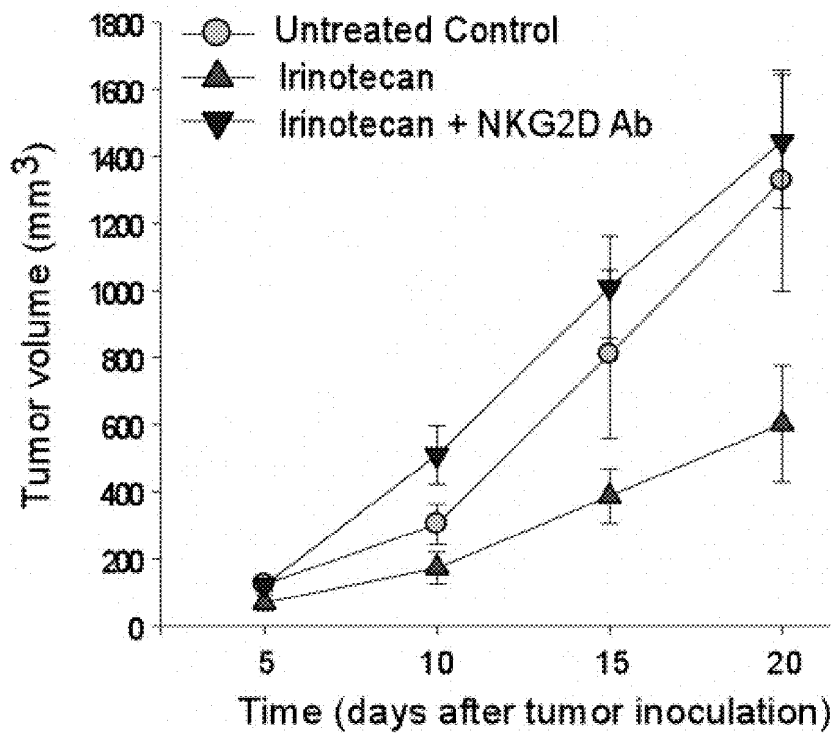


FIG. 57H

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COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation application of U.S. application Ser. No. 17/694,541 filed Mar. 14, 2022, now pending; which is a continuation application of U.S. application Ser. No. 16/601,347 filed Oct. 14, 2019, now issued as U.S. Pat. No. 11,274,156; which is a continuation application of U.S. application Ser. No. 15/362,632 filed Nov. 28, 2016, now issued as U.S. Pat. No. 10,442,860; which is a divisional application of U.S. application Ser. No. 15/231,309 filed Aug. 8, 2016, now issued as U.S. Pat. No. 9,850,306; which is a continuation application of U.S. application Ser. No. 14/645,282 filed Mar. 11, 2015, now issued as U.S. Pat. No. 9,441,044; which is a continuation application of U.S. application Ser. No. 13/582,717 filed Oct. 17, 2012, now issued as U.S. Pat. No. 8,993,524; which is a 35 USC § 371 National Stage application of International Application No. PCT/US2011/027317 filed Mar. 4, 2011, now expired; which claims the benefit under 35 USC § 119 (c) to U.S. Application Ser. No. 61/435,671 filed Jan. 24, 2011 and to U.S. Application Ser. No. 61/311,255 filed Mar. 5, 2010. The disclosure of each of the prior applications is considered part of and is incorporated by reference in the disclosure of this application.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under Grant No. CA123277 awarded by the National Institutes of Health. The government has certain rights in the invention.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

The material in the accompanying sequence listing is hereby incorporated by reference into this application. The accompanying sequence listing text file, named JHU3260-10.xml, was created on Sep. 23, 2024 and is 174,970 bytes in size.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates generally to the field of targeted immunomodulatory antibodies and fusion proteins for cancer therapy and more specifically to composition and methods for targeted immunostimulatory or immunosuppressive antibodies and fusion proteins to counteract or induce immune tolerance of cancer cells.

BACKGROUND INFORMATION

The immune system provides the human body with a means to recognize and defend itself against microorganisms and substances recognized as foreign or potentially harmful. While passive immunotherapy of cancer with monoclonal antibodies and passive transfer of T cells to attack tumor cells have demonstrated clinical efficacy, the goal of active therapeutic vaccination to induce these immune effectors and establish immunological memory

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against tumor cells has remained challenging. Several tumor-specific and tumor-associated antigens have been identified, yet these antigens are generally weakly immunogenic and tumors employ diverse mechanisms to create a tolerogenic environment that allows them to evade immunologic attack. Strategies to overcome such immune tolerance and activating robust levels of antibody and/or T cell responses hold the key to effective cancer immunotherapy.

SUMMARY OF THE INVENTION

The present invention is based on the seminal discovery that targeted immunomodulatory antibodies and fusion proteins can counteract or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. Such immunosuppressive mechanisms include immunosuppressive cytokines (for example, Transforming growth factor beta (TGF- β)) and regulatory T cells and/or immunosuppressive myeloid dendritic cells (DCs). By counteracting tumor-induced immune tolerance, the present invention provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment. The present invention provides strategies to counteract tumor-induced immune tolerance and enhance the antitumor efficacy of chemotherapy by activating and leveraging T cell-mediated adaptive antitumor immunity against resistant or disseminated cancer cells.

In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule, and the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF- β); (ii) Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2); (iii) Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL); (iv) Transforming growth factor-beta receptor (TGF- β R); (v) Programmed death-1 (PD-1); and (vi) Receptor activator of nuclear factor- κ B (RANK).

In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, or CD40.

In one aspect, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2 α receptor; IL-2 α R); (iii) cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); (iv) Interleukin-10 (IL-10); (v) Transforming growth factor-beta receptor (TGF- β R); (vi) Transforming growth factor-beta (TGF- β); (vii) Programmed Death-1 (PD-1); (viii) Programmed death-1 ligand (PD-L1 or PD-L2); (ix) Receptor activator of nuclear factor- κ B (RANK); or (x) Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL).

In one aspect, the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming

growth factor- β (TGF- β); (ii) Programmed death-1 ligand (PD-L1 or PD-L2); or (iii) Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL).

In one aspect, the immunomodulatory moiety includes a molecule that binds TGF- β . In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Transforming growth factor- β receptor TGF- β RII, TGF- β RIIb, or TGF- β RIII. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- β RII. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of TGF- β .

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- β RII. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Programmed Death-1 (PD-1), Programmed death-1 ligand 1 (PD-L1), or Programmed death-1 ligand 2 (PD-L2). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- β RII. In another aspect, the molecule includes PD-1 ectodomain, immunoglobulin Fc region, and TGF β RII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- κ B (RANK) or Receptor activator of nuclear factor- κ B ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- β RII. In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and TGF β RII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Programmed death-1 ligand 1 (PD-L1).

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to

Receptor activator of nuclear factor- κ B (RANK) or Receptor activator of nuclear factor- κ B ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Programmed Death-1 (PD-1). In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 25 or 26.

In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Receptor activator of nuclear factor- κ B ligand (RANKL). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Receptor activator of nuclear factor- κ B ligand (RANKL).

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.

In one aspect, the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the immunomodulatory moiety increases the function of PD-1.

In one aspect, the targeting moiety specifically binds to Tumor Necrosis Factor- α (TNF- α), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the targeting moiety includes an antibody that binds TNF- α , and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 37. In another aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2), and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from PD-L1. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 38 or 39.

In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25, or CD4, and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 40, 41, 42, 43, 44, or 45.

In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 46 or 47.

In one aspect, the targeting moiety includes transforming growth factor- β (TGF- β) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 48 or 49.

In one aspect, the immunomodulatory moiety includes a sequence from transforming growth factor- β (TGF- β). In an additional aspect, the immunomodulatory moiety activates the signaling function of transforming growth factor- β (TGF- β) receptor.

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor- α (TNF- α), and the immunomodulatory moiety includes a sequence from transforming growth factor- β (TGF- β). In an additional aspect, the targeting moiety includes an antibody that binds TNF- α , and the immunomodulatory moiety includes a sequence from TGF- β . In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 50. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from transforming growth factor- β (TGF- β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 51 or 52.

In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25 (IL-2 α receptor; IL-2 α R), or CD4, and the immunomodulatory moiety includes a sequence from transforming growth factor- β (TGF- β). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 53, 54, 55, 56, 57 or 58.

In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes a sequence from transforming growth factor- β (TGF- β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 59 or 60.

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor- α (TNF- α), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In an additional aspect, the targeting moiety includes an antibody that binds TNF- α , and the immunomodulatory moiety includes a sequence from an extracellular RANKL-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 61. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from an extracellular RANK-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 62 or 63.

In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

In one aspect, the targeting moiety includes a sequence from transforming growth factor- β (TGF- β) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

In one aspect, the targeting moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

In various aspects, the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

In another embodiment, the present invention provides a composition including the molecule of the invention and a cell, wherein the cell is a tumor cell, immune cell, or dendritic cell.

In another embodiment, the present invention provides a method of counteracting or overcoming immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof one or more molecule of the invention. In various aspects, the subject is administered one or more molecule of the invention in combination with another anticancer therapy. In one aspect, the anticancer therapy includes a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent or cytotoxic agent. In another aspect, the anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof an antibody that targets and depletes CD4+ regulatory T cells (Tregs) in combination with another cytotoxic anticancer therapy. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In various aspects, the cytotoxic anticancer therapy includes a chemotherapeutic molecule, tumor-targeted antibody, small molecule kinase inhibitor, hormonal agent or tumor-targeted cytotoxic agent. In another aspect, the cytotoxic anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

In another embodiment, the subject is administered one or more molecule of the invention in combination with any vaccine. In another aspect, the vaccine includes a tumor antigen, tumor-associated antigen, tumor epitope, tumor antigen-containing fusion protein, tumor cell, or dendritic cell. In another aspect, the vaccine includes a pathogen antigen, pathogen-associated antigen, pathogen epitope, or pathogen antigen-containing fusion protein.

In another embodiment, the present invention provides a method for treating immune cells wherein the cells are contacted ex vivo or in vitro with a molecule of the invention. In another embodiment, the present invention provides a method of treatment of a neoplastic disease. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

In another embodiment, the present invention provides a method of inducing or promoting immune tolerance. The

method includes administering to a subject in need thereof one or more molecule of the invention.

In another embodiment, the present invention provides a method of preventing or treating an autoimmune or inflammatory disease including administering to a subject in need thereof one or more molecule of the invention. In one aspect, the subject is administered one or more molecule of the invention in combination with another anti-inflammatory or immunosuppressive therapy. In another embodiment, the present invention provides a method of treatment of immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treating an autoimmune or inflammatory disease or preventing rejection of grafted cells or tissue. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1C show exemplary amino acid sequences of transforming growth factor beta receptor type II (TGF- β -RII) or TGF- β -RIIB or a fragment thereof, including (i) Transforming growth factor beta receptor type II (TGF- β -RII) (SEQ ID NO: 79); and (ii) Transforming growth factor beta receptor type IIB (TGF- β -RIIB) (SEQ ID NO: 80).

Also shown in FIGS. 1A-1C are exemplary truncated mutants of Transforming growth factor beta Receptor II (TGF- β -RII) or TGF- β -RIIB including the Extracellular domain (ECD) region that binds TGF- β , including (i) TGF- β R-II (Δ C terminus): TGF β RII lacking the last 38 amino acids from the C-terminus (SEQ ID NO: 81) and TGF- β R-IIB (Δ C terminus): TGF β RIIB lacking the last 38 aa from the C-terminus (SEQ ID NO: 82); (ii) TGF- β R-II (Δ cyt): TGF β RII lacking the kinase domain & juxtamembrane region (SEQ ID NO: 83) and TGF- β R-IIB (Δ cyt): TGF β RIIB lacking the kinase domain & juxtamembrane region (SEQ ID NO: 84); (iii) TGF- β R-II containing the N-terminus region including the extracellular domain (SEQ ID NO: 85) and TGF- β R-IIB containing the N-terminus region including the extracellular domain (SEQ ID NO: 86); (iv) TGF- β R-II containing the extracellular domain that binds TGF- β (SEQ ID NO: 87) and TGF- β R-IIB containing the extracellular domain that binds TGF- β (SEQ ID NO: 88); and (v) TGF- β R-II containing the region of the extracellular domain that binds TGF- β (SEQ ID NO: 89).

In addition, FIGS. 1A-1C also show exemplary kinase-deficient mutants, deletion mutants, or point mutants of Transforming growth factor beta Receptor II (TGF β -RII) or TGF β -RIIB or a fragment thereof which binds TGF- β , including (i) Transforming growth factor beta Receptor II containing point mutations-amino acid sequence of TGF- β R-II (K277R) contains a point mutation in its ATP-binding site and is inactive as a kinase (SEQ ID NO: 90); and (ii) Transforming growth factor beta Receptor II containing deletions in the amino acid sequence (deletion mutants)—Transforming growth factor beta Receptor II (Δ i)-TGF- β R-II (Δ i2) contains a deletion of amino acids 498 to 508 and is inactive as a kinase (SEQ ID NO: 91).

FIG. 2 shows exemplary fusion proteins including anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including anti-HER2/neu heavy chain+TGF β -RII ECD fusion amino acid sequence (SEQ ID NO: 1) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

FIG. 3 shows exemplary fusion proteins including anti-EGFR1 antibody and Transforming growth factor-beta

receptor II (TGF β -RII) Extracellular domain (ECD), including anti-EGFR1 heavy chain+TGF β -RII ECD fusion amino acid sequence (SEQ ID NO: 2) and anti-EGFR1 light chain amino acid sequence (SEQ ID NO: 71).

FIG. 4 shows exemplary fusion proteins including anti-CD20 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including anti-CD20 heavy chain+TGF β -RII ECD fusion amino acid sequence (SEQ ID NO: 3) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

FIG. 5 shows exemplary fusion proteins including anti-VEGF antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including anti-VEGF heavy chain+TGF β -RII ECD fusion amino acid sequence (SEQ ID NO: 4) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

FIG. 6 shows exemplary fusion proteins including anti-human CTLA-4 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including Anti-CTLA-4 heavy chain+TGF β -RII Extracellular domain fusion amino acid sequence (SEQ ID NO: 5) and Anti-CTLA-4 light chain (SEQ ID NO: 74).

FIG. 7 shows exemplary fusion proteins including IL-2, Fc, and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including IL-2+Fc+TGF β -RII Extracellular domain (SEQ ID NO: 6) and TGF β -RII Extracellular domain+Fc+IL-2 (SEQ ID NO: 7). The linker GGGGGGGSGGGGS (SEQ ID NO: 104) is optional and can be replaced with EPKSCDK (SEQ ID NO: 105) or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

FIGS. 8A-8B show exemplary fusion proteins including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including anti-CD25 (Daclizumab) heavy chain and TGF β -RII Extracellular domain (SEQ ID NO: 8) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 8A); and anti-CD25 (Basiliximab) heavy chain and TGF β -RII Extracellular domain (SEQ ID NO: 9) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76).

FIG. 9 shows exemplary fusion proteins including anti-CD4 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including anti-CD4 heavy chain and TGF β -RII Extracellular domain (SEQ ID NO: 10) and anti-CD4 light chain (SEQ ID NO: 77).

FIG. 10 shows exemplary fusion proteins including Programmed Death-1 (PD-1) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ectodomain), including PD-1 ectodomain+Fc+TGF β RII ectodomain (SEQ ID NO: 11) and TGF β RII ectodomain+Fc+PD-1 ectodomain (SEQ ID NO: 12). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

FIG. 11 shows exemplary fusion proteins including Receptor activator of nuclear factor-kB (RANK) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ectodomain), including RANK ectodomain+Fc+TGF β RII ectodomain (SEQ ID NO: 13) and TGF β RII ectodomain+Fc+RANK ectodomain (SEQ ID NO: 14). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

FIG. 12 shows exemplary immunomodulatory moiety that binds Programmed Death-1 ligand 1 (PD-L1 or B7-H1) or Programmed Death-1 ligand 2 (PD-L2 or B7-DC), including full-length PD-1 or fragment thereof (SEQ ID NO: 92),

PD-1 extracellular domain (ectodomain) or fragment thereof (SEQ ID NO: 93), and PD-1 extracellular domain (ectodomain) ligand-binding region (SEQ ID NO: 94).

FIG. 13 shows exemplary fusion proteins including anti-HER2/neu antibody and PD-1 Ectodomain, including anti-HER2/neu heavy chain+PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 15) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

FIG. 14 shows exemplary fusion proteins including anti-EGFR1 antibody and PD-1 Ectodomain, including anti-EGFR heavy chain+PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 16) and anti-EGFR light chain amino acid sequence (SEQ ID NO: 71).

FIG. 15 shows exemplary fusion proteins including anti-CD20 antibody and PD-1 Ectodomain, including anti-CD20 heavy chain+PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 17) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

FIG. 16 shows exemplary fusion proteins including anti-VEGF antibody and PD-1 Ectodomain, including anti-VEGF heavy chain+PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 18) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

FIG. 17 shows exemplary fusion proteins including anti-human CTLA-4 antibody and PD-1 Ectodomain, including anti-CTLA-4 heavy chain+PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 19) and anti-CTLA-4 light chain (SEQ ID NO: 74).

FIGS. 18A-18B show exemplary fusion proteins including anti-CD25 antibody and PD-1 Ectodomain, including anti-CD25 (Daclizumab) heavy chain and PD-1 ectodomain (SEQ ID NO: 20) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 18A), and anti-CD25 (Basiliximab) heavy chain and PD-1 ectodomain (SEQ ID NO: 21) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 18B).

FIG. 19 shows exemplary fusion proteins including IL-2, Fc, and PD-1 ectodomain, including IL-2+Fc+PD-1 ectodomain (SEQ ID NO: 22) and PD-1 ectodomain+Fc+IL-2 (SEQ ID NO: 23). The linker GGGGSGGGSGGGGS (SEQ ID NO: 104) is optional and can be replaced with EPKSCDK (SEQ ID NO: 105) or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

FIG. 20 shows exemplary fusion proteins including anti-CD4 antibody and PD-1 ectodomain, including anti-CD4 heavy chain and PD-1 ectodomain (SEQ ID NO: 24) and anti-CD4 light chain (SEQ ID NO: 77).

FIG. 21 shows exemplary fusion proteins including Receptor activator of nuclear factor-kB (RANK) Ectodomain, Fc, and PD-1 ectodomain, including RANK ectodomain+Fc+PD-1 ectodomain (SEQ ID NO: 25) and PD-1 ectodomain+Fc+RANK ectodomain (SEQ ID NO: 26). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

FIG. 22 shows exemplary immunomodulatory moiety that binds Receptor activator of nuclear factor-kB (RANK) ligand (RANKL) including full-length RANK or fragment thereof (SEQ ID NO: 95), extracellular ligand-binding domain or ectodomain of RANK (SEQ ID NO: 96), RANKL-binding sequences or residues of RANK (SEQ ID NO: 93), or RANKL-binding sequences of Osteoprotegerin (OPG) (SEQ ID NO: 98).

FIG. 23 shows exemplary fusion proteins including anti-HER2/neu antibody and RANK Ectodomain, including anti-HER2/neu heavy chain+RANK ectodomain fusion amino

acid sequence (SEQ ID NO: 27) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

FIG. 24 shows exemplary fusion proteins including anti-EGFR1 antibody and RANK Ectodomain, including anti-EGFR heavy chain+RANK ectodomain fusion amino acid sequence (SEQ ID NO: 28) and anti-EGFR light chain amino acid sequence (SEQ ID NO: 71).

FIG. 25 shows exemplary fusion proteins including anti-CD20 antibody and RANK Ectodomain, including anti-CD20 heavy chain+RANK ectodomain fusion amino acid sequence (SEQ ID NO: 29) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

FIG. 26 shows exemplary fusion proteins including anti-VEGF antibody and RANK Ectodomain, including anti-VEGF heavy chain+RANK ectodomain fusion amino acid sequence (SEQ ID NO: 30) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

FIG. 27 shows exemplary fusion proteins including anti-human CTLA-4 antibody and RANK Ectodomain, including anti-CTLA-4 heavy chain+RANK ectodomain fusion amino acid sequence (SEQ ID NO: 31) and anti-CTLA-4 light chain (SEQ ID NO: 74).

FIGS. 28A-28B show exemplary fusion proteins including anti-CD25 antibody and RANK Ectodomain, including anti-CD25 (Daclizumab) heavy chain and RANK ectodomain (SEQ ID NO: 32) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 28A), and anti-CD25 (Basiliximab) heavy chain and RANK ectodomain (SEQ ID NO: 33) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 28B).

FIG. 29 shows exemplary fusion proteins including IL-2, Fc, and RANK ectodomain, including IL-2+Fc+RANK ectodomain (SEQ ID NO: 34) and RANK ectodomain+Fc+IL-2 (SEQ ID NO: 35). The linker GGGGSGGGSGGGGS (SEQ ID NO: 104) is optional and can be replaced with EPKSCDK (SEQ ID NO: 105) or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

FIG. 30 shows exemplary fusion proteins including anti-CD4 antibody and RANK ectodomain, including anti-CD4 heavy chain and RANK ectodomain (SEQ ID NO: 36) and anti-CD4 light chain (SEQ ID NO: 77).

FIG. 31 shows exemplary immunomodulatory moiety that binds Programmed Death-1 (PD-1) including a PD-1 ligand 1 (PD-L1 or B7-H1) or PD-1 ligand 2 (PD-L2 or B7-DC) or a fragment thereof (for example, SEQ ID NO: 101), full-length human PD-1 ligand 1 (B7-H1; PDCD1L1; PD-L1; or CD274) protein or a fragment thereof (SEQ ID NO: 99), and PD-L1 extracellular binding domain (ectodomain) or fragment thereof (SEQ ID NO: 100).

FIG. 32 shows exemplary fusion proteins including anti-tumor necrosis factor (TNF α) antibody and PD-1 ligand, including anti-TNF α heavy chain+PD-1L (SEQ ID NO: 37) and anti-TNF α light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

FIG. 33 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand, including TNFR2 ECD+IgG C γ 1+PD-L1 (SEQ ID NO: 38) and PD-L1+IgG C γ 1-TNFR2 ECD (SEQ ID NO: 39).

FIG. 34 shows exemplary fusion proteins including anti-CD20 antibody and PD-1 ligand 1 (PD-L1), including anti-CD20 heavy chain+PD-L1 sequence (SEQ ID NO: 40) and anti-CD20 light chain sequence (SEQ ID NO: 72).

FIGS. 35A-35B show exemplary fusion proteins including anti-CD25 antibody and PD-1 ligand 1 (PD-L1), including anti-CD25 (Daclizumab) heavy chain and PD-L1 (SEQ ID NO: 41) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 35A), and anti-CD25 (Basiliximab) heavy chain and PD-1 ectodomain (SEQ ID NO: 42) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 35B).

FIG. 36 shows exemplary fusion proteins including IL-2, Fc, and PD-1 ligand 1 (PD-L1), including fusion protein hPD-1 ligand 1+Fc+IL-2 (SEQ ID NO: 43) and fusion protein IL-2+Fc+PD-1 ligand 1 (SEQ ID NO: 44). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

FIG. 37 shows exemplary fusion proteins including anti-CD4 antibody and PD-1 ligand 1 (PD-L1), including anti-CD4 heavy chain and PD-1 ligand 1 (PD-L1) (SEQ ID NO: 45) and anti-CD4 light chain (SEQ ID NO: 77).

FIG. 38 shows exemplary fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C γ 1), and a sequence from PD-1 ligand (PD-L1) including Oncostatin M signal peptide+CTLA-4 ECD+IgG C γ 1+PD-L1 (SEQ ID NO: 46) and PD-L1+IgG C γ 1+CTLA-4 ECD (SEQ ID NO: 47). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

FIG. 39 shows exemplary fusion proteins including a sequence of transforming growth factor- β (TGF- β), Immunoglobulin Fc (IgG C γ 1), and a sequence of PD-1 ligand (PD-L1) including TGF- β -1+Fc+PD-L1 (SEQ ID NO: 48), and PD-L1+Fc+TGF- β -1 (SEQ ID NO: 49). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

FIG. 40 shows exemplary immunomodulatory moiety that binds Transforming growth factor-beta receptor (TGF- β R) including Transforming growth factor-beta (TGF- β 1, TGF- β 2, or TGF- β 3 or a fragment thereof, TGF- β 1 full sequence (SEQ ID NO: 102), and mature (active) TGF- β 1 sequence (Ala 279-Ser 390; 112 amino acids) (SEQ ID NO: 103).

FIG. 41 shows exemplary fusion proteins including an antibody that binds TNF- α , and a sequence of transforming growth factor- β (TGF- β), including anti-TNF α heavy chain+TGF- β 1 (SEQ ID NO: 50) and anti-TNF α light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

FIG. 42 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain (TNFR2 ECD), immunoglobulin Fc (IgG C γ 1), and a sequence from transforming growth factor- β (TGF- β) including TNFR2 ECD+IgG C γ 1+TGF- β 1 (SEQ ID NO: 51), and TGF- β 1+IgG C γ 1+TNFR2 ECD (SEQ ID NO: 52).

FIG. 43 shows exemplary fusion proteins including anti-CD20 antibody and a sequence from transforming growth factor- β (TGF- β), including anti-CD20 heavy chain+mature TGF β 1 sequence (SEQ ID NO: 53) and anti-CD20 light chain sequence (SEQ ID NO: 72).

FIGS. 44A-44B show exemplary fusion proteins including anti-CD25 antibody and a sequence from transforming

growth factor- β (TGF- β), including anti-CD25 (Daclizumab) heavy chain and TGF- β 1 (SEQ ID NO: 54) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 44A), and anti-CD25 (Basiliximab) heavy chain and TGF- β 1 (SEQ ID NO: 55) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 44B).

FIG. 45 shows exemplary fusion proteins including IL-2, Fc, and a sequence from transforming growth factor- β (TGF- β), including TGF- β 1+Fc+IL-2 (SEQ ID NO: 56) and IL-2+Fc+TGF- β 1 (SEQ ID NO: 57). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

FIG. 46 shows exemplary fusion proteins including anti-CD4 antibody and a sequence from transforming growth factor- β (TGF- β), including anti-CD4 heavy chain and TGF- β (SEQ ID NO: 58) and anti-CD4 light chain (SEQ ID NO: 77).

FIG. 47 shows exemplary Fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C γ 1), and a sequence from transforming growth factor- β (TGF- β) including Oncostatin M signal peptide+CTLA-4 ECD+IgG C γ 1+TGF- β 1 (SEQ ID NO: 59), and TGF- β 1+IgG C γ 1+CTLA-4 ECD (SEQ ID NO: 60). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

FIG. 48 shows exemplary fusion proteins including an antibody that binds TNF- α , and a sequence of RANK ectodomain, including anti-TNF α heavy chain+RANK ectodomain (SEQ ID NO: 61) and anti-TNF α light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

FIG. 49 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain (TNFR2 ECD), immunoglobulin Fc (IgG C γ 1), and a sequence from RANK ectodomain including TNFR2 ECD+IgG C γ 1+RANK ectodomain (SEQ ID NO: 62), and RANK ectodomain+IgG C γ 1+TNFR2 ECD (SEQ ID NO: 63).

FIG. 50 shows exemplary Fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C γ 1), and a sequence from RANK ectodomain including Oncostatin M signal peptide+CTLA-4 ECD+IgG C γ 1+RANK ectodomain (SEQ ID NO: 64), and RANK ectodomain+IgG C γ 1+CTLA-4 ECD (SEQ ID NO: 65). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

FIG. 51 shows exemplary fusion proteins including a sequence from transforming growth factor- β (TGF- β), immunoglobulin Fc (IgG C γ 1), and a sequence from RANK ectodomain including TGF- β +IgG C γ 1+RANK ectodomain (SEQ ID NO: 66), and RANK ectodomain+IgG C γ 1+TGF- β (SEQ ID NO: 67).

FIG. 52 shows exemplary fusion proteins including a sequence from PD-1 ligand (PD-L1), immunoglobulin Fc (IgG C γ 1), and a sequence from RANK ectodomain including PD-L1+IgG C γ 1+RANK ectodomain (SEQ ID NO: 68), and RANK ectodomain+IgG C γ 1+PD-L1 (SEQ ID NO: 69).

FIGS. 53A-53G show Regulatory T cells (Treg) accumulate in the tumor microenvironment and counteract the ability of chemotherapy to activate CD8 $^{+}$ T cell-mediated

antitumor immunity. (FIG. 53A) Surface exposure of calreticulin (CRT) in response to treatment of human (SW780) and murine (MB49) cancer cells with doxorubicin (10 μ M) for 4 h. The surface exposure of CRT was determined by immunofluorescence cytometry of untreated control or doxorubicin-treated cells stained with DyLight-labeled anti-CRT antibody or an isotype control (IgG1) antibody. (FIG. 53B) Priming of tumor-reactive immune responses by MB49 tumor cells treated with doxorubicin ex vivo or in vivo. 5×10^6 MB49 cells that were pre-treated ex vivo with doxorubicin (10 μ M) for 4 h were injected into one flank of syngeneic immunocompetent C57BL/6 mice. Alternatively, C57BL/6 mice were injected with 5×10^5 live MB49 tumor cells and then administered intratumoral doxorubicin (10 μ g) at 10 d following tumor inoculation. Tumor-reactive immune responses were determined by measuring production of IFN- γ by draining lymph node (DLN) cells in response to in vitro re-challenge with either MB49 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone. (FIG. 53C) Vaccination with doxorubicin-treated tumor cells induces CD8 $^+$ T cell-mediated antitumor immunity that prevents tumor formation following re-challenge with live tumor cells. MB49 cells (5×10^6) that were pre-treated in vitro with doxorubicin (10 μ M) for 4 h were injected subcutaneously into one flank of syngeneic immunocompetent C57BL/6 mice. Naïve or vaccinated mice were challenged with untreated live MB49 tumor cells injected into the opposite flank with or without pre-treatment with an anti-CD8 antibody (Clone GK2.43) (5 μ g $\times$ 2 doses, iv) to deplete CD8 $^+$ T cells. (FIG. 53D) Delayed administration of chemotherapy in mice with pre-established tumors decreases its immunogenicity and antitumor efficacy. C57BL/6 mice were injected with 5×10^5 live syngeneic MB49 tumor cells and then administered intratumoral doxorubicin (10 μ g) at d3, d7, or d10 following tumor inoculation. (FIG. 53E) Tumors foster the accumulation of CD4 $^+$ CD25 $^+$ FoxP3 $^+$ cells (Tregs) in their microenvironment. Flow cytometric analyses of the percentage of CD4 $^+$ CD25 $^+$ FoxP3 $^+$ c $^+$ ells (Tregs) among CD4 $^+$ T lymphocytes isolated from the spleen, draining lymph nodes (DLN), and tumors of immunocompetent C57BL/6 mice at d0 and d14 after subcutaneous inoculation of 5×10^5 live MB49 tumor cells. (FIG. 53F) Tregs infiltrating the tumor microenvironment suppress priming of tumor-reactive immune responses by doxorubicin-treated tumor cells. Naïve C57BL/6 mice were vaccinated with 5×10^6 doxorubicin-killed MB49 cells with or without intravenous adoptive transfer of 5×10^6 CD4 $^+$ CD25 $^+$ cells isolated from tumors and DLN of tumor-bearing mice via immunomagnetic separation. Tumor-reactive immune responses were determined by measuring production of IFN- γ by draining lymph node (DLN) cells in response to in vitro re-challenge with either MB49 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone. (FIG. 53G) Tregs infiltrating the tumor microenvironment suppress the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death. Naïve C57BL/6 mice were vaccinated with 5×10^6 doxorubicin-killed MB49 cells (left flank) with or without pre-treatment with either an anti-CD8 antibody (Clone GK2.43) (5 μ g $\times$ 2 doses, iv) to deplete CD8 $^+$ T cells or adoptive transfer of 5×10^6 CD4 $^+$ CD25 $^+$ cells isolated from tumors and DLN of tumor-bearing mice. Protective antitumor immunity in vaccinated mice was determined by assessment of tumor growth upon challenge with untreated live MB49 tumor cells injected into the opposite flank.

FIGS. 54A-54F show inhibition of TGF- β in the tumor microenvironment reduces 'adaptive' FoxP3 $^+$ regulatory T

cells and enhances the antitumor efficacy chemotherapy. (FIG. 54A) Tumor growth results in a progressive increase in the level of serum TGF- β . Levels of TGF- β in serum of mice at d0, d14, and d28 following inoculation of 5×10^5 live MB49 tumor cells were evaluated utilizing ELISA. (FIG. 54B) Tumor cell-autonomous expression of TGF- β is the dominant source of elevated TGF- β in tumor-bearing mice. Tumor cells or draining lymph node cells isolated from either tumor-bearing mice or their tumor-free counterparts were cultured ex vivo in serum-free medium for 24 h and the amount of TGF- β /10 ^{6} cells in supernatants was measured by ELISA. (FIG. 54C) TGF β RII:Fc sequesters TGF- β in supernatants of MB49 tumor cells in a concentration-dependent manner. MB49 tumor cells were cultured in the presence of graded concentrations of TGF β RII:Fc (0-400 ng/ml) for 24 h followed by measurement of TGF- β (μ g/ml/10 ^{6} cells) in supernatants via ELISA. (FIG. 54D) TGF- β induces 'adaptive' FoxP3 $^+$ regulatory T cells in the tumor microenvironment. At 5 d following inoculation of MB49 tumor cells, mice were either left untreated (control) or treated with TGF β RII:Fc (1 μ g intratumoral; twice weekly) for 3 weeks followed by flow cytometric analyses of intracellular FoxP3 expression in CD4 $^+$ CD25 $^+$ T cells infiltrating the tumors. (FIGS. 54E, 54F). Sequestration of intratumoral TGF- β with TGF β RII:Fc reduces CD4 $^+$ CD25 $^+$ FoxP3 $^+$ Tregs in tumor tissue and improves the antitumor efficacy of doxorubicin. MB49 tumor-bearing mice were administered doxorubicin (5 mg/kg i.p. weekly $\times$ 3) with or without twice weekly treatment with TGF β RII:Fc (1 μ g intratumoral). The percentage of CD4 $^+$ CD25 $^+$ FoxP3 $^+$ cells (Tregs) among tumor cells was assessed by flow cytometry (FIG. 54E), and tumor volume was monitored to determine the effect of counteracting tumor-induced TGF- β -mediated immune tolerance on the in vivo antitumor efficacy of doxorubicin (FIG. 54F).

FIGS. 55A-55D show that anti-CD4 antibody-mediated depletion of CD4 $^+$ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive CD8 $^+$ T cells and enhances the antitumor efficacy of chemotherapy. (FIG. 55A) In vivo depletion of tumor-infiltrating CD4 $^+$ CD25 $^+$ FoxP3 $^+$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody. C57BL/6 mice injected with 5×10^5 MB49 tumor cells s.c. were left untreated (control) or administered an anti-CD4 antibody (Clone GK1.5) i.p. at 5 d and 9 d following tumor challenge. CD4 $^+$ CD25 $^+$ FoxP3 $^+$ T cells infiltrating tumors isolated from mice at d16 following tumor challenge were detected by flow cytometry. (FIG. 55B) Target-specific depletion of either CD4 $^+$ T cells, CD4 $^+$ CD25 $^+$ FoxP3 $^+$ T cells, or CD8 $^+$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody or anti-CD8 antibody. C57BL/6 mice injected s.c. with 5×10^5 MB49 tumor cells were left untreated or treated with doxorubicin (5 mg/kg i.p. weekly $\times$ 3) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Flow cytometric analyses of peripheral blood mononuclear cells isolated from mice at d16 following tumor challenge determined the percentage of CD4 $^+$ T cells or CD8 $^+$ T cells among total mononuclear cells, and the percentage of CD4 $^+$ CD25 $^+$ FoxP3 $^+$ T cells among total CD4 $^+$ T cells. (FIG. 55C) Depletion of CD4 $^+$ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive CD8 $^+$ T cells. C57BL/6 mice injected s.c. with 5×10^5 MB49 tumor cells were left untreated or treated with doxorubicin (5 mg/kg i.p. weekly $\times$ 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-

reactive immune responses were determined by flow cytometric analyses of IFN- γ expression in CD8 $^{+}$ T cells from the tumor and draining lymph node in response to in vitro stimulation with MB49 cell lysates. (FIG. 55D) Depletion of CD4 $^{+}$ regulatory T cells augments the in vivo antitumor efficacy of chemotherapy via activation of tumor-reactive CD8 $^{+}$ T cells. C57BL/6 mice injected s.c. with 5×10^5 MB49 tumor cells were left untreated or treated with doxorubicin (5 mg/kg i.p. weekly $\times 3$) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting either CD4 $^{+}$ T cells or CD8 $^{+}$ T cells on the in vivo antitumor efficacy of doxorubicin.

FIGS. 56A-56F show anti-CD4 antibody-mediated depletion of CD4 $^{+}$ regulatory T cells augments and sustains the antitumor effect of chemotherapy by enabling activation of adaptive antitumor immunity. (FIG. 56A) Surface exposure of calreticulin (CRT) in response to treatment of MB49 cancer cells with either cisplatin or the combination of cisplatin and gemcitabine for 4 h. The surface exposure of CRT was determined by immunofluorescence cytometry of untreated control or chemotherapy-treated cells stained with Dylight-labeled anti-CRT antibody or an isotype control (IgG1) antibody. (FIGS. 56B, 56C) Depletion of CD4 $^{+}$ regulatory T cells enables cisplatin-induced activation of tumor-reactive IFN- γ CD8 $^{+}$ T cells and effector memory (CD8 $^{+}$ CD62L $^{-}$) T cells. C57BL/6 mice injected s.c. with 5×10^5 MB49 tumor cells were left untreated or treated with cisplatin (0.5 mg/kg i.p. weekly $\times 4$) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN- γ expression in CD8 $^{+}$ T cells from the tumor and draining lymph node (DLN) in response to in vitro stimulation with MB49 cell lysates (FIG. 56B). The percentage of effector memory T_{EM} cells was determined by flow cytometric analyses of CD8 $^{+}$ CD62L $^{-}$ cells (FIG. 56C). (FIGS. 56D, 56E and 56F) Depletion of CD4 $^{+}$ regulatory T cells augments the in vivo antitumor efficacy of chemotherapy via activation of tumor-reactive CD8 $^{+}$ T cells. C57BL/6 mice injected s.c. with 5×10^5 MB49 tumor cells were left untreated or treated with either cisplatin (0.5 mg/kg) or the combination of cisplatin and gemcitabine (i.p. weekly $\times 4$) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting either CD4 $^{+}$ T cells or CD8 $^{+}$ T cells on the in vivo antitumor efficacy of chemotherapy and the percentage of mice exhibiting complete tumor-regression by d50 following tumor inoculation. Establishment of adaptive antitumor immunity following regression of primary tumors was determined by re-challenging mice with live MB49 tumor cells in the opposite flank.

FIGS. 57A-57H show that Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD4 $^{+}$ regulatory T cells to stimulate CD8 $^{+}$ T cell-mediated tumor regression. (FIG. 57A) Genotoxic chemotherapeutic agents induce expression of mouse NKG2D ligands (Rae-1) on cancer cells. Kinetics of the upregulation of Rae1 transcripts in mouse CT26 colon cancer cells was determined by quantitative real-time PCR following treatment with irinotecan (25 μ g/ml) or oxaliplatin (10 μ g/ml). Quantitative RT-PCR was carried out using

Rae-1 specific primers [sense, 5'-CTAGTGCCACCTGG-GAATTCA-3' (SEQ ID NO: 111); anti-sense, 5'-CATCATT-AGCTGATCTCCAGCTCA-3' (SEQ ID NO: 112)] and probe [5'-6-FAM-CATCAGTGACAGTTACTTCTT-CACCTTCTACACAGAGA-Tamra-3' (SEQ ID NO: 113)]. (FIG. 57B) Genotoxic chemotherapeutic agents induce p53-independent cell surface expression of human NKG2D ligands (MHC-I-related A and B molecules-MICA/MICB) on cancer cells. Isogenic p53-proficient (p53 $^{+/+}$) or p53-deficient (p53 $^{-/-}$) HCT116 cells were treated with irinotecan (25 μ g/ml) for 16 h or left untreated. Irinotecan-induced upregulation of cell surface expression of MICA/B was determined by flow cytometric analysis of tumor cells labeled with an anti-human MICA/B MAb (R&D Systems). (FIG. 57C) and (FIG. 57D) Induction of NKG2D ligands contributes to the antitumor effect of chemotherapy in vivo. Immunocompetent Balb/C mice injected s.c. with 2×10^5 syngeneic CT26 tumor cells were treated with irinotecan (50 mg/kg i.p. weekly $\times 3$) beginning at d5 following tumor inoculation, with or without pre-treatment with an NKG2D blocking antibody (CX5, eBioscience) (200 μ g i.p.) at 16 h before each dose of chemotherapy. Tumor volume was monitored to determine the effect of NKG2D blockade on the in vivo antitumor efficacy of irinotecan. (FIG. 57E) In vivo depletion of CD4 $^{+}$ CD25 $^{+}$ FoxP3 $^{+}$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody. Balb/C mice injected with 2×10^5 CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p. weekly $\times 3$) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. CD4 $^{+}$ CD25 $^{+}$ FoxP3 $^{+}$ T cells in spleen and draining lymph node isolated from mice at d16 following tumor challenge were detected by flow cytometry. (FIG. 57F) Depletion of CD4 $^{+}$ regulatory T cells facilitates irinotecan-induced activation of tumor-reactive IFN- γ CD8 $^{+}$ T cells. Balb/C mice injected with 2×10^5 CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p. weekly $\times 3$) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN- γ expression in CD8 $^{+}$ T cells from the tumor and draining lymph node (DLN) in response to in vitro stimulation with either CT26 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone. (FIG. 57G) and (FIG. 57H) Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD4 $^{+}$ regulatory T cells to stimulate CD8 $^{+}$ T cell-mediated tumor regression. Balb/C mice injected with 2×10^5 CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p. weekly $\times 3$) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) and/or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting CD4 $^{+}$ T cells and/or CD8 $^{+}$ T cells on the in vivo antitumor efficacy of irinotecan.

DETAILED DESCRIPTION OF THE INVENTION

Targeted immunostimulatory antibodies and/or fusion proteins for prevention or treatment of cancer: Chemotherapy is a cornerstone of systemic treatment of patients with most common types of advanced cancers. The vast majority of human cancers harbor genetic alterations and signaling mechanisms that impair the direct death signaling

pathways entrained by chemotherapeutic agents. Although chemotherapeutic agents employ diverse mechanisms to directly kill tumor cells, the present invention provides that these agents have immuno-adjuvant effects which activate innate and adaptive antitumor immune responses that are crucial for their antitumor efficacy in vivo. The present invention also provides that antitumor CD8⁺ T cells play an instrumental role in the in vivo response of tumors to diverse cytotoxic chemotherapeutic agents. Although chemotherapeutic agents can induce "immunogenic" tumor cell death and facilitate cross-presentation of antigens by dendritic cells, tumors create a tolerogenic environment that allows them to suppress the activation of innate and adaptive immune responses and evade immunologic attack by immune effector cells. The present invention provides that strategies to counteract tumor-induced immune tolerance in the tumor microenvironment can enhance the antitumor efficacy of chemotherapy by activating and leveraging T cell-mediated adaptive antitumor immunity against disseminated cancer cells.

The present invention is based on the seminal discovery that targeted immunomodulatory antibodies and fusion proteins can counteract or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. By counteracting tumor-induced immune tolerance, the present invention provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment.

The present invention provides compositions and methods for targeted immunostimulatory antibodies and fusion proteins that counteract immune tolerance in the tumor microenvironment and promote T cell-mediated adaptive antitumor immunity for maintenance of durable long-term protection against recurrent or disseminated cancers. These tumor-targeted immunostimulatory molecules are designed to facilitate effective long term T cell-mediated immune responses against tumor cells by at least one of the following:

- (i) promoting death of tumor cells via enhancement of antibody-dependent cellular cytotoxicity (ADCC);
- (ii) facilitating effective cross-presentation of tumor antigen(s) from dying tumor cells by augmenting maturation of dendritic cells (DCs); and
- (iii) increasing activation and proliferation of antitumor CD8⁺ T cells by negating immune suppression mediated by regulatory T cells and myeloid suppressor cells. These antitumor immune responses may be activated in tandem with the sensitization of tumor cells to immune effector-mediated cytotoxicity, thereby establishing a positive feedback loop that augments tumor cytorreduction and reinforces adaptive antitumor immunity. The tumor-targeted immunostimulatory monoclonal antibodies (mAbs) of the present invention provides the ability to generate and boost antitumor immunity to multiple cross-presented tumor antigens obtained from endogenous tumor cells during the course of therapy (as an in situ tumor vaccine), while simultaneously leveraging the antitumor immune response to eliminate disseminated cancer cells. Accordingly, the targeted immunostimulatory antibodies and fusion proteins of the invention can integrate the hitherto distinct fields of passive and active immunotherapy and provide a novel platform for simultaneously leveraging the synergistic

benefits of these strategies to entrain effective innate and adaptive immune responses against targeted cancers.

While passive immunotherapy of cancer with tumor-targeted monoclonal antibodies has demonstrated clinical efficacy, the goal of active therapeutic vaccination to induce T cell-mediated immunity and establish immunological memory against tumor cells has remained challenging. Several tumor-specific and tumor-associated antigens have been identified, yet tumors employ diverse mechanisms to create a tolerogenic environment that allows them to suppress the activation of a T cell-mediated antitumor immune response. The tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention are designed to overcome such immune tolerance in the tumor microenvironment and activate robust levels of T cell responses for effective cancer immunotherapy or chemo-immunotherapy. Accordingly, the tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention have broad clinical relevance for advancing the treatment of many types of human cancers.

The tumor-targeted immunostimulatory mAbs and/or fusion proteins of the invention provide their ability to generate and boost antitumor immunity to multiple cross-presented tumor antigens obtained from endogenous tumor cells during the course of therapy (as an in situ tumor vaccine), while simultaneously leveraging the antitumor immune response to eliminate disseminated cancer cells. Accordingly, the tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention can integrate the hitherto distinct fields of passive and active immunotherapy and provide a novel platform for simultaneously leveraging the synergistic benefits of these strategies to entrain effective innate and adaptive immune responses against targeted cancers. This approach of the present invention is distinguished from and superior to conventional tumor antigen-, allogeneic tumor cell- or DC-based vaccines in at least one of the following aspects: (i) There is no a priori requirement to define, clone and purify individual tumor antigens, since the patient's tumor itself is the in vivo source of antigens; (ii) Multivalent antitumor immune responses that are naturally tailored against antigens from the patient's own tumor are less likely to allow immune escape than a pre-selected tumor antigen; (iii) The activation of antitumor immune responses by the immuno-adjuvant effects of tumor-targeted immunostimulatory antibodies or fusion proteins occurs in tandem with the sensitization of tumor cells to immune effector-mediated cytotoxicity, thereby establishing a positive feedback loop that augments tumor cytorreduction and reinforces adaptive antitumor immunity; and (iv) The molecules of the invention have broad clinical relevance for advancing the treatment of many types of human cancers.

In addition, the targeted immunostimulatory antibodies and/or fusion proteins of the invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) to counteract immune tolerance in the tumor microenvironment and promote T cell-mediated adaptive antitumor immunity for maintenance of long-term protection against recurrent or disseminated cancers (for prevention or treatment of diverse cancers); (ii) to produce immune cell compositions for adoptive cellular therapy of diverse cancers; and (iii) to serve as immune adjuvants or vaccines for prophylaxis of diverse cancers or infectious diseases.

The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to disrupt immunosuppressive networks in the tumor microenviron-

ment. Tumors employ a wide array of regulatory mechanisms to avoid or suppress the immune response. Cancer cells actively promote immune tolerance in the tumor microenvironment via the expression of cytokines and molecules that inhibit the differentiation and maturation of antigen-presenting dendritic cells. The immunosuppressive cytokines and ligands produced by tumor cells include the following: (i) Transforming growth factor-beta (TGF- β); (ii) Programmed death-1 ligand 1 (PD-L1; B7-H1); (iii) Vascular endothelial growth factor (VEGF); and (iv) Interleukin-10 (IL-10). In addition to blocking dendritic cell (DC) maturation, these molecules promote the development of specialized subsets of immunosuppressive CD4⁺ T cells (regulatory T cells; Treg cells) and myeloid-derived suppressor cells (MDSC). Tregs are a minority sub-population of CD4⁺ T cells that constitutively express CD25 [the interleukin-2 (IL-2) receptor α -chain] and the forkhead box P3 (FOXP3) transcription factor. Tregs (CD4+CD25+FoxP3+ cells) maintain immune tolerance by restraining the activation, proliferation, and effector functions of a wide range of immune cells, including CD4⁺ and CD8⁺ T cells, natural killer (NK) and NKT cells, B cells and antigen presenting cells (APCs) in vitro and in vivo. The accumulation of Treg cells in the tumor microenvironment reinforces tumor immune tolerance and facilitates tumor progression and metastases. The increased expression of immunosuppressive cytokines (TGF- β ; PD-L1) and tumor-infiltrating Tregs is correlated with a reduction of survival of patients with diverse types of cancers. The present invention provides that tumor-induced immune tolerance mediated via Tregs is a crucial determinant of the resistance of cancers to cytotoxic chemotherapeutic agents and tumor-targeted antibodies. The targeted immunostimulatory antibodies and/or fusion proteins of the invention inhibit key immunosuppressive molecules expressed by the targeted tumor cell or tumor-infiltrating Treg cells and myeloid suppressor cells (DCs or MDSC). As such, they provide the targeted ability to inhibit the development or function of Tregs within the tumor microenvironment. In another aspect, they provide the ability to counteract Treg-induced immune suppression in the tumor microenvironment.

The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to inhibit the development or function of Tregs and myeloid suppressor cells (DCs or MDSC) within the tumor microenvironment. Tregs (CD4+CD25+FoxP3+ cells) express an array of immunosuppressive cytokines and molecules which act in concert to induce immune tolerance and promote tumor progression and metastases. These include: (i) Cytotoxic T-lymphocyte associated protein 4 (CTLA-4; CD152), a co-inhibitory receptor that binds to the ligands CD80 (B7-1) or CD86 (B7-2) on the antigen presenting cell (APC) and inhibits co-stimulation of T cells; (ii) Programmed death-1 ligand 1 (PD-L1; B7-H1), a ligand which engages the co-inhibitory receptor Programmed death-1 (PD-1) and inhibits T cell activation and proliferation. (iii) Transforming growth factor-beta (TGF- β), a cytokine which regulates immune responses by restricting the maturation and antigen-presenting function of dendritic cells, inhibiting the proliferation and activation of naive T cells, suppressing the expression of cytotoxic molecules (Granzyme A/B, FasL, Apo2L/TRAIL, IFN- γ) in immune effector cells, and promoting the development and function of Tregs; (iv) Receptor activator of nuclear factor- κ B ligand (RANKL), a ligand which engages Receptor activator of nuclear factor- κ B (RANK) and promotes osteoclast differentiation, Treg development, and tumor metastases. In addition, Tregs

express other surface molecules; (v) LAG-3, a CD4-related molecule that binds MHC class II; (vi) glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); and (vii) IL-10. The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to bind a targeted molecule expressed by Tregs or myeloid suppressor cells while concurrently sequestering and inhibiting one or more immunosuppressive molecule that promotes their development, survival or function. In one aspect, the targeted immunostimulatory antibodies and/or fusion proteins directly deplete the number of Tregs.

In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule on the tumor cell or tumor microenvironment (tumor stroma, tumor vasculature, or tumor infiltrating immune cell), and the immunomodulatory moiety specifically binds an immunosuppressive molecule expressed by the targeted tumor cell or tumor-infiltrating Treg cells and myeloid suppressor cells (DC or MDSC).

In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule expressed by Treg cells, myeloid suppressor cells (MDSC), or dendritic cells (DC), and the immunomodulatory moiety specifically binds an immunosuppressive molecule that promotes their development, survival or function.

In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule, and the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF- β); (ii) Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2); (iii) Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL); (iv) Vascular endothelial growth factor (VEGF); (v) Transforming growth factor-beta receptor (TGF- β R); (vi) Programmed death-1 (PD-1); and (vii) Receptor activator of nuclear factor- κ B (RANK).

In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, or CD40.

In one aspect, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2 α receptor; IL-2 α R); (iii) cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); (iv) Interleukin-10 (IL-10); (v) Transforming growth factor-beta receptor (TGF- β R); (vi) Transforming growth factor-beta (TGF- β); (vii) Programmed Death-1 (PD-1); (viii) Programmed death-1 ligand (PD-L1 or PD-L2); (ix) Receptor activator of nuclear factor- κ B (RANK); (x) Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL); (xi) LAG-3; or (xii) glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18).

In one aspect, the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF- β); (ii) Programmed death-1 ligand (PD-L1 or PD-L2); (iii) Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL); or (iv) vascular endothelial growth factor (VEGF).

In one aspect, the immunomodulatory moiety includes a molecule that binds TGF- β . In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF- β RII, TGF- β RIIb, or TGF- β RIII. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- β RII. In another aspect, the molecule includes a TGF- β -binding amino acid sequence corresponding to SEQ ID NOs: 79-91. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of TGF- β .

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- β RII. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Programmed Death-1 (PD-1), Programmed death-1 ligand 1 (PD-L1), or Programmed death-1 ligand 2 (PD-L2). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- β RII. In another aspect, the molecule includes PD-1 ectodomain, immunoglobulin Fc region, and TGF β RII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- κ B (RANK) or Receptor activator of nuclear factor- κ B ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- β RII. In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and TGF β RII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes a PD-L1-binding amino acid sequence corresponding to SEQ ID NO: 92, 93, or 94. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Programmed death-1 ligand 1 (PD-L1).

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4. In an additional

aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- κ B (RANK) or Receptor activator of nuclear factor- κ B ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Programmed Death-1 (PD-1). In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 25 or 26.

In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Receptor activator of nuclear factor- κ B ligand (RANKL). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes a RANKL-binding amino acid sequence corresponding to SEQ ID NO: 95, 96, 97, or 98. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Receptor activator of nuclear factor- κ B ligand (RANKL).

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.

The present invention provides novel targeted immunosuppressive antibodies and fusion proteins that induce or promote immune tolerance by at least one of the following:

- (i) inhibiting the activation of dendritic cells, T cells, and/or B cells; and
- (ii) promoting the development and/or suppressor function of regulatory T cells and immunosuppressive myeloid DCs. These targeted immunosuppressive molecules of the invention are designed to suppress unwanted or excessive immune or inflammatory responses in order to treat autoimmune or inflammatory diseases or prevent the rejection of a transplanted cell, tissue, or organ.

Targeted immunosuppressive antibodies and/or fusion proteins: The aberrant activation of self-reactive T cells and/or breakdown of the mechanisms of immune tolerance promotes the development of autoimmunity that results in various diseases including type I diabetes mellitus, multiple sclerosis, systemic lupus erythematosus, inflammatory bowel disease, and rheumatoid arthritis. The targeted immunosuppressive antibodies and/or fusion proteins of the invention are designed to suppress unwanted or excessive immune or inflammatory responses and restore or promote immune tolerance. Accordingly, the compositions and methods of the invention have broad clinical relevance for the treatment of diverse autoimmune or inflammatory diseases and preventing the rejection of a transplanted cell, tissue, or organ grafts.

The targeted immunosuppressive antibodies and/or fusion proteins of the invention provides their ability to inhibit the activity of targeted pro-inflammatory cytokines or immune cells while simultaneously promoting immune tolerance via the targeted delivery of immunosuppressive molecules that facilitate the development and/or function of regulatory T cells. These molecules of the present invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) The molecules of the invention enable targeted delivery of immunosuppressive molecules to immune cells or pro-inflammatory molecules in the milieu of the affected cell, tissue or organ; (ii) The molecules of the invention can couple the inhibition of the targeted pro-inflammatory molecule or immune cell with the simultaneous delivery of an immunosuppressive molecule that promotes immune tolerance, thereby improving the suppression of immune effector cells; and (iii) The molecules of the invention can provide a mechanism of simultaneously engaging two independent or synergistic mechanisms of immune tolerance or immune suppression.

Further, the targeted immunosuppressive antibodies and/or fusion proteins of the invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) To suppress unwanted or excessive immune or inflammatory responses in order to treat autoimmune or inflammatory diseases; and (ii) To prevent the rejection of a transplanted cell, tissue, or organ grafts.

In one aspect, the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes a PD-1-binding amino acid sequence corresponding to SEQ ID NO: 99, 100, or 101. In an additional aspect, the immunomodulatory moiety increases the function of PD-1.

In one aspect, the targeting moiety specifically binds to Tumor Necrosis Factor- α (TNF- α), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the targeting moiety includes an antibody that binds TNF- α , and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 37. In another aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2), and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from PD-L1. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 38 or 39.

In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25, or CD4, and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 40, 41, 42, 43, 44, or 45.

In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cy1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 46 or 47.

In one aspect, the targeting moiety includes transforming growth factor- β (TGF- β) and immunoglobulin Fc region (IgG Cy1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 48 or 49.

In one aspect, the immunomodulatory moiety includes a sequence from transforming growth factor- β (TGF- β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 99, 100, or 101. In an additional aspect, the immunomodulatory moiety activates the signaling function of transforming growth factor- β (TGF- β) receptor.

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor- α (TNF- α), and the immunomodulatory moiety includes a sequence from transforming growth factor- β (TGF- β). In an additional aspect, the targeting moiety includes an antibody that binds TNF- α , and the immunomodulatory moiety includes a sequence from TGF- β . In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 50. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from transforming growth factor- β (TGF- β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 51 or 52.

In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25 (IL-2 α receptor; IL-2 α R), or CD4, and the immunomodulatory moiety includes a sequence from transforming growth factor- β (TGF- β). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 53, 54, 55, 56, 57 or 58.

In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cy1), and the immunomodulatory moiety includes a sequence from transforming growth factor- β (TGF- β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 59 or 60.

In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor- α (TNF- α), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK) or Osteoprotegerin (OPG). In an additional aspect, the targeting moiety includes an antibody that binds TNF- α , and the immunomodulatory moiety includes a sequence from an extracellular RANKL-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 61. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from an extracellular RANK-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 62 or 63.

In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cy1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK) or Osteo-

protegerin (OPG). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

In one aspect, the targeting moiety includes a sequence from transforming growth factor- β (TGF- β) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

In one aspect, the targeting moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

In various aspects, the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

In another embodiment, the present invention provides a composition including the molecule of the invention and a cell, wherein the cell is a tumor cell, immune cell, or dendritic cell.

In another embodiment, the present invention provides a method of counteracting or overcoming immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. In one aspect, the neoplastic disease is a non-T cell malignancy which does not express CD4 on the tumor cell. In one embodiment, the method includes administration to a subject in need thereof an antibody that targets and depletes CD4⁺ regulatory T cells (Tregs) in combination with a cytotoxic anticancer therapy. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In various aspects, the cytotoxic anticancer therapy includes a chemotherapeutic molecule, tumor-targeted antibody, small molecule kinase inhibitor, hormonal agent, or tumor-targeted cytotoxic agent, anti-angiogenic agent or any combination thereof. In another aspect, the cytotoxic anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

In another embodiment, the method includes administration to a subject in need thereof an antibody or molecule that targets and depletes CD4⁺ regulatory T cells (Tregs) in combination with an immunostimulatory antibody, fusion protein, peptide or ligand that targets CTLA-4, PD1, PD-1L, RANKL, TGF- β , GITR, 4-1BB, OX-40, or Toll-like receptors (TLR 1-10). In one aspect, the TLR-agonist comprises an activator of TLR-8 or TLR-9. In one aspect, the TLR agonist comprises an immunostimulatory nucleic acid sequence containing CpG nucleotides. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody.

In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof one or more molecule of the invention. In various aspects, the subject is administered one or more molecule of the invention in combination with another anticancer therapy. In one aspect, the anticancer therapy includes a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent, cytotoxic agent, targeted therapeutic agent, or anti-angiogenic agent. In another aspect, the anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

In another aspect, the subject is administered one or more molecule of the invention in combination with an antibody or molecule that inhibits the production or function of regulatory T cells (Tregs) or depletes the number of Tregs.

In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In another aspect, the molecule that counteracts the function of Tregs is an antibody, fusion protein, peptide or ligand that targets CTLA-4, PD1, PD-1L, RANKL, TGF- β , GITR. In another aspect, the molecule that counteracts the function of Tregs is an antibody or fusion protein or ligand that targets 4-1BB or OX-40. In another aspect, the molecule that counteracts the function of Tregs is an agonist of Toll-like receptors (TLR 1-10). In one aspect, the TLR-agonist comprises an activator of TLR-8 or TLR-9. In one aspect, the TLR agonist is an immunostimulatory nucleic acid sequence containing CpG nucleotides.

In one aspect the chemotherapeutic agent is a topoisomerase-interacting agent, anthracycline, doxorubicin, mitoxantrone, camptothecin, camptothecin analogue, irinotecan, epipodophylotoxin, etoposide, alkylating agent, cyclophosphamide, cisplatin, cisplatin analogue, oxaliplatin, antimetabolite, fluoropyrimidine analogue, 5-fluorouracil, gemcitabine, azacytidine, antimicrotubule agent, taxane, paclitaxel, or docetaxel.

In another embodiment, the subject is administered one or more molecule of the invention in combination with any vaccine. In another aspect, the vaccine includes a tumor antigen, tumor-associated antigen, tumor epitope, tumor antigen-containing fusion protein, tumor cell, or dendritic cell. In another aspect, the vaccine includes a pathogen antigen, pathogen-associated antigen, pathogen epitope, or pathogen antigen-containing fusion protein. In one aspect, the vaccine includes a surrogate CD4⁺ T cell helper epitope from tetanus toxin. In one aspect, the CD4⁺ T helper sequence contains a domain of tetanus toxin fragment C (pDOM1). In one aspect, the pDOM sequence is fused to a cell-permeabilizing cationic polypeptide (e.g., Arginine-9). In another aspect, the Arg9-pDOM sequence is fused to a specific antigen comprising the vaccine.

In another embodiment, the present invention provides a method for treating immune cells wherein the cells are contacted ex vivo or in vitro with a molecule of the invention. In another embodiment, the present invention provides a method of treatment of a neoplastic disease. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

In another embodiment, the present invention provides a method of inducing or promoting immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

In another embodiment, the present invention provides a method of preventing or treating an autoimmune or inflammatory disease including administering to a subject in need thereof one or more molecule of the invention. In one aspect, the subject is administered one or more molecule of the invention in combination with another anti-inflammatory or immunosuppressive therapy. In another embodiment, the present invention provides a method of treatment of immune cells wherein the cells are contacted ex vivo or in vitro with a molecule of the invention. In another embodiment, the present invention provides a method of treating an autoimmune or inflammatory disease or preventing rejection of grafted cells or tissue. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. Thus, for example, references to “the method” includes one or more methods, and/or steps of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described.

As used herein “immune cells” or “immune effector cells” include T lymphocytes, B lymphocytes, natural killer (NK) cells, NKT cells, monocytes, macrophages, dendritic cells (DC), antigen presenting cells (APC).

As used herein, “neoplasm” or “tumor” including grammatical variations thereof, means new and abnormal growth of tissue, which may be benign or cancerous. In a related aspect, the neoplasm is indicative of a neoplastic disease or disorder, including but not limited, to various cancers. For example, such cancers can include prostate, pancreatic, biliary, colon, rectal, liver, kidney, lung, testicular, breast, ovarian, pancreatic, brain, and head and neck cancers, melanoma, sarcoma, multiple myeloma, leukemia, lymphoma, and the like.

As used herein, “subject,” including grammatical variations thereof, means a human or vertebrate animal including a dog, cat, horse, cow, pig, sheep, goat, chicken, monkey, rat, and mouse.

As used herein, “targeting moiety” refers to a molecule that has the ability to localize and bind to a specific molecule or cellular component. The targeting moiety can be an antibody, antibody fragment, scFv, Fc-containing polypeptide, fusion antibody, polypeptide, peptide, aptamer, ligand, nucleic acid, or any combination thereof. In one embodiment, a targeting moiety can bind to a molecule present in a cell or tissue. In one aspect, the targeting moiety can bind a molecule in a diseased cell or tissue, such as a cancer cell or tumor. In, another aspect, the targeting molecule can bind a normal cell or tissue, such as an immune cell. In another aspect, the targeting moiety can bind a cellular or extracellular molecule that modulates the immune response. In another aspect, the targeting moiety binds a growth factor receptor, growth factor, cytokine receptor, cytokine, or cell surface molecule.

In another embodiment, the targeting moiety is a tumor-targeting moiety. The tumor-targeting moiety can bind a component of a tumor cell or bind in the vicinity of a tumor cell (e.g., tumor vasculature or tumor microenvironment). In one embodiment, the tumor targeting moiety binds to a component of a tumor cell, tumor microenvironment, tumor vasculature, tumor-associated lymphocyte, tumor antigen, tumor-associated antigen, tumor cell surface molecule, tumor antigenic determinant, tumor antigen-containing fusion protein, tumor-associated cell, tumor-associated immune cell, or tumor vaccine.

For example, in various embodiments, a targeting moiety is specific for or binds to a molecule or component, which includes but is not limited to, epidermal growth factor

receptor (EGFR, EGFR1, ErbB-1, HER1), ErbB-2 (HER2/neu), ErbB-3/HER3, ErbB-4/HER4, EGFR ligand family; insulin-like growth factor receptor (IGFR) family, IGF-binding proteins (IGFBPs), IGFR ligand family (IGF-1R); platelet derived growth factor receptor (PDGFR) family, PDGFR ligand family; fibroblast growth factor receptor (FGFR) family, FGFR ligand family, vascular endothelial growth factor receptor (VEGFR) family, VEGF family; HGF receptor family; TRK receptor family; ephrin (EPH) receptor family; AXL receptor family; leukocyte tyrosine kinase (LTK) receptor family; TIE receptor family, angiopoietin 1, 2; receptor tyrosine kinase-like orphan receptor (ROR) receptor family; discoidin domain receptor (DDR) family; RET receptor family; KLG receptor family; RYK receptor family; MuSK receptor family; Transforming growth factor alpha (TGF- α), TGF- α receptor; Transforming growth factor-beta (TGF- β), TGF- β receptor; Interleukin 13 receptor alpha2 chain (IL13Ralpha2), Interleukin-6 (IL-6), IL-6 receptor, Interleukin-4, IL-4 receptor, Cytokine receptors, Class I (hematopoietin family) and Class II (interferon/IL-10 family) receptors, tumor necrosis factor (TNF) family, TNF- α , tumor necrosis factor (TNF) receptor superfamily (TNFRSF), death receptor family, TRAIL-receptor; cancer-testis (CT) antigens, lineage-specific antigens, differentiation antigens, alpha-actinin-4, ARTC1, breakpoint cluster region-Abelson (Bcr-abl) fusion products, B-RAF, caspase-5 (CASP-5), caspase-8 (CASP-8), beta-catenin (CTNNB1), cell division cycle 27 (CDC27), cyclin-dependent kinase 4 (CDK4), CDKN2A, COA-1, dek-can fusion protein, EFTUD-2, Elongation factor 2 (ELF2), Ets variant gene 6/acute myeloid leukemia 1 gene ETS (ETC6-AML1) fusion protein, fibronectin (FN), GPNMB, low density lipid receptor/GDP-L fucose: beta-Dgalactose 2-alpha-L-fucosyltransferase (LDLR/FUT) fusion protein, HLA-A2, arginine to isoleucine exchange at residue 170 of the alpha-helix of the alpha2-domain in the HLA-A2 gene (HLA-A*201-R170I), MLA-A11, heat shock protein 70-2 mutated (HSP70-2M), KIAA0205, MART2, melanoma ubiquitous mutated 1, 2, 3 (MUM-1, 2, 3), prostatic acid phosphatase (PAP), neo-PAP, Myosin class 1, NFYC, OGT, OS-9, pml-RARalpha fusion protein, PRDX5, PTPRK, K-ras (KRAS2), N-ras (NRAS), HRAS, RBAF600, SIRT2, SNRPD1, SYT-SSX1 or -SSX2 fusion protein, Triosephosphate Isomerase, BAGE, BAGE-1, BAGE-2,3,4,5, GAGE-1,2,3,4,5,6,7,8, GnT-V (aberrant N-acetyl glucosaminyl transferase V, MGAT5), HERV-K-MEL, KK-LC, KM-HN-1, LAGE, LAGE-1, CTL-recognized antigen on melanoma (CAMEL), MAGE-A1 (MAGE-1), MAGE-A2, MAGE-A3, MAGE-A4, MAGE-AS, MAGE-A6, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-3, MAGE-B1, MAGE-B2, MAGE-B5, MAGE-B6, MAGE-C1, MAGE-C2, mucin 1 (MUC1), MART-1/Melan-A (MLANA), gp100, gp100/Pme117 (SILV), tyrosinase (TYR), TRP-1, HAGE, NA-88, NY-ESO-1, NY-ESO-1/LAGE-2, SAGE, Sp17, SSX-1,2,3,4, TRP2-1NT2, carcinoembryonic antigen (CEA), Kallikrein 4, mammaglobin-A, OA1, prostate specific antigen (PSA), prostate specific membrane antigen, TRP-1/gp75, TRP-2, adipophilin, interferon inducible protein absent in melanoma 2 (AIM-2), BING-4, CPSF, cyclin D1, epithelial cell adhesion molecule (Ep-CAM), EpcA3, fibroblast growth factor-5 (FGF-5), glycoprotein 250 (gp250) intestinal carboxyl esterase (iCE), alpha-feto protein (AFP), M-CSF, mdm-2, MUC1, p53 (TP53), PBF, FRAME, PSMA, RAGE-1, RNF43, RU2AS, SOX10, STEAP1, survivin (BIRC5), human telomerase reverse transcriptase (hTERT), telomerase, Wilms' tumor gene (WT1), SYCP1, BRDT, SPANX, XAGE, ADAM2,

PAGE-5, LIP1, CTAGE-1, CSAGE, MMA1, CAGE, BORIS, HOM-TES-85, AF15q14, HCA661, LDHC, MORC, SGY-1, SPO11, TPX1, NY-SAR-35, FTHL17, NXF2 TDRD1, TEX 15, FATE, TPTE, immunoglobulin idiotypes, Bence-Jones protein, estrogen receptors (ER), androgen receptors (AR), CD40, CD30, CD20, CD19, CD33, CD4, CD25, CD3, cancer antigen 72-4 (CA 72-4), cancer antigen 15-3 (CA 15-3), cancer antigen 27-29 (CA 27-29), cancer antigen 125 (CA 125), cancer antigen 19-9 (CA 19-9), beta-human chorionic gonadotropin, 1-2 microglobulin, squamous cell carcinoma antigen, neuron-specific enolase, heat shock protein gp96, GM2, sargramostim, CTLA-4, 707 alanine proline (707-AP), adenocarcinoma antigen recognized by T cells 4 (ART-4), carcinoembryonic antigen peptide-1 (CAP-1), calcium-activated chloride channel-2 (CLCA2), cyclophilin B (Cyp-B), human signet ring tumor-2 (HST-2), Human papilloma virus (HPV) proteins (HPV-E6, HPV-E7, major or minor capsid antigens, others), Epstein-Barr virus (EBV) proteins (EBV latent membrane proteins—LMP1, LMP2; others), Hepatitis B or C virus proteins, and HIV proteins. A composition of the invention can further include the foregoing as a peptide/polypeptide and/or encoding the same.

In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, CD40, or a combination thereof.

Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as trastuzumab (anti-HER2/neu antibody); Pertuzumab (anti-HER2 mAb); cetuximab (chimeric monoclonal antibody to epidermal growth factor receptor EGFR); panitumumab (anti-EGFR antibody); nimotuzumab (anti-EGFR antibody); Zalutumumab (anti-EGFR mAb); Necitumumab (anti-EGFR mAb); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-447 (humanized anti-EGF receptor bispecific antibody); Rituximab (chimeric murine/human anti-CD20 mAb); Obinutuzumab (anti-CD20 mAb); Ofatumumab (anti-CD20 mAb); Tositumumab-I131 (anti-CD20 mAb); Ibritumomab tiuxetan (anti-CD20 mAb); Bevacizumab (anti-VEGF mAb); Ramucirumab (anti-VEGFR2 mAb); Ranibizumab (anti-VEGF mAb); Aflibercept (extracellular domains of VEGFR1 and VEGFR2 fused to IgG1 Fc); AMG386 (angiopoietin-1 and -2 binding peptide fused to IgG1 Fc); Dalotuzumab (anti-IGF-1R mAb); Gemtuzumab ozogamicin (anti-CD33 mAb); Alemtuzumab (anti-Campath-1/CD52 mAb); Brentuximab vedotin (anti-CD30 mAb); Catumaxomab (bispecific mAb that targets epithelial cell adhesion molecule and CD3); Naptumomab (anti-5T4 mAb); Girentuximab (anti-Carbonic anhydrase ix); or Faretuzumab (anti-folate receptor). Other examples include antibodies such as Panorex™ (17-1A) (murine monoclonal antibody); Panorex (@ (17-1A) (chimeric murine monoclonal antibody); BEC2 (ami-idiotypic mAb, mimics the GD epitope) (with BCG); Oncolym (Lym-1 monoclonal antibody); SMART M195 Ab, humanized 13' 1 LYM-1 (Oncolym), Ovarex (B43.13, anti-idiotypic mouse mAb);

3622W94 mAb that binds to EGP40 (17-1A) pancarcinoma antigen on adenocarcinomas; Zenapax (SMART Anti-Tac (IL-2 receptor); SMART M195 Ab, humanized Ab, humanized); NovoMab-G2 (pancarcinoma specific Ab); TNT (chimeric mAb to histone antigens); TNT (chimeric mAb to histone antigens); Gliomab-H (Monoclonals—Humanized Abs); GNI-250 Mab; EMD-72000 (chimeric-EGF antagonist); LymphoCide (humanized IL-2 antibody); and MDX-260 bispecific, targets GD-2, ANA Ab, SMART IDIO Ab, SMART ABL 364 Ab or ImmuRAIT-CEA. Examples of antibodies include those disclosed in U.S. Pat. Nos. 5,736, 167, 7,060,808, and 5,821,337.

In one embodiment, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2 α receptor; IL-2 α R); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF- β R); Transforming growth factor-beta (TGF- β); Programmed Death-1 (PD-1); Programmed death-1 ligand (PD-L1 or PD-L2); Receptor activator of nuclear factor- κ B (RANK); Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R). In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

In one aspect, the targeting moiety binds a specific cytokine, cytokine receptor, co-stimulatory molecule, co-inhibitory molecule, or immunomodulatory receptor that modulates the immune system. In another aspect, the targeting moiety specifically binds one of the following molecules: tumor necrosis factor (TNF) superfamily; tumor necrosis factor- α (TNF- α); tumor necrosis factor receptor (TNFR) superfamily; Interleukin-12 (IL-12); IL-12 receptor; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNFR4); OX40 ligand (OX40L; CD40; CD40 ligand (CD40L); CTLA-4; Programmed death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); or PD-1 ligand 2 (PD-L2; B7-DC); B7 family; B7-1 (CD80); B7-2 (CD86); B7-H3; B7-H4; GITR/AITR; GITRL/AITRL; BTLA; CD70; CD27; LIGHT; HVEM; Toll-like receptor (TLR) (TLR 1,2,3,4,5,6,7,8,9,10). In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide, or peptide that specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a cytokine, cytokine receptor, co-stimulatory molecule, or co-inhibitory molecule that modulates the immune system. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2 α receptor; IL-2 α R); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF- β R); Transforming growth factor-beta (TGF- β); Programmed Death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); PD-1 ligand 2 (PD-L2; B7-DC); Receptor activator of nuclear factor- κ B (RANK); Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18);

Interleukin-4 receptor (IL-4R); tumor necrosis factor (TNF) superfamily; tumor necrosis factor- α (TNF- α); tumor necrosis factor receptor (TNFR) superfamily; Interleukin-12 (IL-12); IL-12 receptor; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNFR4); OX40 ligand (OX40L; CD40; CD40 ligand (CD40L); CTLA-4; B7 family; B7-1 (CD80); B7-2 (CD86); B7-H3; B7-H4; GITR/AITR; GITRL/AITRL; BTLA; CD70; CD27; LIGHT; or HVEM. In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as Zanolimumab (anti-CD4 mAb), Keliximab (anti-CD4 mAb); Ipilimumab (MDX-101; anti-CTLA-4 mAb); Tremilimumab (anti-CTLA-4 mAb); (Dacizumab (anti-CD25/IL-2R mAb); Basiliximab (anti-CD25/IL-2R mAb); MDX-1106 (anti-PD1 mAb); antibody to GITR; GC1008 (anti-TGF- β antibody); metelimumab/CAT-192 (anti-TGF- β antibody); lerdelimumab/CAT-152 (anti-TGF- β antibody); ID11 (anti-TGF- β antibody); Denosumab (anti-RANKL mAb); BMS-663513 (humanized anti-4-1BB mAb); SGN-40 (humanized anti-CD40 mAb); CP870,893 (human anti-CD40 mAb); Infliximab (chimeric anti-TNF mAb); Adalimumab (human anti-TNF mAb); Certolizumab (humanized Fab anti-TNF); Golimumab (anti-TNF); Etanercept (Extracellular domain of TNFR fused to IgG1 Fc); Belatacept (Extracellular domain of CTLA-4 fused to Fc); Abatacept (Extracellular domain of CTLA-4 fused to Fc); Belimumab (anti-B Lymphocyte stimulator); Muromonab-CD3 (anti-CD3 mAb); Otelixizumab (anti-CD3 mAb); Tepilizumab (anti-CD3 mAb); Tocilizumab (anti-IL6R mAb); REGN88 (anti-IL6R mAb); Ustekinumab (anti-IL-12/23 mAb); Briakinumab (anti-IL-12/23 mAb); Natalizumab (anti- α 4 integrin); Vedolizumab (anti- α 4 B7 integrin mAb); T1h (anti-CD6 mAb); Epratuzumab (anti-CD22 mAb); Efalizumab (anti-CD11a mAb); and Atacicept (extracellular domain of transmembrane activator and calcium-modulating ligand interactor fused with Fc).

In one embodiment, the present invention provides a molecule including a targeting moiety fused with an "immunomodulatory moiety". As used herein, "immunomodulatory moiety" refers to a ligand, peptide, polypeptide, or Fc-containing polypeptide that binds a specific component of a regulatory T cell, myeloid suppressor cell, or dendritic cell and modulates the number or function of Tregs or myeloid suppressor cells. In an additional aspect, the "immunomodulatory moiety" specifically binds a cytokine, cytokine receptor, co-stimulatory molecule, or co-inhibitory molecule that modulates the immune system. In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: Transforming growth factor-beta receptor (TGF- β R); Transforming growth factor-beta (TGF- β); Programmed Death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); PD-1 ligand 2 (PD-L2; B7-DC); Receptor activator of nuclear factor- κ B (RANK); or Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL); or vascular endothelial growth factor (VEGF). In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18); GITRL/AITRL; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNFR4); OX40 ligand (OX40L); B7-H3; B7-H4; BTLA; CD40; CD40 ligand (CD40L); CD70; CD27; LIGHT; or HVEM. In another aspect, the immunomodulatory moiety specifically

binds one of the following molecules: tumor necrosis factor- α (TNF- α); Interleukin-12 (IL-12); IL-12R; Interleukin-10 (IL-10); IL-10R. In another aspect, the immunomodulatory moiety comprises an extracellular domain of CTLA-4. In one aspect, the immunomodulatory moiety is an agonist that increases the function of the bound molecule. In another aspect, the immunomodulatory moiety is an antagonist that inhibits the function of the targeted molecule.

In another aspect, the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF- β RII, TGF- β RIIb, or TGF- β RIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor- κ B (RANK); vascular endothelial growth factor receptor (VEGFR1 or VEGFR2); or IL-10R. In another aspect, the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: tumor necrosis factor receptor 2 (TNFR2); 4-1BB (CD137); OX40 (CD134; TNFR4); CD40; IL-12R; or glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18). In an additional aspect, the extracellular domain of the specific receptor binds the cognate ligand and inhibits the interaction of the ligand with its native receptor.

In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: Transforming growth factor-beta (TGF- β); PD-1 ligand 1 (PD-L1); PD-1 ligand 2 (PD-L2); or IL-10. In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL.

In another aspect, the immunomodulatory moiety is fused to the C-terminus of the targeting moiety. In another aspect, the immunomodulatory moiety is fused to the N-terminus of the targeting moiety. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is the targeting moiety, Fc is an immunoglobulin Fc region, and Y is the immunomodulatory moiety. In another aspect, the fusion molecule is represented by Y-Fc-X, wherein X is the targeting moiety, and Y is the immunomodulatory moiety. In one aspect, the targeting moiety may additionally be an immunomodulatory moiety.

In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell, and the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF- β RII, TGF- β RIIb, or TGF- β RIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor- κ B (RANK); vascular endothelial growth factor receptor (VEGFR1 or VEGFR2); or IL-10R.

In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell, and the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL.

In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds a specific component of a regulatory T cell, myeloid suppressor cell, or dendritic cell, and the immunomodulatory moiety comprises an extracellular domain or

ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF- β RII, TGF- β RIIb, or TGF- β RIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor- κ B (RANK); or IL-10R. In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL. In another aspect, the specific targeted component of a regulatory T cell, myeloid suppressor cell, or dendritic cell is one of the following molecules: CD4; CD25 (IL-2 α receptor; IL-2 α R); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta (TGF- β); Programmed Death-1 (PD-1); Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R).

In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds one of the following: CTLA-4; 4-1BB (CD137); OX40 (CD134; TNR4); CD40; IL-12R; or glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18); and the immunomodulatory moiety comprises a different molecule selected from the following: (i) an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF- β RII, TGF- β RIIb, or TGF- β RIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor- κ B (RANK); or IL-10R; or (ii) Fc-containing polypeptide or ligand that binds one of the following: CTLA-4; 4-1BB (CD137); OX40 (CD134; TNR4); CD40; IL-12R; or GITR (AITR; TNFRSF18).

In another aspect, the targeting moiety and immunomodulatory moiety are two different molecules selected from any of the following: an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds TGF- β , CTLA-4, PD-1, 4-1BB (CD137), OX40 (CD134; TNR4), CD40; IL-12R, or GITR/AITR (TNFRSF18), or Toll-like receptor (TLR); an extracellular domain or ligand-binding sequence of Transforming growth factor-beta receptor (TGF- β RII, TGF- β RIIb, or TGF- β RIII), Programmed Death-1 (PD-1), Receptor activator of nuclear factor- κ B (RANK), or IL-10R. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is an immunomodulatory targeting moiety and Y is a different immunomodulatory moiety.

In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds one of the following molecules: CD4; CD25 (IL-2 α receptor; IL-2 α R); or CD20; and the immunomodulatory moiety comprises one of the following ligands or active ligand fragments: Transforming growth factor-beta (TGF- β); PD-1 ligand 1 (PD-L1); PD-1 ligand 2 (PD-L2); or IL-10.

In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds tumor necrosis factor- α (TNF- α), Interleukin-12 (IL-12), IL-6R, B-lymphocyte stimulator, CD11a, CD6, or CD22; and the immunomodulatory moiety comprises one of the following: (i) ligands or active ligand fragments of Transforming growth factor-beta (TGF- β), PD-1 ligand 1 (PD-L1), or IL-10; or (ii) an extracellular domain or ligand-binding fragment of RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18).

In another aspect, the targeting moiety comprises the extracellular domain of CTLA-4 fused to immunoglobulin Fc, and the immunomodulatory moiety comprises one of the following: (i) ligands or active ligand fragments of Transforming growth factor-beta (TGF- β), PD-1 ligand 1 (PD-L1), or IL-10; or (ii) ligand-binding fragment of TNFR2, RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18).

In another aspect, the targeting moiety and immunomodulatory moiety are two different molecules selected from any of the following: an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds tumor necrosis factor- α (TNF- α), Interleukin-12 (IL-12), IL-6R, B-lymphocyte stimulator, CD11a, CD6, or CD22; a ligand-binding fragment of TNFR2, RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18); ligands or active ligand fragments of Transforming growth factor-beta (TGF- β), PD-1 ligand 1 (PD-L1), or IL-10; or CTLA-4-Fc. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is the immunomodulatory targeting moiety and Y is a different immunomodulatory moiety.

Antibodies: In one embodiment, the targeting moiety or fusion protein is an immunoglobulin. As used herein, the term "immunoglobulin" includes natural or artificial mono- or polyclonal antibodies including, but not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin ion can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) or subclass of immunoglobulin molecule.

An antibody as disclosed herein includes an antibody fragment, such as, but not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdfv) and fragments including either a VL or VH domain. In one embodiment, the targeting moiety is an antibody or scFv.

An antigen-binding antibody fragment, including single-chain antibody, may include the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. An antigen-binding fragment can also include any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. Also included is a Fc fragment, antigen-Fc fusion proteins, and Fc-targeting moiety. The antibody may be from any animal origin including birds and mammals. In one aspect, the antibody is, or derived from, a human, murine (e.g., mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken. Further, such antibody may be a humanized version of an antibody. The antibody may be monospecific, bispecific, trispecific, or of greater multispecificity.

The antibody herein specifically include a "chimeric" antibody in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another

species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816, 567; and Morrison et al. (1984) Proc. Natl. Acad. Sci. USA. 81:6851-6855). A chimeric antibody of interest herein includes "primatized" antibodies including variable domain antigen-binding sequences derived from a non-human primate (e.g., Old World Monkey, Ape etc.) and human constant region sequences.

Various methods have been employed to produce antibodies. Hybridoma technology, which refers to a cloned cell line that produces a single type of antibody, uses the cells of various species, including mice (murine), hamsters, rats, and humans. Another method to prepare an antibody uses genetic engineering including recombinant DNA techniques. For example, antibodies made from these techniques include, among others, chimeric antibodies and humanized antibodies. A chimeric antibody combines DNA encoding regions from more than one type of species. For example, a chimeric antibody may derive the variable region from a mouse and the constant region from a human. A humanized antibody comes predominantly from a human, even though it contains nonhuman portions. Like a chimeric antibody, a humanized antibody may contain a completely human constant region. But unlike a chimeric antibody, the variable region may be partially derived from a human. The nonhuman, synthetic portions of a humanized antibody often come from CDRs in murine antibodies. In any event, these regions are crucial to allow the antibody to recognize and bind to a specific antigen.

In one embodiment, a hybridoma can produce a targeted fusion protein comprising a targeting moiety and an immunomodulatory moiety. In one embodiment, a targeting moiety comprising an antibody, antibody fragment, or polypeptide is linked or fused to an immunomodulatory moiety consisting of a polypeptide, with a linker or without a linker. The linker can be an amino acid linker. In one embodiment, a linker is (GGGGS)_n (SEQ ID NO: 123) wherein n is 1, 2, 3, 4, 5, 6, 7, or 8. For example, GGGSGGGSGGGGS (SEQ ID NO: 104). In another embodiment, a linker is EPKSCDK (SEQ ID NO: 105). In another embodiment, a linker is IEGRDMD (SEQ. ID. NO: 106). In various aspects, the length of the linker may be modified to optimize binding of the target moiety or the function of the immunomodulatory moiety. In various aspects, the immunomodulatory moiety is a polypeptide that is fused to the C-terminus of the Fc region of the heavy chain of a targeting antibody or Fc-containing fusion protein. In another aspect, the immunomodulatory moiety is a polypeptide that is fused to the C-terminus of the light chain of a targeting antibody. In another aspect, the fusion protein comprises an X-Fc-Y sequence, wherein X is a targeting polypeptide and Y is an immunomodulatory polypeptide.

For example, a hybridoma can produce the polypeptides corresponding to SEQ. ID. NO: 1-69.

An antibody fragment can include a portion of an intact antibody, e.g., including the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; Fc fragments or Fc-fusion products; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragment(s).

An intact antibody is one which includes an antigen-binding variable region as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (e.g., human native sequence constant

domains) or amino acid sequence variant thereof for any other modified Fc (e.g., glycosylation or other engineered Fc).

The intact antibody may have one or more "effector functions" which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region or any other modified Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor (BCR)); and cross-presentation of antigens by antigen presenting cells or dendritic cells. In one embodiment, the targeting antibody or Fc-containing fusion protein facilitates focused or preferential delivery of a immunomodulatory moiety to a target cell. In another aspect, a targeting antibody can induce death of the targeted cell or sensitize it to immune cell-mediated cytotoxicity. In another aspect, the Fc-fusion protein or antibody can facilitate delivery of the immunomodulatory moiety or immunogenic apoptotic material from antibody-bound tumor targets, or both, to an antigen presenting cells (APC) via interactions between their Fc and Fc receptors (on APC).

Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different "classes." There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), e.g., IgG1, IgG2, IgC3, IgG4, IgA, and IgA2. The heavy-chain constant domains (that correspond to the different classes of antibodies are called alpha (α), delta (δ), epsilon (ε), gamma (γ), and mu (μ) respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

Peptides: In some aspects of the invention the targeting moiety or immunomodulatory moiety is a peptide or polypeptide. A peptide includes any analog, fragment or chemical derivative of a peptide whose amino acid residue sequence is shown herein. Therefore, a present peptide can be subject to various changes, substitutions, insertions, and deletions where such changes provide for certain advantages in its use. In this regard, a peptide of this invention corresponds to, rather than is identical to, the sequence of a recited peptide where one or more changes are made and it retains the ability to function as the unmodified peptide in one or more of the assays.

The term "analog" includes any peptide having an amino acid residue sequence substantially identical to a sequence specifically shown herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the activity as described herein. Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another.

The term "fragment" refers to any subject polypeptide having an amino acid residue sequence shorter than that of a polypeptide whose amino acid residue sequence is disclosed herein.

As used herein "a tumor targeting peptide" includes polymers containing fewer than 100 amino acids, where the

polymer specifically binds to a cellular component of a tumor cell, tumor vasculature, and/or a component of a tumor microenvironment.

A peptide of the present invention can be synthesized by any of the techniques that are known to those skilled in "the polypeptide art, including recombinant DNA techniques. Synthetic chemistry techniques, such as a solid-phase Merrifield-type synthesis, are preferred for reasons of purity, antigenic specificity, freedom from undesired side products, ease of production and the like. An excellent summary of the many techniques available can be found in Steward et al., "Solid Phase Peptide Synthesis" W. H. Freeman Co., San Francisco, 1969; Bodanszky, et al., "Peptide Synthesis", John Wiley & Sons, Second Edition, 1976; J. Meienhofer, "Hormonal Proteins and Peptides". Vol. 2. p. 46, Academic Press (New York), 1983; Merrifield, *Adv. Enzymol.*, 32:221-96, 1969; Fields et al. *Int. J. Peptide Protein Res.*, 35:161-214, 1990; and U.S. Pat. No. 4,244,946 for solid phase peptide synthesis, and Schroder et al., "The Peptides", Vol. 1, Academic Press (New York), 1965 for classical solution synthesis. Appropriate protective groups usable in such synthesis are described in the above texts and in J. F. W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, New York, 1973.

Aptamers: In one aspect of the invention, the targeting moiety is an aptamer. In various embodiments, an aptamer is specific for a molecule on a tumor cell, tumor vasculature, and/or a tumor microenvironment. The term "aptamer" includes DNA, RNA or peptides that are selected based on specific binding properties to a particular molecule. For example, an aptamer(s) can be selected for binding a particular gene product in a tumor cell, tumor vasculature, tumor microenvironment, and/or an immune cell, as disclosed herein, where selection is made by methods known in the art and familiar to one of skill in the art. Subsequently, said aptamer(s) can be administered to a subject to modulate or regulate an immune response.

Some aptamers having affinity to a specific protein, DNA, amino acid and nucleotides have been described (e.g., K. Y. Wang, et al., *Biochemistry* 32:1899-1904 (1993); Pitner et al., U.S. Pat. No. 5,691,145; Gold, et al., *Ann. Rev. Biochem.* 64:763-797 (1995); Szostak et al., U.S. Pat. No. 5,631,146). High affinity and high specificity binding aptamers have been derived from combinatorial libraries (supra, Gold, et al.). Aptamers may have high affinities, with equilibrium dissociation constants ranging from micromolar to sub-nanomolar depending on the selection used, aptamers may also exhibit high selectivity, for example, showing a thousand fold, discrimination between 7-methyl G and G (Haller and Sarnow, *Proc. Natl. Acad. Sci. USA* 94:8521-8526 (1997)) or between D and L-tryptophan (supra, Gold et al.). An aptamer can be selected based on the particular molecule targeted (e.g., aptamer targeting EGFR or other cancer markers). Standard procedures for in vitro selection are known, such as SELEX experiments, described at *Science* 249 (4968) 505-510 (1990), and *Nature* (London), 346 (6287) 818-822 (1990) which can be followed throughout, or with modifications and improvements known in the art.

The term "therapeutically effective amount" means the amount of the subject compound that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

By "pharmaceutically acceptable" it is meant the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The terms "administration of" and or "administering" should be understood to mean providing a composition in a therapeutically effective amount to the individual in need of treatment. Administration can be intratumoral or systemic (intravenous) administration. Furthermore, in conjunction with vaccination of recipient with pathogen antigen vaccine (e.g., tetanus toxoid). In addition, in conjunction with agent to deplete or inactivate regulatory T cells (e.g., cyclophosphamide) or myeloid suppressor cells (e.g., gemcitabine). In a further example, ex vivo treatment of immune cells and tumor cells for generation of tumor reactive or pathogen antigen reactive immune cells—for adoptive cellular immunotherapy. Administration can be intradermal or subcutaneous.

Furthermore, administration can be in combination with one or more additional therapeutic agents deplete or inactivate regulatory T cells (cyclophosphamide) or myeloid suppressor cells (e.g., gemcitabine). The pharmaceutical compositions of the invention identified herein are useful for parenteral, topical, oral, nasal (or otherwise inhaled), rectal, or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic treatment of one or more of the pathologies/indications described herein (e.g., cancer, pathogenic infectious agents, associated conditions thereof). The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. Suitable unit dosage forms, include, but are not limited to powders, tablets, pills, capsules, lozenges, suppositories, patches, nasal sprays, injectables, implantable sustained-release formulations, lipid complexes, etc.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, as it will be understood that modifications and variations are encompassed within the spirit and scope of the instant disclosure.

The following examples are provided to further illustrate the embodiments of the present invention, but are not intended to limit the scope of the invention. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

Example 1

Counteracting Tumor Immune Tolerance via Antibody-mediated Depletion of CD4⁺ Regulatory T Cells Facilitates the Activation of Tumor-Reactive CD8⁺ T Cells and Enhances the In Vivo Antitumor Efficacy of Cytotoxic Anticancer Agents.

Immunogenic death of tumor cells by chemotherapeutic agents can induce CD8⁺ T cell-mediated antitumor immunity. In response to specific chemotherapeutic agents, tumor cells exhibit the rapid translocation of intracellular calreticulin (CRT) to the cell surface where its aggregation provides a signal for the recognition and engulfment of dying tumor cells by antigen presenting dendritic cells (DCS). Treatment of mouse MB49 or human SW780 bladder cancer cells with doxorubicin, an anthracycline chemotherapeutic agent, induced rapid surface exposure of CRT that was detected by immunofluorescence cytometry of cells stained with Dylight 488-labeled anti-CRT antibody (FIG. 53A). To determine whether ex vivo treatment with doxorubicin induced an immunogenic death of tumor cells, either untreated live MB49 cells or an equivalent number of MB49

cells that were pre-treated in vitro with doxorubicin were injected into one flank of syngeneic immunocompetent C57BL/6 mice. Unlike mice injected with live tumor cells, mice injected with doxorubicin-treated tumor cells exhibited increased production of IFN- γ by draining lymph node (DLN) cells in response to in vitro re-challenge with MB49 cell lysates (FIG. 53B). Vaccination with doxorubicin-killed MB49 cells generated a tumor-specific immune response since no corresponding increase in IFN- γ secretion by DLN cells was observed following in vitro exposure to an irrelevant peptide (Hemagglutinin-HA). Injection of doxorubicin-treated MB49 tumor cells protected mice against tumor growth upon challenge with untreated live MB49 tumor cells injected into the opposite flank. (FIG. 1C). The protection against tumor growth by vaccination with doxorubicin-treated tumor cells was not observed in mice that were depleted of CD8 $^{+}$ T cells with an anti-CD8 antibody before challenge with live tumor cells (FIG. 53C). These observations indicate that ex vivo treatment with chemotherapeutic agents can induce an immunogenic death of tumor cells that generates CD8 $^{+}$ T cell-mediated adaptive antitumor immunity.

Tumor-induced immune tolerance inhibits activation of CD8 $^{+}$ T cells in response to chemotherapy. To examine whether in vivo treatment with chemotherapeutic agents can activate CD8 $^{+}$ T cell-mediated immune responses in mice with pre-established tumors, C57BL/6 mice were injected with live syngeneic MB49 tumor cells and then administered intratumoral doxorubicin at various time points following tumor inoculation. In contrast to vaccination of naïve mice with doxorubicin-killed MB49 cells, in vivo treatment of mice with established MB49 tumors at d10 following tumor inoculation failed to induce a corresponding increase in IFN- γ secretion by DLN cells in response to in vitro re-challenge with MB49 cell lysates (FIG. 53B). Whereas treatment with doxorubicin on d3 following tumor inoculation was able to arrest tumor growth, delayed administration of the same dose of doxorubicin on d10 failed to inhibit the progressive growth of established MB49 tumors (FIG. 53D). These results indicate that tumor-induced immune tolerance in the microenvironment of established cancers counteracts the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death.

Regulatory T cells (Treg) accumulate in the tumor microenvironment and counteract the ability of chemotherapy to activate CD8 $^{+}$ T cell mediated antitumor immunity. To investigate whether FoxP3 $^{+}$ Tregs are involved in enforcing immune tolerance in the tumor microenvironment, we examined the percentage of CD4 $^{+}$ CD25 $^{+}$ FoxP3 $^{+}$ cells (Tregs) among CD4 $^{+}$ T lymphocytes in the spleen, draining lymph nodes (DLN), and tumors of immunocompetent C57BL/6 mice at d0 and d14 after tumor inoculation. Whereas tumor-bearing mice exhibited only a minor increase in the percentage of Tregs among CD4 $^{+}$ T cells in the spleen and DLN at d14 following tumor inoculation, a majority of tumor-infiltrating CD4 $^{+}$ T cells at this time were CD4 $^{+}$ CD25 $^{+}$ FoxP3 $^{+}$ cells (FIG. 53E). To investigate whether Tregs infiltrating the tumor microenvironment can suppress the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death, CD4 $^{+}$ CD25 $^{+}$ cells isolated from tumors and DLN of tumor-bearing mice were adoptively transferred into syngeneic C57BL/6 naïve mice before vaccination with doxorubicin-killed MB49 cells. The adoptive transfer of tumor-infiltrating CD4 $^{+}$ CD25 $^{+}$ cells into naïve mice inhibited the ability of subsequent in vivo vaccination with doxorubicin-treated MB49 tumor cells to increase production of IFN- γ by

draining lymph node (DLN) cells in response to in vitro re-challenge with MB49 cell lysates (FIG. 53F). Consistent with the ability of tumor-infiltrating CD4 $^{+}$ CD25 $^{+}$ cells to suppress the tumor-specific immune response, the adoptive transfer of these cells counteracted the protection conferred by vaccination with doxorubicin-treated MB49 cells against tumor growth upon challenge with untreated live MB49 tumor cells (FIG. 53G). These results indicate that the tumor microenvironment fosters the accumulation of FoxP3 $^{+}$ Tregs which counteract the activation of CD8 $^{+}$ T cell mediated antitumor immunity in response to chemotherapy-induced tumor cell death.

Inhibition of TGF- β in the tumor microenvironment reduces tumor-infiltrating FoxP3 $^{+}$ regulatory T cells and enhances the antitumor efficacy chemotherapy. TGF- β induces FoxP3 expression in naïve peripheral CD4 $^{+}$ CD25 $^{-}$ FoxP3 $^{-}$ T cells and facilitates their conversion into 'adaptive' FoxP3 $^{+}$ Tregs that share the immunosuppressive ability of natural FoxP3 $^{+}$ Tregs generated in the thymus. Since human cancers frequently become refractory to the growth-inhibitory effect of TGF- β and acquire an ability to increase expression and secretion of TGF- β , we investigated whether this switch enables tumor cells to increase the number of adaptive Tregs in the tumor microenvironment. Examination of serum levels of TGF- β in mice at d0, d14, and d28 following inoculation of live MB49 tumor cells demonstrated that tumor growth resulted in a progressive increase in the level of serum TGF- β (FIG. 54A). To assess the precise source of TGF- β in tumor-bearing mice, the total amount of TGF- β in supernatants of tumor cells or draining lymph node cells isolated from tumor-bearing mice were measured following ex vivo culture in serum-free medium for 24 h. Measurement of the level of TGF- β /10 ^{6} cells showed that tumor cells were the dominant source of the increased level of TGF- β in tumor-bearing mice (FIG. 54B). In addition to tumor cell-autonomous expression of TGF- β , T cells from tumor-bearing mice also expressed higher levels of TGF- β compared to their counterparts from tumor-free mice (FIG. 54B). To determine whether the elevation of TGF- β is responsible for the upregulation of Tregs in the tumor microenvironment, tumor-bearing mice were treated with a soluble chimeric protein comprising the extracellular domain of TGF β RII and the Fc portion of the murine IgG1 heavy chain (TGF β RII:Fc). This fusion protein interferes with the binding of TGF- β to endogenous TGF β RII and functions as a stable TGF- β antagonist. ELISA assays confirmed the ability of TGF β RII:Fc to sequester TGF- β in supernatants of MB49 tumor cells in a concentration-dependent manner (FIG. 54C). At 5 d following inoculation of MB49 tumor cells, mice were either left untreated or treated with TGF β RII:Fc (1 μ g intratumoral; twice weekly) for 3 weeks followed by flow cytometric analyses of intracellular FoxP3 expression in CD4 $^{+}$ CD25 $^{+}$ T cells infiltrating the tumors. In vivo treatment of tumors with TGF β RII:Fc resulted in a significant decline in FoxP3 expression in tumor-infiltrating CD4 $^{+}$ T cells (FIG. 54D) and a dramatic reduction of CD4 $^{+}$ CD25 $^{+}$ FoxP3 $^{+}$ Tregs in tumor tissue (FIG. 54E). To determine whether inhibition of TGF- β in the tumor microenvironment can improve the antitumor efficacy of chemotherapy, MB49 tumor-bearing mice were administered doxorubicin (5 mg/kg i.p. weekly $\times$ 3) with or without twice weekly treatment with TGF β RII:Fc (1 μ g intratumoral). In contrast to treatment with either doxorubicin or TGF β RII:Fc alone, combined treatment with both agents was able to arrest the growth of MB49 tumors. These results indicate that tumor cell autonomous expression of TGF- β in the tumor microenvironment induces 'adaptive' FoxP3 $^{+}$

Tregs and that counteracting tumor-induced TGF- β -mediated immune tolerance enhances the antitumor efficacy of chemotherapy.

Anti-CD4 antibody-mediated depletion of CD4⁺ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive CD8⁺ T cells and enhances the antitumor efficacy of chemotherapy. To determine whether depletion of CD4⁺ regulatory T cells can improve the antitumor efficacy of chemotherapy by enhancing the activity of CD8⁺ T cells in the tumor microenvironment, immunocompetent mice bearing syngeneic tumors were administered an anti-CD4 antibody (Clone GK1.5) to deplete CD4⁺ T cells or an anti-CD8 antibody (Clone GK2.43) to deplete CD8⁺ T cells and then treated with specific chemotherapeutic agents. Flow cytometric analyses of peripheral blood mononuclear cells from MB49 tumor-bearing mice at d7 following administration of anti-CD4 antibody or anti-CD8 antibody confirmed the target-specific depletion of either CD4⁺ T cells or CD8⁺ T cells, respectively (FIG. 55A). Mice treated with anti-CD4 antibody showed loss of CD4⁺CD25⁺FoxP3⁺ T cells in the peripheral blood as well as among tumor-infiltrating cells (FIGS. 55A, 55B). To determine whether antibody-mediated depletion of CD4⁺CD25⁺FoxP3⁺ cells facilitates chemotherapy-induced activation of tumor-reactive CD8⁺ T cells in the tumor microenvironment, we evaluated the expression of IFN- γ in CD8⁺ T cells extracted from the tumor and draining lymph node of MB49 tumor-bearing mice that were left untreated or treated with doxorubicin (with or without anti-CD4 antibody). Flow cytometric analyses showed that CD8⁺ T cells from untreated mice did not express IFN- γ in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 55C). Whereas IFN- γ ⁺CD8⁺ T cells became evident in mice treated with doxorubicin alone, antibody-mediated depletion of CD4⁺ T cells further enhanced the percentage of tumor-reactive CD8⁺ T cells that expressed IFN- γ in doxorubicin-treated animals (FIG. 55C). To directly evaluate whether the activation of tumor-reactive CD8⁺ T cells determines the *in vivo* antitumor efficacy of chemotherapy, we examined the effect of antibody-mediated depletion of CD8⁺ T cells or CD4⁺ T cells on the response of MB49 tumor-bearing mice to systemic treatment with doxorubicin (5 mg/kg). Treatment with doxorubicin alone inhibited the growth of MB49 tumors but failed to arrest tumor progression. Whereas depletion of CD8⁺ T cells completely impaired the *in vivo* antitumor efficacy of doxorubicin, depletion of CD4⁺ T cells enhanced the response to doxorubicin and resulted in tumor regression (FIG. 55D).

Anti-CD4 antibody-mediated depletion of CD4⁺ regulatory T cells augments and sustains the antitumor effect of chemotherapy by enabling activation of adaptive antitumor immunity. Whereas tumor cells treated with anthracyclins, such as doxorubicin, are particularly effective in eliciting an antitumor immune response, other chemotherapeutic agents are less effective in inducing immunogenic tumor cell death. The surface exposure of calreticulin is a key determinant of the immunogenicity of tumor cell death in response to chemotherapeutic agents. Compared to the efficient translocation of CRT to the cell surface in response to treatment with doxorubicin (FIG. 1A), treatment of MB49 tumor cells with equitoxic doses of either cisplatin or the combination of cisplatin and gemcitabine was less effective in increasing CRT exposure (FIG. 56A). Whereas tumor-reactive IFN- γ ⁺CD8⁺ T cells were evident in tumors of MB49 tumor-bearing mice treated with doxorubicin (FIG. 55C), treatment with cisplatin was unable to induce a corresponding elevation of IFN- γ expression in CD8⁺ T cells in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 56B). To

examine whether counteraction of Treg-mediated immune tolerance enables the activation of antitumor immunity by cisplatin, immunocompetent tumor-bearing mice were treated with cisplatin following depletion of Tregs with anti-CD4 antibody. Antibody-mediated depletion of CD4⁺ T cells enhanced the percentage of tumor-reactive IFN- γ ⁺CD8⁺ T cells as well as CD8⁺CD62L⁺ T cells in cisplatin-treated animals (FIGS. 56B, 56C). Treatment of MB49 tumor-bearing mice with cisplatin partially inhibited tumor growth but failed to arrest tumor progression. Whereas depletion of CD8⁺ T cells completely negated the *in vivo* antitumor effect of cisplatin, depletion of CD4⁺ T cells enhanced the response to cisplatin and arrested tumor growth (FIG. 56D). Although treatment of tumor-bearing mice with the combination of cisplatin and gemcitabine was also able to arrest tumor growth, tumor growth rapidly resumed following termination of therapy with none of the animals (0/8) being tumor-free at d50 following tumor inoculation (FIG. 56E). In contrast, mice depleted of CD4⁺ T cells exhibited a more sustained response to either single agent or combination chemotherapy, with 7/16 mice exhibiting complete tumor regression. The complete regression of tumors was attended with establishment of adaptive antitumor immunity since none of the cured mice (7/7) developed tumors when re-challenged with live MB49 tumor cells in the opposite flank.

Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD4⁺ regulatory T cells to stimulate CD8⁺ T cell-mediated tumor regression. NKG2D (NK group 2, member D) is a lectin-like type II transmembrane stimulatory receptor used by NK cells, $\gamma\delta$ -TCR⁺ T cells and $\alpha\beta$ -TCR⁺ T cells for immune surveillance of tumors. Expression of mouse and human ligands for NKG2D is upregulated in transformed epithelial cell lines in response to genotoxic stress or stalled DNA replication, via activation of a DNA damage checkpoint pathway initiated by ATM (ataxia telangiectasia, mutated) or ATR (ATM- and Rad3-related) protein kinases. Treatment of CT26 mouse colon cancer cells with genotoxic chemotherapeutic agents resulted in upregulation of mouse NKG2D ligands of the retinoic acid inducible gene family (Rae1) (FIG. 57A). RT-PCR showed that Rae1 mRNA was induced in CT26 cells by 2-4 h, reached a plateau after 16-24 h, and began to decline after 48 h of treatment with either the irinotecan or oxaliplatin (FIG. 57A). Flow cytometric analysis demonstrated that cell surface expression of human NKG2D ligands (MHC-I-related A and B molecules—MICA, MICB) was also upregulated on human colorectal cancer cells (HCT116) in response to treatment with irinotecan (FIG. 53B). Isogenic HCT116 cells that differ only in their p53 status demonstrated that p53 is not required for irinotecan-induced upregulation of MICA/B (FIG. 53B). To examine whether the induction of NKG2D ligands contributes to the antitumor effect of chemotherapy *in vivo*, immunocompetent Balb/C mice inoculated with syngeneic CT26 tumor cells were treated with irinotecan (50 mg/kg i.p.) with or without pre-treatment with an NKG2D blocking antibody (200 μ g i.p.). Whereas treatment with irinotecan alone inhibited the growth of CT26 tumors, the antitumor effect of irinotecan was negated by pre-treatment with the NKG2D blocking antibody (FIG. 1C). Since engagement of NKG2D by its ligands provides a costimulatory signal for the activation of CD8⁺ T cells, we investigated whether DNA damage-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD4⁺ regulatory T cells to stimulate CD8⁺ T cell-mediated tumor regression. Balb/C mice bearing CT26 tumors were administered an anti-CD4

antibody (Clone GK1.5) to deplete CD⁴⁺ T cells and/or an anti-CD8 antibody (Clone GK2.43) to deplete CD⁸⁺ T cells and then treated with irinotecan. Flow cytometric analyses confirmed the loss of CD⁴⁺CD2⁵⁺FoxP³⁺ T cells in the spleen and draining lymph node of mice treated with anti-CD4 antibody (FIG. 57D). Antibody-mediated depletion of CD⁴⁺ T cells enhanced the percentage of tumor-reactive IFN- γ CD⁸⁺ T cells in irinotecan-treated animals (FIG. 57E). Whereas treatment of CT26 tumor-bearing mice with irinotecan only slowed tumor growth, depletion of CD⁴⁺ T cells enhanced the response to irinotecan and arrested tumor growth (FIG. 57F). The ability of CD⁴⁺ T cell depletion to augment the antitumor efficacy of irinotecan was mediated by CD⁸⁺ T cells since antibody-mediated depletion of CD⁸⁺ T cells completely negated the in vivo antitumor effect of chemotherapy in CD⁴⁺ T cell-depleted mice (FIG. 57F).

These data provide the following insights: (i) activation of tumor-reactive CD8⁺ T cells in response to immunogenic tumor cell death is a crucial determinant of the antitumor efficacy of chemotherapy in vivo; (ii) tumor-induced Tregs impair the antitumor efficacy of chemotherapy by inhibiting the activation of CD8⁺ T cells in the tumor microenvironment; and (iii) Counteracting tumor-induced immune tolerance via antibody-mediated depletion of CD4⁺ regulatory T cells facilitates chemotherapy-induced activation of antitumor immunity with memory, thereby enhancing the antitumor efficacy of chemotherapy; (iv) Strategies to decrease the number or function of CD4⁺ regulatory T cells in the tumor microenvironment can increase the activation of CD8⁺ T cells and improve the response of tumors to cytotoxic anticancer agents (chemotherapy, tumor-targeted antibodies, targeted therapeutics, kinase inhibitors) or chemoimmunotherapy (combination of chemotherapeutic agent with immunotherapeutic agents).

Example 2

Exemplary Targeted Immunomodulatory Antibodies and Fusion Proteins

A targeting moiety, including an antibody, can be coupled to an immunomodulatory moiety including a polypeptide derived from the extracellular domain of TGFBR2. Cross-linkers or activating agents for such coupling or conjugation are well known in the art. Alternatively, the fusion proteins of the invention can be synthesized using recombination DNA technology well known in the art where the coding sequences of various portions of the fusion proteins can be linked together at the nucleic acid level. Subsequently the fusion proteins of the invention can be produced using a host cell well known in the art. Examples of targeted immunomodulatory antibodies and fusion proteins are shown in FIGS. 1-33 and briefly described below.

In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety specifically binds to Transforming growth factor-beta (TGF- β). SEQ ID NO: 1 provides a fusion protein including anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD) (FIG. 2). SEQ ID NO: 2 provides a fusion protein including anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD) (FIG. 3). SEQ ID NO: 3 provides a fusion protein including anti-CD20 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD) (FIG. 4). SEQ ID NO: 4 provides a fusion

protein including anti-VEGF antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD) (FIG. 5). SEQ ID NO: 5 provides a fusion protein including anti-human CTLA-4 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD) (FIG. 6). SEQ ID NO: 6 provides a fusion protein including IL-2, Fc, and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD) (FIG. 7). SEQ ID NO: 7 provides a fusion protein including Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), Fc, and IL-2 (FIG. 7). SEQ ID NO: 8 provides a fusion protein including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD) (FIG. 8A). SEQ ID NO: 9 provides a fusion protein including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD) (FIG. 8B). SEQ ID NO: 10 provides a fusion protein including anti-CD4 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD) (FIG. 9). SEQ ID NO: 11 provides a fusion protein including PD-1 Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ectodomain) (PD-1 ectodomain+Fc+TGF β RII ectodomain; FIG. 10). SEQ ID NO: 12 provides a fusion protein including Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ectodomain), Fc, and PD-1 Ectodomain (TGF β RII ectodomain+Fc+PD-1 ectodomain; FIG. 10). SEQ ID NO: 13 provides a fusion protein including RANK Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ectodomain) (RANK ectodomain+Fc+TGF β RII ectodomain; FIG. 11). SEQ ID NO: 14 provides a fusion protein including Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ectodomain), Fc, and RANK Ectodomain (TGF β RII ectodomain+Fc+RANK ectodomain; FIG. 11).

In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Programmed Death-1 ligand 1 (PD-L1 or B7-H1) or Programmed Death-1 ligand 2 (PD-L2 or B7-DC). SEQ ID NO: 15 provides a fusion protein including anti-HER2/neu antibody and PD-1 Ectodomain (FIG. 13). SEQ ID NO: 16 provides a fusion protein including anti-EGFR1 antibody and PD-1 Ectodomain (FIG. 14). SEQ ID NO: 17 provides a fusion protein including anti-CD20 antibody and PD-1 Ectodomain (FIG. 15). SEQ ID NO: 18 provides a fusion protein including anti-VEGF antibody and PD-1 Ectodomain (FIG. 16). SEQ ID NO: 19 provides a fusion protein including anti-human CTLA-4 antibody and PD-1 Ectodomain (FIG. 17). SEQ ID NO: 20 provides a fusion protein including anti-CD25 antibody and PD-1 Ectodomain (FIG. 18A). SEQ ID NO: 21 provides a fusion protein including anti-CD25 antibody and PD-1 Ectodomain (FIG. 18B). SEQ ID NO: 22 provides a fusion protein including IL-2, Fc, and PD-1 ectodomain (IL-2+Fc+PD-1 ectodomain; FIG. 19). SEQ ID NO: 23 provides a fusion protein including PD-1 ectodomain, Fc, and IL-2 (PD-1 ectodomain+Fc+IL-2; FIG. 19). SEQ ID NO: 24 provides a fusion protein including anti-CD4 antibody and PD-1 Ectodomain (FIG. 20). SEQ ID NO: 25 provides a fusion protein including RANK Ectodomain, Fc, and PD-1 ectodomain (RANK ectodomain+Fc+PD-1 ectodomain; FIG. 21). SEQ ID NO: 26 provides a fusion protein including PD-1 ectodomain, Fc, and RANK Ectodomain (PD-1 ectodomain+Fc+RANK ectodomain; FIG. 21).

In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Receptor activator of NF- κ B ligand (RANKL). SEQ ID NO: 27 provides a fusion protein including anti-HER2/neu antibody and RANK Ectodomain (FIG. 23). SEQ ID NO: 28 provides a fusion protein including anti-EGFR1 antibody and RANK Ectodomain (FIG. 24). SEQ ID NO: 29 provides a fusion protein including anti-CD20 antibody and RANK Ectodomain (FIG. 25). SEQ ID NO: 30 provides a fusion protein including anti-VEGF antibody and RANK Ectodomain (FIG. 26). SEQ ID NO: 31 provides a fusion protein including anti-human CTLA-4 antibody and RANK Ectodomain (FIG. 27). SEQ ID NO: 32 provides a fusion protein including anti-CD25 antibody and RANK Ectodomain (FIG. 28A). SEQ ID NO: 33 provides a fusion protein including anti-CD25 antibody and RANK Ectodomain (FIG. 28B). SEQ ID NO: 34 provides a fusion protein including IL-2, Fc, and RANK ectodomain (IL-2+Fc+RANK ectodomain; FIG. 29). SEQ ID NO: 35 provides a fusion protein including RANK ectodomain, Fc, and IL-2 (RANK ectodomain+Fc+IL-2; FIG. 29). SEQ ID NO: 36 provides a fusion protein including anti-CD4 antibody and RANK Ectodomain (FIG. 30).

In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 (PD-1). SEQ ID NO: 37 provides a fusion protein including anti-tumor necrosis factor (TNF α) antibody and PD-1 ligand 1 (FIG. 32). SEQ ID NO: 38 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand: (TNFR2 ECD+IgG C γ 1+PD-L1; FIG. 33). SEQ ID NO: 39 provides a fusion protein including PD-1 ligand, Fc, and TNFR2 Extracellular ligand binding domain: (PD-L1+IgG C γ 1-TNFR2 ECD; FIG. 33). SEQ ID NO: 40 provides a fusion protein including anti-CD20 antibody and PD-1 ligand 1 (PD-L1) (FIG. 34). SEQ ID NO: 41 provides a fusion protein including anti-CD25 antibody and PD-1 ligand 1 (PD-L1) (FIG. 35A). SEQ ID NO: 42 provides a fusion protein including anti-CD25 antibody and PD-1 ligand 1 (PD-L1) (FIG. 35B). SEQ ID NO: 43 provides a fusion protein including PD-1 ligand 1 (PD-L1), Fc, and IL-2 (PD-L1-Fc-IL2; FIG. 36). SEQ ID NO: 44 provides a fusion protein including IL-2, Fc, and PD-1 ligand 1 (PD-L1) (IL-2-Fc-PD-L1; FIG. 36). SEQ ID NO: 45 provides a fusion protein including anti-CD4 antibody and PD-1 ligand 1 (PD-L1) (FIG. 37). SEQ ID NO: 46 provides a fusion protein including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C γ 1), and a sequence from PD-1 ligand (PD-L1) (Oncostatin M signal peptide+CTLA-4 ECD+IgG C γ 1+PD-L1; FIG. 38). SEQ ID NO: 47 provides a fusion protein including the extracellular domain of PD-1 ligand (PD-L1), immunoglobulin Fc (IgG C γ 1), and a sequence from the extracellular domain of CTLA-4: (PD-L1+IgG C γ 1+CTLA-4 ECD; FIG. 38). SEQ ID NO: 48 provides a fusion protein including Transforming growth factor- β (TGF- β), immunoglobulin Fc (IgG C γ 1), and a sequence from PD-1 ligand 1 (PD-L1) (TGF- β -1+Fc+PD-L1; FIG. 39). SEQ ID NO: 49 provides a fusion protein including a sequence from PD-1 ligand 1 (PD-L1), immunoglobulin Fc (IgG C γ 1), and Transforming growth factor beta (TGF- β) (PD-L1+Fc+TGF- β -1; FIG. 39).

In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety includes a molecule that specifically binds to Transforming growth factor-beta receptor (TGF- β R). SEQ ID NO: 50 provides a fusion protein including an antibody that binds TNF- α , and a sequence from Transforming growth factor- β (TGF- β) (FIG. 41). SEQ ID NO: 51 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and a sequence from Transforming growth factor- β (TGF- β) (TNFR2 ECD+IgG C γ 1+TGF- β ; FIG. 42). SEQ ID NO: 52 provides a fusion protein including a sequence from Transforming growth factor- β (TGF- β), Fc, and TNFR2 Extracellular ligand binding domain: (TGF- β +IgG C γ 1+TNFR2 ECD; FIG. 42). SEQ ID NO: 53 provides a fusion protein including anti-CD20 antibody and a sequence from Transforming growth factor- β (TGF- β) (FIG. 43). SEQ ID NO: 54 provides a fusion protein including anti-CD25 antibody and a sequence from transforming growth factor- β (TGF- β) (FIG. 44A). SEQ ID NO: 55 provides a fusion protein including anti-CD25 antibody and a sequence from transforming growth factor- β (TGF- β) (FIG. 44B). SEQ ID NO: 56 provides a fusion protein including a sequence from Transforming growth factor- β (TGF- β), Fc, and IL-2 (TGF- β +Fc+IL-2; FIG. 45). SEQ ID NO: 57 provides a fusion protein including IL-2, Fc, and Transforming growth factor- β (TGF- β) (IL-2+Fc+TGF- β ; FIG. 45). SEQ ID NO: 58 provides a fusion protein including anti-CD4 antibody and a sequence from transforming growth factor- β (TGF- β) (FIG. 46). SEQ ID NO: 59 provides a fusion protein including the extracellular domain of CTLA-4, immunoglobulin Fc (IgG C γ 1), and a sequence from a sequence from transforming growth factor- β (TGF- β) (Oncostatin M signal peptide+CTLA-4 ECD+IgG C γ 1+TGF- β -1; FIG. 47). SEQ ID NO: 60 provides a fusion protein including a sequence from Transforming growth factor- β (TGF- β), immunoglobulin Fc (IgG C γ 1), and a sequence from the extracellular domain of CTLA-4: (TGF- β -1+IgG C γ 1+CTLA-4 ECD) (FIG. 47).

In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Receptor activator of NF- κ B ligand (RANKL). SEQ ID NO: 61 provides a fusion protein including an antibody that binds TNF- α , and a sequence from RANK Ectodomain (FIG. 48). SEQ ID NO: 62 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and a sequence from RANK Ectodomain (TNFR2 ECD+IgG C γ 1+RANK Ectodomain; FIG. 49). SEQ ID NO: 63 provides a fusion protein including a sequence from RANK Ectodomain, Fc, and TNFR2 Extracellular ligand binding domain: (RANK Ectodomain+IgG C γ 1+TNFR2 ECD; FIG. 49). SEQ ID NO: 64 provides a fusion protein including the extracellular domain of CTLA-4, immunoglobulin Fc (IgG C γ 1), and a sequence from a sequence from RANK Ectodomain (Oncostatin M signal peptide+CTLA-4 ECD+IgG C γ 1+RANK Ectodomain; FIG. 50). SEQ ID NO: 65 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG C γ 1), and a sequence from the extracellular domain of CTLA-4: (RANK Ectodomain+IgG C γ 1+CTLA-4 ECD) (FIG. 50). SEQ ID NO: 66 provides a fusion protein including a sequence from transforming growth factor- β (TGF- β), immunoglobulin Fc region (IgG C γ 1), and an extracellular ligand-binding domain or ectodo-

main of Receptor activator of nuclear factor- κ B (RANK) (TGF- β +IgG C γ 1+RANK Ectodomain; FIG. 51). SEQ ID NO: 67 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG C γ 1), and a sequence from transforming growth factor- β (TGF- β); (RANK Ectodomain+IgG C γ 1+TGF- β) (FIG. 51). SEQ ID NO: 68 provides a fusion protein including a sequence from Programmed death-1 ligand 1 (PD-L1), immunoglobulin Fc region (IgG C γ 1), and an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK) (PD-L1+IgG C γ 1+RANK Ectodomain;

FIG. 52). SEQ ID NO: 69 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG C γ 1), and a sequence from Programmed death-1 ligand 1 (PD-L1): (RANK Ectodomain+IgG C γ 1+PD-L1) (FIG. 52).

Although the invention has been described with reference to the above example, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims.

SEQUENCE LISTING

Sequence total quantity: 124

SEQ ID NO: 1 moltype = AA length = 602

FEATURE Location/Qualifiers

source 1..602

mol_type = protein

organism = synthetic construct

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ASTKGPSVFAP LAPSSKSTSG GTAALGCLVK DYFPEPTVTS WNSGALTSGV HTFPAVLQSS 180
GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG 240
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QQGNVFSCSV MHEALHNHYT QKSLSLSPGK GGGGSGGGGS GGGGSTIPPH VQKSVNNDMI 480
VTDNNGAVKF PQCKFCFQDVR FSTCDNQKSC MSNCSITSIC EKQPQEVCAV WRKNDENITL 540
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PD 602

SEQ ID NO: 2 moltype = AA length = 601

FEATURE Location/Qualifiers

source 1..601

mol_type = protein

organism = synthetic construct

SEQUENCE: 2

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STKGPSVFPAL APSSKSTSGG TAALGCLVKD YFPEPTVTSW NSGALTSGVH TFPAPVLQSSG 180
LYSLSSSVTV PSSSLGTQTY IICNVNHKPSN TKVDKRVPEK SCDKTHTCPP CPAPELLGGP 240
SVFLFPPPKP DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS 300
TYRVVSVLT LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL 360
TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVV LDSDGSFFLYS KLTVDKSRWQ 420
QQNVFSCSV MHEALHNHYT QKSLSLSPGK GGGGSGGGGS GGGGSTIPPHV QKSVNNDMIV 480
TDNNGAVKFP QLCKFCFQDVR FSTCDNQKSCM SNCSITSICE KPQEVCAVAV RKNDENITL 540
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D 601

SEQ ID NO: 3 moltype = AA length = 603

FEATURE Location/Qualifiers

source 1..603

mol_type = protein

organism = synthetic construct

SEQUENCE: 3

QVQLQQPGAE LVKPGASVKM SCKASGYTFT SYNHWVVKQT PGRGLEWIGA IYPNGDTSY 60
NQKFKGKATL TADKSSSTAY MQLSSLTSED SAVYYCARST YYGGDWYFNV WGAGTTVTVS 120
AASSTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPTV WNSGALTSG VHTFPAVLQS 180
SGLYSLSSVV TVPSSSLGTQ TYICNVNHKPS NTKVDKKAE PKSCDKTHTC PPCAPELLG 240
GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY 300
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKT I SKAKGQPREP QVYTLPPSRD 360
ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR 420
WQQGNVFSCS VMHEALHNHY TQKSLSLSPG KGGGSGGGGS GGGGSTIPP HVQKSVNNDM 480
IVTDNNGAVK FPQLCKFCDV RFSTCDNQKS CMSNCSITSI CEKPQEVCAV VWRKNDENIT 540
LETVCHDPKL PYHDFILED A ASPKCIMEK KKPGETFFMC SCSSDECNDN IIFSEYNTS 600
NPD 603

SEQ ID NO: 4 moltype = AA length = 605

FEATURE Location/Qualifiers

source 1..605

mol_type = protein

organism = synthetic construct

SEQUENCE: 4

EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWRQA PGKGLEWVGW INTYTGEPTY 60
AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGLTVT 120
VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL 180

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QSSGLYSLSS	VVTVPSSSLG	TQTYICNVNH	KPSNTKVDKK	VEPKSCDKTH	TCPPCPAPEL	240
LGGPSVFLFP	PKPKDTLMIS	RTPEVTCVVV	DVSHEDPEVK	FNWYVDGVEV	HNAKTKPREE	300
QYNSTYRVVS	VLTVLHQDWL	NGKEYKCKVS	NKALPAPIEK	TISKAKGQPR	EPQVYTLPPS	360
REEMTKNQVS	LTCLVKGFYP	SDIAVEWESN	GQPENNYKTT	PPVLSDGSF	FLYSKLTVDK	420
SRWQQGNVFS	CSVMHEALHN	HYTQKSLSL	PGKGGGSGG	GGSGGGGSTI	PPHVQKSVNN	480
DMIVTDNNGA	VKFPQLCKFC	DVRFSTCDNQ	KSCMSNCSIT	SICEKPQEV	VAVWRKNDEN	540
ITLETVCHDP	KLPYHDFILE	DAASPKCIMK	EKKKPGETFF	MCSCSSDECN	DNIIFSEEYN	600
TSNPD						605

SEQ ID NO: 5 moltype = AA length = 600
 FEATURE Location/Qualifiers
 source 1..600
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 5

QVQLVESGGG	VVQPGSRSLRL	SCAASGFTFS	SYTMHWVRQA	PGKGLEWVTF	ISYDGNKKYY	60
ADSVKGRFTI	SRDNSLRAD	LQMNSLRAD	TAIYYCARTG	WLGPFYDYGQ	GLTVTVSSAS	120
TKGPSVFLPLA	PSSKSTSGGT	AALGCLVKDY	FPEPVTVSWN	SGALTSGVHT	FPAVLQSSGL	180
YSLSSVTVTP	SSSLGTQTYI	CNVNHHKPSNT	KVDKRVPEPKS	CDKTHTCPPC	PAPELLGGPS	240
VFLFPPKPKD	TLMISRTPEV	TCVVVDVSHE	DPEVKFNWYV	DGVEVHNAKT	KPREEQYNST	300
YRVVSVLTVL	HQDWLNGKEY	KCKVSNKALP	APIEKTISKA	KQPREPQVY	TLPPSRDEL	360
KNQVSLTCLV	KGFYPSDIAV	EWESNGQPEN	NYKTPPVLD	SDGSFFLYSK	LTVDKSRWQ	420
GNVPSCSVMH	EALHNHYTQK	SLSLSPGKGG	GGSGGGSGG	GGSTIPPHVQ	KSVNNDMIVT	480
DNMGAVKFPQ	LCKFCDFRFS	TCDNQKSCMS	NCSITSICEK	PQEVCAVAVR	KNDENITLET	540
VCHDPKLPYH	DFILEDAAAP	KCIMKEKKKP	GETFFMCSS	SDECNNDNII	SEEYNTSNPD	600

SEQ ID NO: 6 moltype = AA length = 525
 FEATURE Location/Qualifiers
 source 1..525
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 6

APTSSSTKKT	QLQLEHLLLD	LQMILNGINN	YKNPKLTRML	TFKFYMPKKA	TELKHLQCLE	60
ELKPLEEVL	NLAQSKNPHL	RPRDLISNIN	VIVLELKGSE	TTFMCEYADE	TATIVEFLNR	120
WITFCQSIIS	TLTGGGGSGG	GGSGGGSGST	TCPPCPAPEL	LGGPSVFLFP	PKPKDTLMIS	180
RTPEVTCVVV	DVSHEDPEVK	FNWYVDGVEV	HNAKTKPREE	QYNSTYRVVS	VLTVLHQDWL	240
NGKEYKCKVS	NKALPAPIEK	TISKAKGQPR	EPQVYTLPPS	REEMTKNQVS	LTCLVKGFYP	300
SDIAVEWESN	GQPENNYKTT	PPVLSDGSF	FLYSKLTVDK	SRWQQGNVFS	CSVMHEALHN	360
HYTQKSLSL	PGKGGGSGG	GGSGGGGSTI	PPHVQKSVNN	DMIVTDNNGA	VKFPQLCKFC	420
DVRFSTCDNQ	KSCMSNCSIT	SICEKPQEV	VAVWRKNDEN	ITLETVCHDP	KLPYHDFILE	480
DAASPKCIMK	EKKKPGETFF	MCSCSSDECN	DNIIFSEEYN	TSNPD		525

SEQ ID NO: 7 moltype = AA length = 525
 FEATURE Location/Qualifiers
 source 1..525
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 7

TIPPHVQKSV	NNDMIVTDNN	GAVKFPQLCK	FCDVRFSTCD	NQKSCMSNCS	ITSICEKPQE	60
VCAVAVWRKND	ENITLETVCH	DPKLPYHDFI	LEDAASPKCI	MKEKKKPGET	FFMCSSSDE	120
CNDNIIIFSEE	YNTSNPDGGG	GGSGGGSGGG	GSTHTCPPCP	APPELLGGPSV	FLFPPKPKDT	180
LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD	GVEVHNAKTK	PREEQYNSTY	RVVSVLTVLH	240
QDWLNGKEYK	CKVSNKALPA	PIEKTISKAK	GQPREPQVYT	LPPSREEMTK	NQVSLTCLVK	300
GFYPSDIAVE	WESNGQPENN	YKTPPVLDSD	DGSFFLYSKL	TVDKSRWQQG	NVFSQSVNME	360
ALHNHYTQKS	LSLSPGKGGG	GGSGGGSGGG	GSAPTSSSTK	KTQLQLEHLL	LDLQMLNGI	420
NNYKNPKLTR	MLTFKFYMPK	KATELKHLC	LEEELKPLEE	VNLQAQSKNF	HLRPRDLISN	480
INVIVLELKG	SETTFMCEYA	DETATIVEFL	NRWITFCQSI	ISTLT		525

SEQ ID NO: 8 moltype = AA length = 598
 FEATURE Location/Qualifiers
 source 1..598
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 8

QVQLVQSGAE	VKKPGSSVKV	SKKASGYTFT	SYRMHWVRQA	PQGLEWIGY	INPSTGYTEY	60
NQKFQDKATI	TADESTNTAY	MELSSLRSED	TAVYYCARGG	GVFDYWGQGT	LTVSSASTK	120
GPSVFPLAPS	SKSTSGGTAA	LGCLVKDYFP	EPVTVSWNSG	ALTSGVHTFP	AVLQSSGLYS	180
LSSVTVVPSS	SLGTQTYICN	VNHKPSNTKV	DKKVEPKSCD	KTHTCPPCPA	PELLGGPSVF	240
LFPPKPKDTL	MISRTPEVTC	VVVDVSHEDP	EVKFNWYVDG	VEVHNAKTKP	REEQYNSTYR	300
VVSVLTVLHQ	DWLNGKEYKC	KVSNKALPAP	IEKTISKAKG	QPREPQVYTL	PPSRDELTKN	360
QVSLTCLVKG	FYPSPDIAVE	ESNGQPENNY	KTPPVLDSD	GSFFLYSKLT	VDKSRWQQGN	420
VFSCSVNMEA	LHNHYTQKSL	SLSPGKGGG	GGGGSGGGG	STIPPHVQKS	VNNDMIVTDN	480
NGAVKFPQLC	KFCDFRFS	DNQKSCMSNC	SITSICEKPQ	EVCAVAVWRK	DENITLETVC	540
HDPKLPYHDF	ILEDAAAPK	IMKEKKKPG	ETFFMCSSSD	ECNDNIIIFE	EYNTSNPD	598

SEQ ID NO: 9 moltype = AA length = 597
 FEATURE Location/Qualifiers
 source 1..597
 mol_type = protein

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organism = synthetic construct

SEQUENCE: 9
QLQQSGTTLA  RPGASVKMSC  KASGYSFTRY  WMHWIKQRPQ  QGLEWIGAIY  PGNSDTSYNQ  60
KFEGKAKLTA  VTSASTAYME  LSSLTHEDSA  VYVCSRDYGY  YDFWQGQTT  LTVSSASTKG  120
PSVPPLAPSS  KSTSGGTAAL  GCLVKDYFPE  PVTVSWNSGA  LTSGVHTFPA  VLQSSGLYSL  180
SSVVTVPSSS  LGTQTYICNV  NHKPSNTKVD  KRVEPKSCDK  THTCPPCPAP  ELLGGPSVFL  240
FPPKPKDTLM  ISRTPPEVTC  VVDVSHEDPE  VKFNWYVDGV  EVHNAKTKPR  EEQYNSTYRV  300
VSVLTVLHQD  WLNQKEYKCK  VSNKALPAPI  EKTISKAKGQ  PREPQVYTL  PSRDELTKNQ  360
VSLTCLVKGF  YPSDIAVEWE  SNGQPENNYK  TTPPVLDSDG  SFFLYSKLTV  DKSRWQQGNV  420
FSCSVMHEAL  HNHYTQKSL  LSPGKGGGGS  GGGGSGGGGS  TIPPHVQKSV  NNDMIVTDNN  480
GAVKFPQLCK  FCDVRFSTCD  NQKSCMSNCS  ITSICEKPQE  VCVAVWRKND  ENITLETVCH  540
DPKLPYHDFI  LEDAASPKCI  MKEKKKPGET  FFMCSCSSDE  CNDNIIFSEE  YNTSNPD     597

SEQ ID NO: 10      moltype = AA  length = 603
FEATURE            Location/Qualifiers
source              1..603
                    mol_type = protein
                    organism = synthetic construct

SEQUENCE: 10
QVQLQEAAGPG  LVKPSSETLSL  TCSVSGGSIS  GDYYWFWIRQ  SPKGLEWIG  YIYGSGGGTN  60
YNPSLNNRVS  ISIDTSKNLF  SLKLRSVTAA  DTAVYYCASN  ILKYLHWLLY  WQQGLVTVS   120
SASTKGPSVF  PLAPSSKSTS  GGTAAAGCLV  KDYFPEPVT  SWNSGALTSG  VHTFPAVLQS  180
SGLYSLSSVV  TTPSSSLGTQ  TYICNVNHKP  SNTKVDKRV  PKSCDKTHTC  PPCPAPELLG  240
GPSVFLFPPK  PKDTLMISRT  PEVTCVVVDV  SHEDPEVKFN  WYVDGVEVHN  AKTKPREEQY  300
NSTYRVVSVL  VTLHQDWLNG  KEYKCKVSNK  ALPAPIEKTI  SKAKGQPREP  QVYTLPPSRD  360
ELTKNQVSLT  CLVKGFYPSD  IAVEWESNGQ  PENNYKTPP  VLDSDGSFFL  YSKLTVDKSR  420
WQQGNVFCSS  VMHEALHNHY  TQKSLSLSPG  KGGGSGGGGS  SGGGGSTIPP  HVQKSVNNDM  480
IVTDNNGAVK  FPQLCKFCDV  RFSTCDNQKS  CMSNCSITSI  CEKPQEVCA  VWRKNDENIT  540
LETVCHDPKL  PYHDFILED  ASPKICIMKE  KPGETFFMC  SCSSDECNDN  IIFSEYNTS   600
NPD
NP
NP

SEQ ID NO: 11      moltype = AA  length = 542
FEATURE            Location/Qualifiers
source              1..542
                    mol_type = protein
                    organism = synthetic construct

SEQUENCE: 11
PGWFLDSPDR  PWNPPFTFSPA  LLVVTEGDNA  TFTCSFSNTS  ESFVLNWMYR  SPSNQTDKLA  60
APPEDRSQPG  QDCRFRTVL  PNGRDFHMSV  VRARRNDSTG  YLCGAISLAP  KAIKESLRA   120
ELRVTERRAE  VPTAHPSPP  RPAGQFQTLV  GGGGSGGGGS  GGGGSTHTCP  PCPAPELLGG  180
PSVFLFPPKP  KDTLMISRT  EVTCVVVDVS  HEDPEVKFNW  YVDGVEVHNA  KTKPREEQYN  240
STYRVVSVLT  VLHQDWLNGK  EYKCKVSNKA  LPAPIEKTIS  KAKGQPREPQ  VYTLPPSREE  300
MTKNQVSLTC  LVKGFYPSDI  AVEWESNGQP  ENNYKTPPV  LDSDGSFFLY  SKLTVDKSRW  360
QQGNVFCSSV  MHEALHNHYT  QKSLSLSPGK  GGGGSGGGGS  GGGGSTIPPH  VQKSVNNDMI  420
VTDNNGAVKP  PQLCKFCFDR  FSTCDNQKSC  MSNCSITSIC  EKPQEVCAV  WRKNDENITL  480
ETVCHDPKLP  YHDFILEDAA  SPKICIMKEK  KPGETPFMCS  CSSDECNDNI  IFSEYNTSN   540
PD
PD

SEQ ID NO: 12      moltype = AA  length = 542
FEATURE            Location/Qualifiers
source              1..542
                    mol_type = protein
                    organism = synthetic construct

SEQUENCE: 12
TIPPHVQKSV  NNDMIVTDNN  GAVKFPQLCK  FCDVRFSTCD  NQKSCMSNCS  ITSICEKPQE  60
VCVAVWRKND  ENITLETVCH  DPKLPYHDFI  LEDAASPKCI  MKEKKKPGET  FFMCSCSSDE  120
CNDNIIFSEE  YNTSNPDGGG  GSGGGSGGGG  GSTHTCPPCP  APELLGGPSV  FLFPPKPKDT  180
LMISRTPEVT  CVVDVSHED  PEVKFNWYVD  GVEVHNAKTK  PREEQYNSTY  RVVSVLTVLH  240
QDWLNGKEYK  CKVSNKALPA  PIEKTISKAK  GQPREPQVYT  LPPSREEMTK  NQVSLTCLVK  300
GFYPSDIAVE  WESNGQPENN  YKTPPVLDSD  DGSFFLYSKL  TVDKSRWQQG  NVFSCSVMHE  360
ALHNHYTQKS  LSLSPGKGGG  GSGGGSGGGG  GSPGWFLDSP  DRPWNPPPTS  PALLVTEGD   420
NATFTCSFSN  TSESVFLNMY  RMSPSNQTDK  LAAPEDRSQ  PQGDCRFRT  QLPNGRDFHM  480
SVVRARRNDS  GTYLCGAISL  APKAQIKESL  RAEALRVTER  AEVPTAHPS  SPRPAQGFQT  540
LV
LV

SEQ ID NO: 13      moltype = AA  length = 577
FEATURE            Location/Qualifiers
source              1..577
                    mol_type = protein
                    organism = synthetic construct

SEQUENCE: 13
TIPPHVQKSV  NNDMIVTDNN  GAVKFPQLCK  FCDVRFSTCD  NQKSCMSNCS  ITSICEKPQE  60
VCVAVWRKND  ENITLETVCH  DPKLPYHDFI  LEDAASPKCI  MKEKKKPGET  FFMCSCSSDE  120
CNDNIIFSEE  YNTSNPDGGG  GSGGGSGGGG  GSTHTCPPCP  APELLGGPSV  FLFPPKPKDT  180
LMISRTPEVT  CVVDVSHED  PEVKFNWYVD  GVEVHNAKTK  PREEQYNSTY  RVVSVLTVLH  240
QDWLNGKEYK  CKVSNKALPA  PIEKTISKAK  GQPREPQVYT  LPPSREEMTK  NQVSLTCLVK  300
GFYPSDIAVE  WESNGQPENN  YKTPPVLDSD  DGSFFLYSKL  TVDKSRWQQG  NVFSCSVMHE  360
ALHNHYTQKS  LSLSPGKGGG  GSGGGSGGGG  GSQIAPPCTS  EKHYEHLGRC  CNKCEPKYKM  420
SSKCTTSDS  VCLPCGPDEY  LDSWNEEDKC  LLHKVCDTGK  ALVAVVAGNS  TTPRRCACTA  480

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GYHWSQDCEC	CRRNTECAPG	LGAQHPLQLN	KDTVCKPCLA	GYFSDAFSST	DKCRPWTNCT	540
FLGKRVRHHG	TEKSDAVCSS	SLPARKPPNE	PHVYLPG			577

SEQ ID NO: 14	moltype = AA	length = 577
FEATURE	Location/Qualifiers	
source	1..577	
	mol_type =	protein
	organism =	synthetic construct

SEQUENCE: 14						
QIAPPCTSEK	HYEHLGRCCN	KCEPGKYMSS	KCTTSDSVC	LPCGPDEYLD	SWNEEDKCLL	60
HKVCDTGKAL	VAVVAGNSTT	PRRCACTAGY	HWSQDCECCR	RNTECAPGLG	AQHPLQLNKD	120
TVCKPCLAGY	FSDAFSSTDK	CRPWTNCTFL	GKRVEHHGTE	KSDAVCSSSL	PARKPPNEPH	180
VYLPGGGGGS	GGGGGGGGGS	THTCPPCPAP	ELLGGPSVFL	FPPKPKDTLM	ISRTPEVTCV	240
VVDVSHEDPE	VKFNWYVDGV	EVHNAKTKPR	EEQYNSTYRV	VSVLTVLHQD	WLNGKEYKCK	300
VSNKALPAPI	EKTISKAKGQ	PREPQVYTLF	PSREEMTKNQ	VSLTCLVKGF	YPSDIAVEWE	360
SNGQPEMNYK	TTTPVLDSDG	SFFLYSKLTV	DKSRWQQGNV	FSCSVMHEAL	HNHYTQKSL	420
LSPGKGGGGS	GGGGGGGGGS	TIPPHVQKSV	NNDMIIVTDNN	GAVKFPQLCK	FCDVRFSTCD	480
NQKSCMSNCS	ITSICEKPQE	VCVAVWRKND	ENITLETVCH	DPKLPYHDFI	LEDAASPKCI	540
MKEKKKPGET	FFMCSCSSDE	CNDNIIFSEE	YNTSNPD			577

SEQ ID NO: 15	moltype = AA	length = 615
FEATURE	Location/Qualifiers	
source	1..615	
	mol_type =	protein
	organism =	synthetic construct

SEQUENCE: 15						
EVQLVESGGG	LVQPGGSLRL	SCAASGFNIK	DTYIHWVRQA	PGKGLEWVAR	IYPTNGYTRY	60
ADSVKGRFTI	SADTSKNTAY	LQMNSLRAED	TAVYYCSRWG	GDGFYAMDYW	GQGLVTVSS	120
ASTKGPSVF	LAPSSKSTSG	GTAAAGCLVK	DYFPEPVTVS	WNSGALTSGV	HTFPAVLQSS	180
GLYSLSSVVT	VPSSSLGTQT	YICNVNHPKS	NTKVDKKVEP	KSCDKTHTCP	PCPAPELLGG	240
PSVFLFPPPK	KDTLMISRT	EVTCVVVDVS	HEDPEVKFNW	YVDGVEVHNA	KTKPREEQYN	300
STYRVVSVLT	VLHQDWLNGK	EYKCKVSNKA	LPAPIEKTIS	KAKGQPREPQ	VYTLPPSREE	360
MTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPV	LDSDGSFFLY	SKLTVDKSRW	420
QQGNVFSCSV	MHEALHNHYT	QKSLSLSPGK	GGGGSGGGGS	GGGGSPGWFL	DSPDRPWNPP	480
TFSPALLVVT	EGDNATFTCS	FSNTSESVFL	NWYRMSPSNQ	TDKLAAPFED	RSQPGQDCRF	540
RVTQLPNGRD	FHMSVVRARR	NDSGTYLCGA	ISLAPKAQIK	ESLRAELRVT	ERRAEVPTAH	600
PSPSPRPAGQ	FQTLV					615

SEQ ID NO: 16	moltype = AA	length = 614
FEATURE	Location/Qualifiers	
source	1..614	
	mol_type =	protein
	organism =	synthetic construct

SEQUENCE: 16						
QVQLKQSGPG	LVQPSQSLSI	TCTVSGFSLT	NYGVHWRQ	PGKGLEWLVG	IWSGGNTDYN	60
TPFTSRSLIN	KDNSKSQVFF	KMNSLQSN	DTYIHWVRQA	YDYEFAYWG	QGLTVTVSAA	120
STKGPSVFPL	APSSKSTSGG	TAALGCLVKD	YFPEPVTVS	NSGALTSGVH	TFPAVLQSSG	180
LYSLSSVVT	PSSSLGTQTY	ICNVNHPSP	TKVDKRVPEK	SCDKTHTCPP	CPAPELLGGP	240
SVFLFPPPK	DTLMISRTPE	VTCVVVDVSH	EDPEVKFNWY	VDGVEVHNAK	TKPREEQYNS	300
TYRVVSVLTV	LHQDWLNGKE	YKCKVSNKAL	PAPIEKTISK	AKGQPREPQV	YTLPPSRDEL	360
TKNQVSLTCL	VKGFPYSDIA	VEWESNGQPE	NNYKTTPPVL	DSGDSFFLYS	KLTVDKSRWQ	420
QGNVFSCSV	HEALHNHYTQ	KSLSLSPGKG	GGGGSGGGSG	GGGGSPGWFLD	SPDRPWNPP	480
FSPALLVVT	EGDNATFTCSF	SNTESESVLN	WYRMSPSNQT	DKLAAPFEDR	SQPGQDCRFR	540
VTQLPNGRDF	HMSVVRARRN	DSGTYLCGAI	SLAPKAQIKE	SLRAELRVTE	RRAEVPTAHP	600
SPSPRPAGQF	QTLV					614

SEQ ID NO: 17	moltype = AA	length = 616
FEATURE	Location/Qualifiers	
source	1..616	
	mol_type =	protein
	organism =	synthetic construct

SEQUENCE: 17						
QVQLQQPGAE	LVKPGASVKM	SCKASGYTFT	SYNMHWVKQT	PGRGLEWIGA	IYPNGDTSY	60
NQKFKGKATL	TADKSSSTAY	MQLSSLTSED	SAVYYCARST	YYGGDWYFNV	WGAGTTVTVS	120
AASTKGPSVF	PLAPSSKSTS	GGTAALGCLV	KDYFPEPVT	SWNSGALTSG	VHTFPAVLQS	180
SGLYSLSSVV	TVPSSSLGTQ	TYICNVNHPK	SNTKVDKKA	PKSCDKTHTC	PPCPAPELLG	240
GPSVFLFPPK	PKDTLMISRT	PEVTCVVVDV	SHEDPEVKFN	WYVDGVEVHN	AKTKPREEQY	300
NSTYRVVSVL	TVLHQDWLNG	KEYKCKVSNK	ALPAPIEKT	SKAKGQPREP	QVYTLPPSRD	360
ELTKNQVSLT	CLVKGFPYSD	IAVEWESNGQ	PENNYKTTP	VLDSDGSFFL	YSKLTVDKSR	420
WQQGNVFSCS	VMHEALHNHY	TQKSLSLSPG	KGGGGSGGGG	SGGGSPGWFL	LDSPPDRPWN	480
PTFSALLVTV	TEGDNATFTC	SFSNTSESV	LNWYRMSPSN	QTDKLAAPFE	DRSQPGQDCR	540
FRVTQLPNGR	DFHMSVVRAR	RNDSGTYLCG	AISLAPKAQ	KESLRAELRV	TERRAEVPTA	600
HPSPSPRPAG	QFQTLV					616

SEQ ID NO: 18	moltype = AA	length = 618
FEATURE	Location/Qualifiers	
source	1..618	
	mol_type =	protein
	organism =	synthetic construct

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SEQUENCE: 18
EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWRVQA PGKGLEWVGW INTYTGEPTY 60
AADFKRRFTI SLDTSKSTAY LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGTLLVT 120
VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL 180
QSSGLYSLSL VVTVPSSSLG TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL 240
LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE 300
QYNSTYRVVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS 360
REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK 420
SRWQQGNVFS CSVMHEALHN HYTKSLSL SLS PGKGGGGSGG GSGGGGGSPG WFLDSPDRPW 480
NPPTFSALL VVTEGDNATF TCSFNTSES FVLNWRMSP SNQTDKLAAP PEDRSQPGQD 540
CRFRVTQLPN GRDFHMSVVR ARRNDSGTYL CGAISLAPKA QIKESLRAEL RVTERRAEVP 600
TAHPSPSPRP AGQFQTLV

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SEQ ID NO: 19      moltype = AA length = 613
FEATURE            Location/Qualifiers
source             1..613
                   mol_type = protein
                   organism = synthetic construct

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SEQUENCE: 19
QVQLVESGGG VVQPGSLRL SCAASGTFPS SYTMHWVRQA PGKGLEWVTF ISYDGNKKY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGQ GTLVTVSSAS 120
TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL 180
YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVPEKS CDKTHTCPCC PAPELLGGPS 240
VFLPPKPKD TLMISRTPEV TCVVDVSHD DPEVKFNWYV DGEVHNNAKT KPREEQYNST 300
YRVVSVLTLP HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPSPRDEL 360
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ 420
GNVFSCSVMH EALHNHYTQK SLSLSPGKGG GSGGGGGSGG GSGPGWFLDS PDRPWNPTF 480
SPALLVVTGE DNATFTCSFS NTSEFVLNW YRMSPSNQTD KLAAPFEDRS QPGQDCRFRV 540
TQLPNGRDFH MSVVRARRND SGTLYCGAIS LAPKAQIKES LRAELRVTER RAEVPTAHP 600
PSPRPAQGFQ TLV

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SEQ ID NO: 20      moltype = AA length = 611
FEATURE            Location/Qualifiers
source             1..611
                   mol_type = protein
                   organism = synthetic construct

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SEQUENCE: 20
QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY 60
NQKFKDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT GTLVTVSSASTK 120
GPSVFPLAPS SKTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS 180
LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPCCPA PELLGGPSVF 240
LFPKPKDITL MISRTPEVTC VVDVSHEDP EVKFNWYVDG VEVHNNAKTP REEQYNSTYR 300
VVSVLTVLHQ DWLNGKEYKC KVSNNKALP IEKTISKAKG QPREPQVYTL PPSRDELTKN 360
QVSLTCLVKG FYPSDIAVEW ESNQGPENNY KTTTPPVLDSD GSFFLYSKLT VDKSRWQQGN 420
VFSCSVMH EA LHNHYTQKSL SLSPGKGGGG GSGGGGGSGG SPGWFLDSPD RPNWNPPTFSP 480
ALLVVTGEDN ATFTCSFSNT SESFVLNWR MSPSNQTDKL AAFPEDRSQP QDCRFRVTQ 540
LPNGRDFHMS VVRARRND SG TYLCAISLA PKAQIKESLR AELRVTERRA EVPTAHPSPS 600
PRPAGQFQTL V

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SEQ ID NO: 21      moltype = AA length = 610
FEATURE            Location/Qualifiers
source             1..610
                   mol_type = protein
                   organism = synthetic construct

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SEQUENCE: 21
QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNO 60
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YDFWQGTGT LTIVSSASTKG 120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL 180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPCCPAP ELLGGPSVFL 240
FPPKPKDITL ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNNAKTKPR EEQYNSTYRV 300
VSVLTVLHQD WLNKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTL PPSRDELTKN 360
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRRWQQGNV 420
FSCSVMEAL HNHYTQKSL SLSPGKGGGG GSGGGGGSGG PGWFLDSPDR PWNPTFSPA 480
LLVVTGEDNA TFTCSFSNTS ESFVLNWRM SPSNQTDKLA APPEDRSQPG QDCRFRVTQL 540
PNGRDFHMSV VRARRND SG TYLCAISLAP KAIKESLRA ELRVTERRAE VPTAHPSPSP 600
RPAGQFQTLV

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SEQ ID NO: 22      moltype = AA length = 538
FEATURE            Location/Qualifiers
source             1..538
                   mol_type = protein
                   organism = synthetic construct

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SEQUENCE: 22
APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA TELKHLQCLE 60
EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR 120
WITFCQSIIS TLTGGGGSGG GSGGGGGSTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS 180
RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL 240
NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP 300
SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN 360

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HYTQKSLSL	PGKGGGGSGG	GGSGGGGSPG	WFLDSPDRPW	NPPTFSPALL	VVTEGDNATF	420
TCSFSNTSES	FVLNWMYRMS	SNQTDKLA	PEDRSQPGQD	CRFRVTQLPN	GRDFHMSVVR	480
ARRNDSGTYL	CGAISLAPKA	QIKESLRAEL	RVTERRAEVP	TAHPSPSPRP	AGQFQTLV	538

SEQ ID NO: 23 moltype = AA length = 538
 FEATURE Location/Qualifiers
 source 1..538
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 23

PGWFLDSPDR	PWNPTTFSPA	LLVVTEGDNA	TFTCSFSNTS	ESFVLNWMYR	SPSNQTDKLA	60
AFPEDRSQPG	QDCRFRVTQL	PNGRDFHMSV	VRARRNDSGT	YLCGAISLAP	KAQIKESLRA	120
ELRVTERRAE	VPTAHPSPPSP	RPAGQFQTLV	GGGGSGGGGS	GGGGSTHTCP	PCPAPELLGG	180
PSVFLFPPKP	KDTLMISRTP	EVTCTVVVDVS	HEDPEVKFNW	YVDGVEVHNA	KTKPREEQYN	240
STYRVVSVLT	VLHQDWLNGK	EYKCKVSNKA	LPAPIEKTIS	KAKGQPREPQ	VYTLPPSREE	300
MTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPV	LDSDGSFFLY	SKLTVDKSRW	360
QQGNVFSCSV	MHEALHNHYT	QKSLSLSPGK	GGGGSGGGGS	GGGGSAPTSS	STKKTQLQLE	420
HLLLDLQML	NGINNYKNPK	LTRMLTFKFY	MPKKATELKH	LQCLEEELKP	LEEVLNLAQS	480
KNFHLRPRDL	ISNINVIIVLE	LKGSSETTFC	EYADETATIV	EPLNRWITFC	QSIISTLT	538

SEQ ID NO: 24 moltype = AA length = 616
 FEATURE Location/Qualifiers
 source 1..616
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 24

QVQLQEAGPG	LVKPSETLSL	TCSVSGGSIS	GDYVFWIRQ	SPGKGLEWIG	YIYGSGGGTN	60
YNPSLNNRVS	ISIDTSKNLF	SLKLRSVTAA	DTAVYYCASN	ILKYLHWLLY	WGQGVLVTVS	120
SASTKGPSVF	PLAPSSKSTS	GGTAALGCLV	KDYFPEPVTV	SWNSGALTSG	VHTFPAVLQS	180
SGLYSLSSVV	TVPSSSLGTQ	TYICNVNHKP	SNTKVDKRVE	PKSCDKTHTC	PPCPAPELLG	240
GPSVFLFPPK	PKDTLMISRTP	PEVTCVVVDV	SHEDPEVKFN	WYVDGVEVHN	AKTKPREEQY	300
NSTYRVVSVL	TVLHQDWLNG	KEYKCKVSNK	ALPAPIEKT	SKAKGQPREP	QVYTLPPSRD	360
BLTKNQVSLT	CLVKGFYPSD	IAVEWESNGQ	PENNYKTTPP	VLDSGGSFFL	YSKLTVDKSR	420
WQQGNVFSCSV	VMHEALHNHY	TQKSLSLSPG	KGGGGSGGGG	SGGGSGPGWF	LDSPDRPWNP	480
PTFSPALLVV	TEGDNATFTC	SFSNTSESFV	LNWYRMSPSN	QTDKLAAPPE	DRSQPGQDCR	540
FRVTQLPNGR	DFHMSVVRAR	RNDSGTYLCG	AILAPKAQI	KESLRAELRV	TERRAEVPTA	600
HPSPSPRPAG	QFQTLV					616

SEQ ID NO: 25 moltype = AA length = 590
 FEATURE Location/Qualifiers
 source 1..590
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 25

QIAPPCTSEK	HYEHLGRCCN	KCEPGKYMSS	KCTTSDSVC	LPCGPDEYLD	SWNEEDKCLL	60
HKVCDTGKAL	VAVVAGNSTT	PRRCCTAGY	HWSQDCECCR	RNTECAPGLG	AQHPLQLNKD	120
TVCKPCLAGY	FSDAFSSTDK	CRPWTNCTFL	GKRVEHHGTE	KSDAVCSSSL	PARKPPNEPH	180
VYLPGGGGGS	GGGGSGGGGS	THTCPPCPAP	ELLGGPSVFL	FPPKPKDTLM	ISRTPEVTCV	240
VVDVSHEDPE	VKFNWYVDG	EVHNAKTKPR	EEQYNSTYRV	VSVLTVLHQD	WLMGKEYCKK	300
VSNKALPAPI	EKTISKAKGQ	PREPQVYTL	PSREEMTKNQ	VSLTCLVKGF	YPSDIAVEWE	360
SNQGPENNYK	TTPPVLDSDG	SFFLYSKLTV	DKSRWQQGNV	FSCSVMHEAL	HNHYTQKSL	420
LSPGKGGGGS	GGGGSGGGGS	PGWFLDSPDR	PWNPTTFSPA	LLVVTEGDNA	TFTCSFSNTS	480
ESFVLNWMYR	SPSNQTDKLA	AFPEDRSQPG	QDCRFRVTQL	PNGRDFHMSV	VRARRNDSGT	540
YLCGAISLAP	KAQIKESLRA	ELRVTERRAE	VPTAHPSPPSP	RPAGQFQTLV		590

SEQ ID NO: 26 moltype = AA length = 590
 FEATURE Location/Qualifiers
 source 1..590
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 26

PGWFLDSPDR	PWNPTTFSPA	LLVVTEGDNA	TFTCSFSNTS	ESFVLNWMYR	SPSNQTDKLA	60
AFPEDRSQPG	QDCRFRVTQL	PNGRDFHMSV	VRARRNDSGT	YLCGAISLAP	KAQIKESLRA	120
ELRVTERRAE	VPTAHPSPPSP	RPAGQFQTLV	GGGGSGGGGS	GGGGSTHTCP	PCPAPELLGG	180
PSVFLFPPKP	KDTLMISRTP	EVTCTVVVDVS	HEDPEVKFNW	YVDGVEVHNA	KTKPREEQYN	240
STYRVVSVLT	VLHQDWLNGK	EYKCKVSNKA	LPAPIEKTIS	KAKGQPREPQ	VYTLPPSREE	300
MTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPV	LDSDGSFFLY	SKLTVDKSRW	360
QQGNVFSCSV	MHEALHNHYT	QKSLSLSPGK	GGGGSGGGGS	GGGGSQIAPP	CTSEKHYEHL	420
GRCCNKCEPG	KYMSSKCTTT	SDSVCLPCGP	DEYLDWNEE	DKCLLHKVCD	TGKALVAVVA	480
GNSTTPRRCA	CTAGYHWSQD	CECCRRNTEC	APGLGAQHPL	QLNKDITVCKP	CLAGYFSDAF	540
SSTDKCPWT	NCTPLGKRVE	HHGTEKSDAV	CSSSLPARKP	PNEPHVYLP		590

SEQ ID NO: 27 moltype = AA length = 650
 FEATURE Location/Qualifiers
 source 1..650
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 27

EVQLVESGGG	LVPQGGSLRL	SCAASGFNIK	DTYIHWVRQA	PGKGLEWVAR	IYPTNGYTRY	60
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ADSVKGRFTI	SADTSKNTAY	LQMNSLRAED	TAVYYCSRWG	GDGFYAMDYV	GQGTLLTVTVSS	120
ASTKGPSVFP	LAPSSKSTSG	GTAALGCLVK	DYFPEPVTVS	WNSGALTSGV	HTFPAVLQSS	180
GLYSLSSVVT	VPSSSLGTQT	YICNVNHKPS	NTKVDKKVEP	KSCDKTHTCP	PCPAPELLGG	240
PSVFLFPPPK	KDTLMSRTP	EVTCTVVDVS	HEDPEVKFNW	YVDGVEVHNA	KTKPREEQYN	300
STYRVVSVLT	VLHQDWLNGK	EYKCKVSNKA	LPAPIEKTIS	KAKGQPREPQ	VYTLPPSREE	360
MTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPV	LDSGGSFFLY	SKLTVDKSRW	420
QQGNVVFCSV	MHEALHNHYT	QKSLSLSPGK	GGGSGGGGGS	GGGGSQIAPP	CTSEKHYEHL	480
GRCCNKCEPG	KYMSKCTTTT	SDSVCLPCGP	DEYLDSWNEE	DKCLLHKVCD	TGKALVAVVA	540
GNSTTPRRCA	CTAGYHWSQD	CECCRRNTEC	APGLGAQHPL	QLNKDTCVCK	CLAGYFSDAF	600
SSTDKCRPWT	NCTFLGKRVE	HGTEKSDAV	CSSSLPARKP	PNEPHVYLPG		650

SEQ ID NO: 28 moltype = AA length = 649
 FEATURE Location/Qualifiers
 source 1..649
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 28

QVQLKQSGPG	LVQPSQSLSI	TCTVSGFSLT	NYGVHWVRQS	PGKGLEWLGV	IWSGGNTDYN	60
TPFTSRLSIN	KDNSKSQVFF	KMNSLQSNLT	AIYYCARALT	YDYEFAYWG	QGTLLTVSAA	120
STKGPSVFP	APSSKSTSGG	TAALGCLVKD	YFPEPVTVS	NSGALTSVGH	TTPAVLQSSG	180
LYSLSSVVT	PSSSLGTQTY	ICNVNHKPSN	TKVDKRVKPK	SCDKTHTCPP	CPAPELLGGP	240
SVFLFPPKPK	DTLMISRTP	VTCVVVDVSH	EDPEVKFNWY	VDGVEVHNAK	TKPREEQYNS	300
TYRVVSVLTV	LHQDWLNGKE	YKCKVSNKAL	PAPIEKTISK	AKGQPREPQV	YTLPPSRDEL	360
TKNQVSLTCL	VKGFPYSDIA	VEWESNGQPE	NNYKTTPPVL	DSGGSFFLYS	KLTVDKSRWQ	420
QGNVVFSCVM	HEALHNHYTQ	KSLSLSPGKG	GGSGGGGGSG	GGGSQIAPPC	TSEKHYEHLG	480
RCCNKCEPGK	YMSKCTTTTS	DSVCLPCGPD	EYLDSWNEED	KCLLHKVCDT	GKALVAVVAG	540
NSTTPRRCAC	TAGYHWSQDC	ECCRRNTECA	PGLGAQHPLQ	LNKDTVCCKP	LAGYFSDAFS	600
STDKCRPWTN	CTFLGKRVEH	HGTEKSDAVC	SSSLPARKPP	NEPHVYLPG		649

SEQ ID NO: 29 moltype = AA length = 651
 FEATURE Location/Qualifiers
 source 1..651
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 29

QVQLQQPGAE	LVKPGASVKM	SKKASGYTFT	SYNMHWVKQT	PGRGLEWIGA	IYPNGDTSY	60
NQKFKGKATL	TADKSSSTAY	MLSSSLTSED	SAVYYCARST	YGGDWYFNV	WGAGTTVTVS	120
AATSKGPSVF	PLAPSSKSTS	GGTAALGCLV	KDYFPEPVTV	SWNSGALTSG	VHTFPAVLQS	180
SGLYSLSSVV	TVPSSSLGTQ	TYICNVNHKP	SNTKVDKKA	PKSCDKTHTC	PPCPAPELLG	240
GPSVFLFPPK	PKDTLMISR	PEVTCVVVDV	SHEDPEVKFN	WYVDGVEVHN	AKTKPREEQY	300
NSTYRVVSVL	TVLHQDWLNG	KEYKCKVSNK	ALPAPIEKT	SKAKGQPREP	QVYTLPPSRD	360
ELTKNQVSLT	CLVKGFPYSD	IAVEWESNGQ	PENNYKTTPP	VLDSDGSFFL	YSKLTVDKSR	420
WQQGNVFCSS	VMHEALHNHY	TQKSLSLSPG	KGGGGSGGGG	SGGGGSQIAP	PCTSEKHYEH	480
LGRCCKNKEP	GKYMSSKCTT	TSDSVCLPCG	PDEYLDSWNE	EDKCLLHKVC	DTGKALVAVV	540
AGNSTTPRRC	ACTAGYHWSQ	DCECCRRNTE	CAPGLGAQHP	LQLNKDTCVCK	PCLAGYFSDA	600
FSSTDKCRPW	TNCTFLGKR	EHHGTEKSDA	VCSSSLPARK	PNNEPHVYLP	G	651

SEQ ID NO: 30 moltype = AA length = 653
 FEATURE Location/Qualifiers
 source 1..653
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 30

EVQLVESGGG	LVQPGGSLRL	SCAASGYTFT	NYGMNWRQA	PGKGLEWVGW	INTYTGEPTY	60
AADFRRFTF	SLDTSKSTAY	LQMNSLRAED	TAVYYCAKYP	HYGSSHWYF	DVWGQGTLLV	120
VSSASTKGPS	VFPLAPSSKS	TSGGTAALGC	LVKDYFPEPV	TVSWNSGALT	SGVHTFPAVL	180
QSSGLYSLSS	VVTVPSSSLG	TQTYICNVNH	KPSNTKVDK	VEPKSCDKTH	TCPPCPAPEL	240
LGGPSVFLFP	PKPKDTLMIS	RTPEVTCVVV	DVSHEDPEVK	FNWYVDGVEV	HNAKTTPREE	300
QYNSTYRVVS	VLTVLHQDWL	NGKEYKCKVS	NKALPAPIEK	TISKAKGQPR	EPQVYTLPPS	360
REEMTKNQVS	LTCLVKGFYP	SDIAVEWESN	GQPENNYKTT	PPVLDSDGSF	FLYSKLTVDK	420
SRWQQGNVFS	CSVMHEALHN	HYTQKSLSL	PGKGGGGSGG	GGSGGGGSQI	APPCTSEKHY	480
EHLGRCCNKC	EPGKYMSSKC	TTTSDSVCLP	CGPDEYLDSW	NEEDKCLLHK	VCDTGKALVA	540
VVAGNSTTPR	RCAC TAGYHW	SQDCECCRRN	TECAPGLGAQ	HPLQLNKDTC	CKPCLAGYFS	600
DAFSSDCKCR	PWTNCTFLGK	RVEHHGTEKS	DAVCSSSLPA	RKPPNEPHVY	LPG	653

SEQ ID NO: 31 moltype = AA length = 648
 FEATURE Location/Qualifiers
 source 1..648
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 31

QVQLVESGGG	VVQPGSRSLR	SCAASGFTFS	SYTMHWVRQA	PGKGLEWVTF	ISYDGNKKY	60
ADSVKGRFTI	SRDNSKNTLY	LQMNSLRAED	TAIYYCARTG	WLGPPFDYWG	GTLTVTVSSAS	120
TKGPSVFPLA	PSSKSTSGGT	AALGCLVKDY	FPEPVTVSWN	SGALTSGVHT	FPAVLQSSGL	180
YSLSSVVTVP	SSSLGTQTYI	CNVNHKPSNT	KVDKRVKPKS	CDKTHTCPPC	PAPELLGGPS	240
VFLFPPKPKD	TLMISRTP	TCVVVDVSH	DPEVKFNWYV	DGVEVHNAKT	KPREEQYNST	300
YRVVSVLTVL	HQDWLNGKEY	KCKVSNKALP	APIEKTISKA	KGQPREPQVY	TLPPSRDEL	360
KNQVSLTCLV	KGFYPSDIAV	EWESNGQPEN	NYKTTPPVLD	SDGSFFLYSK	LTVDKSRWQQ	420
GNVVFSCVMH	EAALHNHYTQ	SLSLSPGKGG	GGSGGGGGSG	GGSQIAPPCT	SEKHYEHLGR	480

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CCNKCEPGKY	MSSKCTTTSD	SVCLPCGPDE	YLDWNEEDK	CLLHKVCDTG	KALVAVVAGN	540
STTPRRCACT	AGYHWSQDCE	CCRRNTECAP	GLGAQHPLQL	NKDTVCKPCL	AGYFSDAFSS	600
TDKCRPWTNC	TFLGKRVEHH	GTEKSDAVCS	SSLPARKPPN	EPHVYLP		648

SEQ ID NO: 32 moltype = AA length = 646
 FEATURE Location/Qualifiers
 source 1..646
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 32

QVQLVQSGAE	VKKPGSSSVK	SKKASGYTFT	SYRMHWVRQA	PGQGLEWIGY	INPSTGYTEY	60
NQKPKDKATI	TADESTNTAY	MELSSLRSED	TAVYYCARGG	GVFDYWGQGT	LTVSSASTK	120
GPSVFPLAPS	SKSTSGGTAA	LGCLVKDYFP	EPVTVSWNSG	ALTSGVHTFP	AVLQSSGLYS	180
LSSVVTVPSS	SLGTQTYICN	VNHKPSNTKV	DKKVEPKSCD	KTHTCPKCPA	PELLGGPSVF	240
LFPPKPKDTL	MISRTPEVTC	VVDVSHEDP	EVKFNWYVDG	VEVHNAKTKP	REEQYNSTYR	300
VVSVLTVLHQ	DWLNKEYKCK	KVSNKALPAP	IEKTISKAKG	QPREPQVYTL	PPSRDELTKN	360
QVSLTCLVKG	FYPSDIAVEW	ESNGQPENNY	KTPPVLDSD	GSFFLYSKLT	VDKSRWQQGN	420
VFSCVMHEA	LHNHYTQKSL	SLSPGKGGGG	SGGGSGGGG	SQIAPPCTSE	KHYEHLGRCC	480
NKCEPGKYM	SKCTTSDSV	CLPCGPDEYL	DSWNEEDKCL	LHKVCDTGKA	LVAVVAGNST	540
TPRRCACTAG	HWSQDCECC	RRNTECAPGL	GAQHPLQLNK	DTVCKPCLAG	YFSDAFSSST	600
KCRPWTNCTF	LGKRVEHHGT	EKSDAVCSSS	LPARKPPNEP	HVYLP		646

SEQ ID NO: 33 moltype = AA length = 645
 FEATURE Location/Qualifiers
 source 1..645
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 33

QLQQSGTVLA	RPGASVKMSC	KASGYSFTRY	WMHWIKQRP	QGLEWIGAIY	PGNSDTSYNQ	60
KFEGKAKLTA	VTSASTAYME	LSSLTHEDSA	VYVCSRDIY	YFDWFQGGT	LTVSSASTKG	120
PSVPPLAPSS	KSTSGGTAA	GCLVKDYFPE	PVTVSWNSGA	LTSVGHVTFP	VLQSSGLYS	180
SSVVTVPSSS	LGTQTYICNV	NHKPSNTKVD	KRVEPKSCDK	THTCPKCPAP	ELLGGPSVFL	240
FPPKPKDTLM	ISRTPEVTCV	VVDVSHEDPE	VKFNWYVDGV	EVHNAKTKPR	EEQYNSTYRV	300
VSVLTVLHQD	WLNKEYKCK	VSNKALPAPI	EKTISKAKGQ	PREPQVYTL	PPSRDELTKNQ	360
VSLTCLVKGF	YPSDIAVEWE	SNQGPENNYK	TTPPVLDSDG	SFFLYSKLTV	DKSRWQQGNV	420
FSCVMHEAL	HNHYTQKSL	LSPGKGGGGS	GGGGSGGGGS	QIAPPCTSEK	HYEHLGRCCN	480
KCEPGKYMSS	KCTTSDSVC	LPCGPDEYLD	SWNEEDKCLL	HKVCDTGKAL	VAVVAGNSTT	540
PRRCACTAGY	HWSQDCECCR	RNTECAPGLG	AQHPLQLNKD	TVCKPCLAGY	FSDAFSSSTDK	600
CRPWTNCTFL	GKRVEHHGTE	KSDAVCSSSL	PARKPPNEPH	VYLP		645

SEQ ID NO: 34 moltype = AA length = 573
 FEATURE Location/Qualifiers
 source 1..573
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 34

APTSSSTKKT	QLQLEHLLLD	LQMILNGINN	YKNPKLTRML	TFKFYMPKKA	TELKHLQCLE	60
EELKPLEEVL	NLAQSKNFHL	RPRDLISNIN	VIVLELKGSE	TTFMCEYADE	TATIVEFLNR	120
WITFCQSIIS	TLTGSGGSGG	GGSGGGGSG	TCPPCPAPEL	LGGPSVFLFP	PKPKDTLMIS	180
RTPEVTCVVV	DVSHEDPEVK	FNWYVDGVEV	HNAKTKPRE	QYNSTYRVVS	VLTVLHQDWL	240
NGKEYKCKVS	NKALPAPIEK	TISKAKGQPR	EPQVYTLPPS	REEMTKNQVS	LTCLVKGFYP	300
SDIAVEWESN	GPPENNYKTT	PPVLDSDGSF	FLYSKLTVDK	SRWQQGNVFS	CSVMHEALHN	360
HYTKSLSLSL	PGKGGGSGG	GGSGGGGSI	APPCTSEKHY	EHLGRCCNKC	EPGKYMSSKC	420
TTTSDSVCLP	CGPDEYLDW	NEEDKCLLHK	VCDTGKALVA	VVAGNSTTPR	RCACTAGYHW	480
SQDCECCRRN	TECAPGLGAG	HPLQLNKDTV	CKPCLAGYFS	DAFSSSTDKCR	PWTNCTFLGK	540
RVEHHGTEKS	DAVCSSSLPA	RKPPNEPHVY	LPG			573

SEQ ID NO: 35 moltype = AA length = 573
 FEATURE Location/Qualifiers
 source 1..573
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 35

QIAPPCTSEK	HYEHLGRCCN	KCEPGKYMSS	KCTTSDSVC	LPCGPDEYLD	SWNEEDKCLL	60
HKVCDTGKAL	VAVVAGNSTT	PRRCACTAGY	HWSQDCECCR	RNTECAPGLG	AQHPLQLNKD	120
TVCKPCLAGY	FSDAFSSSTDK	CRPWTNCTFL	GKRVEHHGTE	KSDAVCSSSL	PARKPPNEPH	180
VYLPGGGSGG	GGGGSGGGG	THTCPKCPAP	ELLGGPSVFL	FPPKPKDTLM	ISRTPEVTCV	240
VVDVSHEDPE	VKFNWYVDGV	EVHNAKTKPR	EEQYNSTYRV	VSVLTVLHQD	WLNKEYKCK	300
VSNKALPAPI	EKTISKAKGQ	PREPQVYTL	PSREEMTKNQ	VSLTCLVKGF	YPSDIAVEWE	360
SNQGPENNYK	TTPPVLDSDG	SFFLYSKLTV	DKSRWQQGNV	FSCVMHEAL	HNHYTQKSL	420
LSPGKGGGGS	GGGGSGGGG	APTSSSTKKT	QLQLEHLLLD	LQMILNGINN	YKNPKLTRML	480
TFKFYMPKKA	TELKHLQCLE	EELKPLEEVL	NLAQSKNFHL	RPRDLISNIN	VIVLELKGSE	540
TTFMCEYADE	TATIVEFLNR	WITFCQSIIS	TLT			573

SEQ ID NO: 36 moltype = AA length = 651
 FEATURE Location/Qualifiers
 source 1..651
 mol_type = protein
 organism = synthetic construct

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SEQUENCE: 36
 QVQLQEAGPG LVKPSSETLSL TCSVSGGSGS GDYYWFWIRQ SPKGKLEWIG YIYGSGGGTN 60
 YNPSLNNRVS ISIDTSKNLF SLKLRSVTAA DTAVYYCASN ILKYLHWLLY WGQGLVLTVS 120
 SASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYPPEPVTV SWNSGALTSG VHTFPAVLQS 180
 SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKRVK PKSCDKTHTC PPCPAPELLG 240
 GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY 300
 NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD 360
 ELTKNQVSLT CLVKGFPYPSD IAVEWESNGQ PENNYKTPPP VLDSDGSFPL YSKLTVDKSR 420
 WQQGNVFCSS VMHEALHNHY TQKSLSLSPG KGGGSGGGG SGGGSGQIAP PCTSEKHYEH 480
 LGRCCNKCEP GKYMSSKCTT TSDSVCLPCG PDEYLDWNE EDKCLLHKVC DTGKALVAVV 540
 AGNSTTPRRR ACTAGYHNSQ DCECCRRNTE CAPGLGAQHP LQLNKDITVCK PCLAGYPSDA 600
 FSTDKCRPW TNCTFLGKRV EHHGTEKSDA VCSSSLPARK PPNEPHVLP G 651

SEQ ID NO: 37 moltype = AA length = 755
 FEATURE Location/Qualifiers
 source 1..755
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 37
 EVQLVESGGG LVQPGSRSLRL SCAASGFTFD DYAMHWVRQA PGKGLEWVSA ITWNSGHIDY 60
 ADSVEGFRFTI SRDNNAKNSLY LQMNSLRAED TAVYYCALVS YLSTASSLDY WGQGLVLTVS 120
 SASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYPPEPVTV SWNSGALTSG VHTFPAVLQS 180
 SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKKA E PKSCDKTHTC PPCPAPELLG 240
 GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY 300
 NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD 360
 ELTKNQVSLT CLVKGFPYPSD IAVEWESNGQ PENNYKTPPP VLDSDGSFPL YSKLTVDKSR 420
 WQQGNVFCSS VMHEALHNHY TQKSLSLSPG KGGGSGGGG SGGGSGRIFA VFIFMTYWHL 480
 LNAFTVTVPK DLYVVEYGSN MTIECKFPVE KQLDLAALIV YWEMEDKNII QFVHGEEEDLK 540
 VQHSYSYRQRA RLLKQDLSLG NAALQITDVK LQDAGVYRCM ISYGGADYKR ITVKVNAPYN 600
 KINQRILVVD PVTSEHETC QAEQYKAEV IWTSSDHQVL SGKTTTNSK REEKLFNVT 660
 TLRINTTNE IFYCTFRRLD PEENHTAELV IPELPLAHP NERTHLVILG AILLCLGVAL 720
 TFIPLRLKGR MMDVKKCGIQ DNTSKKQSDT HLEET 755

SEQ ID NO: 38 moltype = AA length = 771
 FEATURE Location/Qualifiers
 source 1..771
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 38
 LPAQVAFPTY APEPGSTCRL REYYDQTAQM CCSKCSGQGH AKVFCTKTS TVCDSCEDST 60
 YTQLWNWVPE CLSCGSRCSS DQVETQACTR EQNRICTRP GWYCALSKQE GCRLCAPLRK 120
 CRPGFGVARP GTETSDVCK PCAPGTFSNT TSSTDICRPH QICNVVAIPG NASMDAVCTS 180
 TSPTSRMAPG AVHLPPQVST RSQHTQPTPE PSTAPSTSFL LPMGPSPPAE GSTGDEPKSC 240
 DKHTTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD 300
 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK 360
 GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLD 420
 DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGKGGG GSGGGSGGGG 480
 GSRIFAVFIF MTYVHLLNAF TVTVPKDLYV VEYGSNMTIE CKFPVEKQLD LAALIVYWE 540
 EDKNIIQFVH GEEDLKVQHS SYRQRARLLK DQLSLGNAAL QITDVKLQDA GVYRCMISY 600
 GADYKRITVK VNAPYNKINQ RILVVDPVTS EHELTQAEQ YPKAEVIWTS SDHQVLSGKT 660
 TTTNSKREEK LFNVTSTLRI NTTTNEIFYC TFRRLDPEEN HTAELVIPEL PLAHPNERT 720
 HLVLGAILL CLGVALTFIF RLRKGRMDV KCGIQDTNS KQSDTHLEE T 771

SEQ ID NO: 39 moltype = AA length = 787
 FEATURE Location/Qualifiers
 source 1..787
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 39
 MRIFAVFIFM TYWHLLNAFT VTVPKDLYV VEYGSNMTIE KFPVEKQLD AALIVYWE 60
 DKNIQFVHG EEDLKVQHS YRQRARLLK DQLSLGNAALQ ITDVKLQDAG VYRCMISY 120
 ADYKRITVKV NAPYNKINQ RILVVDPVTS EHELTQAEQ YPKAEVIWTS DHQVLSGKT 180
 TTNSKREEK FNVSTLRI NTTTNEIFYC FRLDPEENH TAEVLIPEL LAHPNERT 240
 LVILGAILL LGVALTFIF RLRKGRMDV KCGIQDTNS KQSDTHLEE GGGGSGGGG 300
 GGGGSEPKSC DKHTTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED 360
 PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA 420
 PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN 480
 YKTPPVLD DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGKGGG 540
 GSGGGSGGGG GSLPAQVAF PYAPEPGSTC RLREYYDQTA QMCCSKCSG QHAKVFCTKT 600
 SDTVCDSCED STYTQLWNV PECLSCGSR SSQVETQAC TREQNRICR RPWGICALSK 660
 QEGCRLCAPL RRCRPGFVA RGTETSDV CKCAPGTFS NTTSTSDICR PHQICNVVAI 720
 PGNASMDAVC TSTSPTRSM PGAVHLPQPV STRSQHTQPT PEPSTAPSTS FLLPMGSPFP 780
 AEGSTGD 787

SEQ ID NO: 40 moltype = AA length = 755
 FEATURE Location/Qualifiers
 source 1..755
 mol_type = protein
 organism = synthetic construct

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SEQUENCE: 40
 QVQLQQPGAE LVKPGASVKM SCKASGYTFT SYNMHWVKQT PGRGLEWIGA IYPNGDTSY 60
 NQKFKGKATL TADKSSSTAY MQLSSLTSED SAVYYCARST YYGWDWYFNV WGAGTTVTVS 120
 AASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSV VHTFPAVLQS 180
 SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKKA E PKSCDKTHTC PPCPAPELLG 240
 GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY 300
 NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD 360
 ELTKNQVSLT CLVKGFPYSD IAEWESNGQ PENNYKTPP VLDSDGSFPL YSKLTVDKSR 420
 WQQGNVFPSC VMHEALHNHY TQKSLSLSPG KGGGSGGGG SGGGSRIFA VFIFMTYWHL 480
 LNAFTVTPK DLYVVEYGSN MTIECKFPVE KQLDLAALIV YWEMEDKNI QFVHGDEEDLK 540
 VQHSYSYRQRA RLLKQQLSLG NAALQITDVK LQDAGVYRCM ISYGADYKR ITVKVNAPYN 600
 KINQRILVVD PVTSEHELTC QAEGYPKAEV IWTSSDHQVL SGKTTTNSK REEKLFNVT 660
 TLRINTTNE IFYCTFRRLD PEENHTAELV IPELPLAHP NERTHLVILG AILLCLGVAL 720
 TFIFRLKGR MMDVKCKGIQ DTNSKKQSDT HLEET 755

SEQ ID NO: 41 moltype = AA length = 750
 FEATURE Location/Qualifiers
 source 1..750
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 41
 QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY 60
 NQKFKDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LVTVSSASTK 120
 GPSVFPLAPS SKTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS 180
 LSSVTVTPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPEPA PELLGGPSVF 240
 LFPPKPKDTL MISRTPEVTC VVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR 300
 VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN 360
 QVSLTCLVKG FYPSDIAVEW ESNQGPPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN 420
 VFSCVMHEA LHNHYTQKSL SLSPGKGGGG SGGGSGGGG SRIFAVFIFM TYWHLLNAFT 480
 VTPVKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG EEDLVQHS 540
 YRQARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV NAPYNKINQR 600
 ILVVDPTSE HELTCQAEY PKAEVIWTSS DHQVLSGKTT TNSKREEKL FNVSTLRIN 660
 TTTNEIFYCT FRRLDPEENH TAEVLPELPL LAHPPNERTH LVILGAILLC LGVALTFIFR 720
 LRKGRMDVK KCGIQDTNSK QSDTHLEET 750

SEQ ID NO: 42 moltype = AA length = 749
 FEATURE Location/Qualifiers
 source 1..749
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 42
 QLQSGTVLA RPGASVKMCS KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ 60
 KFEGKAKLTA VTSASTAYME LSSLTTHEDSA VYYCSRDIYGY YDFWQGGTT LTVSSASTKG 120
 PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL 180
 SSVVTVPPSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL 240
 FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKP REEQYNSTYR 300
 VSVLTVLHQD WLNNGKEYCK VSNKALPAPI EKTISKAKGQ PREPQVYTL PPSRDELTKN 360
 VSLTCLVKGF YPSDIAVEWE SNGQPPENNY KTTTPVLDSDG SFFLYSKLTV DKSRLVQQNV 420
 PSCSVMHEAL HNHYTQKSL LSPGKGGGG SGGGSGGGG RIFAVFIFMT YWHLLNAFTV 480
 TVPKDLYVVE YGSNMTIECK FVPEKQLDLA ALIVYWEMED KNI IQFVHGE EDLVQHS 540
 RQARLLKQD LSLGNAALQI TDVKLQDAGV YRCMISYGGG DYKRITVKVN APYNKINQRI 600
 LVVDPTSEH ELTCQAEYGP KAEVIWTSS DHQVLSGKTT TNSKREEKL FNVSTLRINT 660
 TTTNEIFYCT FRRLDPEENH AELVIPELPL AHPPNERTHL VILGAILLCL GVALTFIFRL 720
 RKGRMDVK KCGIQDTNSK QSDTHLEET 749

SEQ ID NO: 43 moltype = AA length = 678
 FEATURE Location/Qualifiers
 source 1..678
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 43
 MRIFAVFIFM TYWHLLNAFT VTPKDLVYV EYGSNMTIEC KFPVEKQLDL AALIVYWEME 60
 DKNIIQFVHG EEDLVQHS YRQARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG 120
 ADYKRITVKV NAPYNKINQR ILVVDPTSE HELTCQAEY PKAEVIWTSS DHQVLSGKTT 180
 TNSKREEKL FNVSTLRIN TTTNEIFYCT FRRLDPEENH TAEVLPELPL LAHPPNERTH 240
 LVILGAILLC LGVALTFIFR LRKGRMDVK KCGIQDTNSK QSDTHLEET GGGSGGGG 300
 GGGSTHTCP PCPAPELLGG PSVFLFPPK KDTLMISRT PEVTCVVVDV HEDPEVKFNW 360
 YVDGVEVHN KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNK ALPAPIEKTI 420
 KAKGQPREPQ VYTLPPSREE MTKNQVSLTCL LVKGFYPSDI AVEWESNGQ PENNYKTPPV 480
 LDDSGSFPLY SKLTVDKSRW QQGNVFPSCV MHEALHNHYT QKSLSLSPGK GGGSGGGG 540
 GGGGAPTSS STTKTQLQLE HLLLDLQML NGINNYKNPK LTRMLTTFKY MPKATELKH 600
 LQCLEELKP LEEVLNLQAS KNFHLRPRDL ISNINVIVLE LKGETTFM C EYADETATIV 660
 EFLNRWITFC QSIISTLT 678

SEQ ID NO: 44 moltype = AA length = 677
 FEATURE Location/Qualifiers
 source 1..677
 mol_type = protein
 organism = synthetic construct

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SEQUENCE: 44
 APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA TELKHLQCLE 60
 BELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR 120
 WITPCQSIIS TLTGGGGSGG GSGGGGGSTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS 180
 RTEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL 240
 NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCVLKGFYP 300
 SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN 360
 HYTKSLSLS PGKGGGGSGG GSGGGGSRI FAVFIFMTYW HLLNAFTVTV PKDLYVVEYG 420
 SNMTIECKFP VEKQLDLAAL IVYWEMEDKN IIQFVHGEED LKVQHSSYRQ RARLLKDQLS 480
 LGNAALQITD VKLQDAGVYR CMISYGGADY KRITVKVNAP YNKINQRILV VDPVTSEHEL 540
 TCQAEGYPKA EVIWTSSDHQ VLSGKTTTIN SKREEKLFNV TSTLRINTTT NEIFYCTFRR 600
 LDPEENHTAE LVIPELPLAH PPNERTHLVI LGAILLCLGV ALTFIFRLRK GRMMDVKKCG 660
 IQDTNSKKQS DTHLEET 677

SEQ ID NO: 45 moltype = AA length = 755
 FEATURE Location/Qualifiers
 source 1..755
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 45
 QVQLQEAGPG LVKPSSETLSL TCSVSGGSIS GDYYWFIRQ SPKGLEWIG YIYSGGGGTN 60
 YNPSLNRRVS ISIDTSKNLF SLKLRSVTAA DTAVYYCASN ILKYLHWLLY WGQGLVLTVS 120
 SASTKGPSVP PLAPSSKSTS GGTAALGCLV KDYPPEPVTV SWNSGALTSG VHTFPAVLQS 180
 SGLYSLSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKRVK PKSCDKTHTC PPCPAPELLG 240
 GPSVFLPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY 300
 NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD 360
 ELTKNQVSLT CLVKGFPSPD IAVEWESNGQ PENNYKTPPP VLDSDGSFPFL YSKLTVDKSR 420
 WQQGNVFSKS VMHEALHNHY TQKSLSLSPG KGGGGSGGGG SGGGGSRIFA VFIFMTYWHL 480
 LNAFTVTVPK DLYVVEYGSN MTIECKFPVE KQLDLAALIV YWEMEDKNII QFVHGEEDLK 540
 VQHSYRQRA RLLKDQLSLG NAALQITDVK LQDAGVYRCM ISYGGADYKR ITVKVNAPYN 600
 KINQRILVVD PVTSEHELTC QAEGYPKAEV IWTSSDHQVL SGKTTTNSK REEKLNVTS 660
 TLRINTTTNE IFYCTFRRLD PEENHTAELV IPELPLAHP NERTHLVILG AILLCLGVAL 720
 TFIFRLRKGR MMDVKKCGIQ DTNSKKQSDT HLEET 755

SEQ ID NO: 46 moltype = AA length = 687
 FEATURE Location/Qualifiers
 source 1..687
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 46
 MGVLITQRTL LSLVLALLFP SMASAMHVA QPAVVLASSR GIASVCEYA SPGKATEVRV 60
 TVLRQADSQV TEVCAATYMM GNELTFLDDS ICTGTSSGNQ VNLTIOGLRA MDTGLYICKV 120
 ELMYPPPYL GIGNGTQIYV IDPEPCPDS QEPKSCDKTH TCPPCPAPEL LGGPSVFLFP 180
 PKPKDTLMIS RTEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS 240
 VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS RDELTKNQVS 300
 LTCVLKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS 360
 CSVMHEALHN HYTKSLSLS PGKGGGGSGG GSGGGGSRI FAVFIFMTYW HLLNAFTVTV 420
 PKDLYVVEYG SNMTIECKFP VEKQLDLAAL IVYWEMEDKN IIQFVHGEED LKVQHSSYRQ 480
 RARLLKDQLS LGNAALQITD VKLQDAGVYR CMISYGGADY KRITVKVNAP YNKINQRILV 540
 VDPVTSHEL TCQAEGYPKA EVIWTSSDHQ VLSGKTTTIN SKREEKLFNV TSTLRINTTT 600
 NEIFYCTFRR LDPEENHTAE LVIPELPLAH PPNERTHLVI LGAILLCLGV ALTFIFRLRK 660
 GRMMDVKKCG IQDTNSKKQS DTHLEET 687

SEQ ID NO: 47 moltype = AA length = 678
 FEATURE Location/Qualifiers
 source 1..678
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 47
 MRIFAVFIFM TYWHLLNAFT VTVPKDLYVV EYGSNMTEIC KFPVEKQLDL AALIVWEME 60
 DKNIQFVHG EEDLKVQHSS YRQARLLKD QLSLGNALQ ITDVKLQDAG VYRCMISYGG 120
 ADYKRITVKV NPYNKINQR ILVVDPTVSE HELTCQAEGY PKAEVIWTS DHQVLSGKTT 180
 TTNSKREEKL FNVSTLRIN TTTNEIFYCT FRRLDPEENH TAEVPELPL LAHPNERTH 240
 LVILGAILLC LGVALTFIFR LRKGRMMDVK KCGIQDTNSK KQSDTHLEET GGGGGGGGGS 300
 GGGGSQEPKS CDKTHTCPPC PAPELLGGPS VFLPPPCKPD TLMISRTPEV TCVVVDVSH 360
 DPEVKFNWYV DGEVHNNAK KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP 420
 APIEKTISKA KGQPREPQVY TLPPSRDEL TKNQVSLTCLV KGFYPSDIAV EWESNGQPEN 480
 NYKTPPVLD SDGSFPLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGKGG 540
 GSGGGGGSGG GGSAMHVAQP AVVLASSRGI ASFVCEYASP GKATEVRVTV LRQADSQVTE 600
 VCAATYMMGN ELTFLDDSDIC TGTSSGNQVN LTIQGLRAMD TGLYICKVEL MYPPPYLGI 660
 GNGTQIYVID PEPCPDS 678

SEQ ID NO: 48 moltype = AA length = 656
 FEATURE Location/Qualifiers
 source 1..656
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 48
 ALDTNYCFSS TEKNCCVRQL YIDFRKDLGW KWIHEPKGYH ANFLGPGCPY IWSLDTQYSK 60

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VLALYNQHNP	GASAAPCCVP	QALEPLPIVY	YVGRKPKVEQ	LSNMIVRSCK	CSGGGSGGGG	120
GGGGGGSTHT	CPPCPAPELL	GGPSVFLFPP	KPKDTLMISR	TPEVTCVVVD	VSHEDPEVKF	180
NWYVDGVEVH	NAKTKPREEQ	YNSTYRVVSV	LTVLHQDWLN	GKEYKCKVSN	KALPAPIEKT	240
ISKAKGQPRE	PQVYTLPPSR	EEMTKNQVSL	TCLVKGFYPS	DIAEWESNG	QPENNYKTPP	300
PVLDSGGSFF	LYSKLTVDKS	RWQQGNVFSC	SVMHEALHNH	YTQKSLSLSP	GKGGGSGGGG	360
GGGGGSRIF	AVFIFMTYWH	LLNAFTVTVP	KDLYVVEYGS	NMTIECKFPV	EKQLDLAALI	420
VYWEMEDKNI	IQFVHGEEDL	KVQHSSYRQR	ARLLKDQLSL	GNAALQITDV	KLQDAGVYRC	480
MISYGGADYK	RITVKVNAPY	NKINQRILVV	DPVTSEHFLT	CQAEGYPKAE	VIWTSSDHQV	540
LSGKTTTNS	KREEKLFNVT	STLRINTTTN	EIFYCTFRRL	DPEENHTAEL	VIPELPLAHP	600
PNERTHLVIL	GAILLCLGVA	LTFIFRLRKG	RMDVKKCGI	QDTNSKKQSD	THLEET	656

SEQ ID NO: 49 moltype = AA length = 657
 FEATURE Location/Qualifiers
 source 1..657
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 49

MRIFAVFIM	TYWHLNNAFT	VTVPKDLVYV	EYGSNMTIEC	KFPVEKQLDL	AALIVYWEME	60
DKNIIQFVHG	EEDLKVQHSS	YRQARLLKD	QLSLGNAALQ	ITDVKLQDAG	VYRCMISYGG	120
ADYKRITVKV	NAPYKNINQR	ILVVDPTVSE	HELTCAEAGY	PKAEVIWTSS	DHQLVLSGKT	180
TTNSKREEKL	FNVTSTLRIN	TTTNEIFYCT	FRRLDPEENH	TAEVLVPELP	LAHPPNERTH	240
LVILGAILLC	LGVALTFFIFR	LKGRMMDVK	KCGIQDTNSK	KQSDTHLEET	GGGSGGGGGS	300
GGGGSTHTCP	PCPAPELLGG	PSVFLFPPKP	KDTLMISRT	EVTCTVVVDV	HEDPEVKFNW	360
YVDGVEVHNA	KTKPREEQYN	STYRVVSVLT	VLHQDWLNGK	EYKCKVSNKA	LPAPIEKTIS	420
KAKGQPREPQ	VYTLPPSREE	MTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPV	480
LDSDGSFFLY	SKLTVDKSRW	QQGNVFSCSV	MHEALHNHYT	QKSLSLSPGK	GGGSGGGGGS	540
GGGGSALDTN	YCFSSSTEKNC	CVRQLYIDFR	KDLGWKWIHE	PKGYHANFCL	GPCPYIWSLD	600
TQYSKVLALY	NQHNPGASAA	PCCVPQALEP	LPIVYVYGRK	PKVEQLSNMI	VRSCCKCS	657

SEQ ID NO: 50 moltype = AA length = 578
 FEATURE Location/Qualifiers
 source 1..578
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 50

EVQLVESGGG	LVPQGRSLRL	SCAASGFTFD	DYAMHWVRQA	PGKGLEWVSA	ITWNSGHIDY	60
ADSVGEGRFTI	SRDNAKNSLY	LQMNSLRAED	TAVYYCALVS	YLSTASSLDY	WGQGLTVTVS	120
SASTKGPSVF	PLAPSSKSTS	GGTAALGCLV	KDYFPEPVTV	SWNSGALTSG	VHTFPAVLQS	180
SGLYSLSSVV	TPSSSLGTQ	TYICNVNHKP	SNTKVDKKA	EYKCKVSNKA	LPAPIEKTIS	240
GPSVFLFPPK	PKDTLMISRT	PEVTCVVVDV	SHEDPEVKFN	WYVDGVEVHN	AKTKPREEQY	300
NSTYRVVSVL	TVLHQDWLNG	KEYKCKVSNK	ALPAPIEKT	ISKAKGQPRE	QVYTLPPSRD	360
ELTKNQVSLT	CLVKGFYPSD	IAVEWESNGQ	PENNYKTPP	VLDSDGSPFL	YSKLTVDKSR	420
WQQGNVFSCS	VMHEALHNHY	TQKSLSLSPG	KGGGSGGGGG	SGGGGSALDT	NYCFSSSTEKN	480
CCVRQLYIDF	RKDLGWKWIH	EPKGYHANFC	LGPCPYIWSL	DTQYSKVLAL	YNQHNPGASA	540
APCCVPQALE	PLPIVYVYGR	KPKVEQLSNM	IVRSCCKCS			578

SEQ ID NO: 51 moltype = AA length = 594
 FEATURE Location/Qualifiers
 source 1..594
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 51

LPAQVAPTPY	APEPGSTCR	REYYDQTAQM	CCSKCSPGQH	AKVFCTKTS	TVCDSCEDST	60
YTQLWNWVPE	CLSCSSRCSS	DQVETQACTR	EQNRICTCRP	GWYCALSKQE	GCRLCAPLRK	120
CRPGFGVARP	GTETSDVVC	PCAPGTFSNT	TSSTDICRPH	QICNVVAIPG	NASMDAVCTS	180
TSPTSRMAPG	AVHLPPQVST	RSQHTQPTPE	PSTAPSTSFL	LPMGPSPPAE	GSTGDEPKSC	240
DKHTCTPPCP	APELLGGPSV	FLFPPKPKDT	LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD	300
GVEVHNAKTK	PREEQYNSTY	RVVSVLTVLH	QDWLNGKEYK	CKVSNKALPA	PIEKTISKAK	360
GQPREPQVYT	LPPSREEMTK	NQVSLTCLVK	GFYPSDIAVE	WESNGQPENN	YKTPPVLD	420
DGSPFLYSKL	TVDKSRWQQG	NVPSCSVMHE	ALHNHYTQKS	LSLSPGKGGG	SGGGGSGGGG	480
GSALDTNYCF	SSTEKNCCVR	QLYIDFRKDL	GWKWIHEPKG	YHANFCLGPC	PYIWSLDTQY	540
SKVLALYNQH	NPGASAAPCC	VPQALEPLPI	VYVYGRKPKV	EQLSNMIVRS	CKCS	594

SEQ ID NO: 52 moltype = AA length = 602
 FEATURE Location/Qualifiers
 source 1..602
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 52

ALDTNYCFSS	TEKNCCVRQL	YIDFRKDLGW	KWIHEPKGYH	ANFCLGPCPY	IWSLDTQYSK	60
VLALYNQHNP	GASAAPCCVP	QALEPLPIVY	YVGRKPKVEQ	LSNMIVRSCK	CSGGGSGGGG	120
GGGGGGSTHT	CPPCPAPELL	GGPSVFLFPP	KPKDTLMISR	TPEVTCVVVD	VSHEDPEVKF	180
NWYVDGVEVH	NAKTKPREEQ	YNSTYRVVSV	LTVLHQDWLN	GKEYKCKVSN	KALPAPIEKT	240
ISKAKGQPRE	PQVYTLPPSR	EEMTKNQVSL	TCLVKGFYPS	DIAEWESNG	QPENNYKTPP	300
PVLDSGGSFF	LYSKLTVDKS	RWQQGNVFSC	SVMHEALHNH	YTQKSLSLSP	GKGGGSGGGG	360
SGGGGSLPA	QVAFPTYAPE	PGSTCRRLREY	YDQTAQMCCS	KCSPGQHAKV	FCTKTSDTV	420
DSCEDSTYTP	LWNWVPECLS	CGSRCSDDQV	ETQACTREQN	RICTCRPGWY	CALSKQEGCR	480
LCAPLRKCRP	FGGVARPGTE	TSADVCKPCA	PGTFSNTTSS	TDICRPHQIC	NVVAIPGNAS	540
MDAVCTSTSP	TRSMAPGAVH	LQPVSTRSQ	HTQPTPEPST	APSTSFLPLM	GPSPPAEGST	600

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GD 602

SEQ ID NO: 53 moltype = AA length = 578
 FEATURE Location/Qualifiers
 source 1..578
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 53

QVQLQQPGAE	LVKPGASVKM	SKKASGYTFT	SYNMHWVKQT	PGRGLEWIGA	IYPGNQDTSY	60
NQKFQKQATL	TADKSSSTAY	MLSSSLTSED	SAVYYCARST	YYGGDWYFNV	WGAGTTVTVS	120
AASTKGPSVF	PLAPSSKSTS	GGTAALGCLV	KDYFPEPVTV	SWNSGALTSG	VHTFPAVLQS	180
SGLYSLSSVV	TVPSSSLGTQ	TYICNVNHKP	SNTKVDKKAE	PKSCDKTHTC	PPCPAPELLG	240
GPSVFLFPPK	PKDTLMISRT	PEVTCVVVDV	SHEDPEVKFN	WYVDGVEVHN	AKTKPREEQY	300
NSTYRVVSVL	TVLHQDWLNG	KEYKCKVSNK	ALPAPIEKTI	SKAKGQPREP	QVYTLPPSRD	360
ELTKNQVSLT	CLVKGFPYSD	IAVEWESNGQ	PENNYKTTTP	VLDSDGSFFL	YSKLTVDKSR	420
WQQGNVFCSS	VMHEALHNHY	TQKSLSLSPG	KGGGGSGGGG	SGGGGSALDT	NYCFSSTEKN	480
CCVRQLYIDF	RKDLGWKWIH	EPKGYHANFC	LGPCPYIWSL	DTQYSKVLAL	YNQHNPGASA	540
APCCVPQALE	PLPIVYVGR	KPKVEQLSNM	IVRSCKCS			578

SEQ ID NO: 54 moltype = AA length = 573
 FEATURE Location/Qualifiers
 source 1..573
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 54

QVQLVQSGAE	VKKPGSSVKV	SKKASGYTFT	SYRMHWVRQA	PQGLEWIGY	INPSTGYTEY	60
NQKFQDKATI	TADESTNTAY	MELSSLRSED	TAVYYCARGG	GVFDYWGQGT	LTVSSASTK	120
GPSVFLPLAPS	SKSTSGGTAA	LGCLVKDYFP	EPVTVSWNSG	ALTSGVHTFP	AVLQSSGLYS	180
LSSVVTVPSS	SLGTQTYICN	VNHKPSNTKV	DKKVEPKSCD	KTHTCPPCPA	PELLGGPSVF	240
LFPPKPKDTL	MISRTPEVTC	VVDVSHEDP	EVKFNWYVDG	VEVHNAKTKP	REEQYNSTYR	300
VVSVLTVLHQ	DWLNGKEYKC	KVSNKALPAP	IEKTISKAKG	QPREPQVYTL	PPSRDELTKN	360
QVSLTCLVKG	FYPSDIAVEW	ESNGQPENNY	KTPPVVLDSD	GSFFLYSKLT	VDKSRWQQGN	420
VFSCSVMHAE	LHNHYTQKSL	SLSPGKGGGG	SGGGSGGGGG	SALDTNYCFS	STEKNCCVRQ	480
LYIDFRKDLG	WKWIHEPKGY	HANFCLGPCP	YIWSLDTQYS	KVLALYNQHN	PGASAAPCCV	540
PQALEPLPIV	YVGRKPKVE	QLSNMIVRSC	KCS			573

SEQ ID NO: 55 moltype = AA length = 572
 FEATURE Location/Qualifiers
 source 1..572
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 55

QLQQSGTVLA	RPGASVKMSC	KASGYSFTRY	WMHWIKQRP	QGLEWIGAIY	PGNSDTSYNQ	60
KFEGKAKLTA	VTSASTAYME	LSSLTHEDSA	VYYCSRDIYGY	YFDYWGQGT	LTVSSASTK	120
PSVFPLAPSS	KSTSGGTAAL	GCLVKDYFPE	PVTVSWNSGA	LTSVGHVTFPA	VLQSSGLYSL	180
SSVVTVPSSS	LGTQTYICNV	NHKPSNTKVD	KRVEPKSCDK	THTCPPCPAP	ELGGGPSVFL	240
FPPKPKDTLM	ISRTPEVTCV	VVDVSHEDPE	VKFNWYVDGV	EVHNAKTKPR	EEQYNSTYRV	300
VSVLTVLHQD	WLNGKEYKCK	VSNKALPAPI	EKTISKAKGQ	PREPQVYTL	PSRDELTKNQ	360
VSLTCLVKGF	YPSDIAVEWE	SNGQPENNYK	TTPPVVLDSDG	SFFLYSKLTV	DKSRWQQGNV	420
FSCSVMHAEAL	HNHYTQKSL	LSPGKGGGG	GGGGSGGGGG	ALDTNYCFSS	TEKNCCVRQL	480
YIDFRKDLGW	KWIHEPKGYH	ANFCLGPCPY	IWSLDTQYSK	VLALYNQHN	GASAAPCCVP	540
QALEPLPIVY	YVGRKPKVEQ	LSNMIVRSCK	CS			572

SEQ ID NO: 56 moltype = AA length = 500
 FEATURE Location/Qualifiers
 source 1..500
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 56

ALDTNYCFSS	TEKNCCVRQL	YIDFRKDLGW	KWIHEPKGYH	ANFCLGPCPY	IWSLDTQYSK	60
VLALYNQHN	GASAAPCCVP	QALEPLPIVY	YVGRKPKVEQ	LSNMIVRSCK	CSGGGGSGGG	120
GGGGGGSTHT	CPCPAPELL	GGPSVFLFPP	KPKDTLMISR	TPEVTCVVVD	VSHEDPEVKF	180
NWYVDGVEVH	NAKTKPREEQ	YNSTYRVVSV	LTVLHQDWLN	GKEYKCKVSN	KALPAPIEKT	240
ISKAKGQPRE	PQVYTLPPSR	EEMTKNQVSL	TCLVKGFYPS	DIAVEWESNG	QPENNYKTTTP	300
PVLDSGSPFF	LYSKLTVDKS	RWQQGNVFSC	SVMHEALHNH	YTQKSLSLSP	GKGGGGSGGG	360
GGGGGGSAPT	SSSTKKTQLQ	LEHLLLDLQM	ILNGINNYKN	PKLTRMLTFK	FYMPKKATEL	420
KHLQCLEEEL	KPLEEVLNLA	QSKNFHLRPR	DLISINIVIV	LELKGSETTF	MCEYADETAT	480
IVEFLNRWIT	FCQSIISTLT					500

SEQ ID NO: 57 moltype = AA length = 500
 FEATURE Location/Qualifiers
 source 1..500
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 57

APTSSTKKT	QLQLEHLLLD	LQMILNGINN	YKNPKLTRML	TFKFYMPKKA	TELKHLQCLE	60
BEKPLEEVL	NLAQSKNFHL	RPRLDISNIN	VIVLELKGSE	TTFMCEYADE	TATIVEFLNR	120
WITFCQSIIS	TLTGGGGSGG	GGSGGGGSGG	TCPPCPAPEL	LGGPSVFLFP	KPKDTLMIS	180
RTPEVTCVVV	DVSHEDPEVK	FNWYVDGVEV	HNAKTKPREE	QYNSTYRVVS	VLTVLHQDWL	240

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NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP 300
SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMEALHN 360
HYTKSLSLS PGKGGGSGG GSGGGGGSAL DTNYCFSSTE KNCCVRQLYI DFRKDLGWKW 420
IHEPKGYHAN FCLGPCPYIW SLDTQYSKVL ALYNQHNPQA SAAPCCVPQA LEPLPIVYV 480
GRKPKVEQLS NMIVRSCKCS 500

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SEQ ID NO: 58      moltype = AA length = 578
FEATURE           Location/Qualifiers
source            1..578
                  mol_type = protein
                  organism = synthetic construct

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SEQUENCE: 58
QVQLQEAGPG LVKPSSETLSL TCSVSGGSIS GDYYWFIRQ SPKGLEWIG YIYGSGGGTN 60
YNPSLNNRVS ISIDTSKNLF SLKLRSVTAA DTAVYYCASN ILKYLHWLLY WGQGVLTVTS 120
SASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYPPEPVTV SWNSGALTSG VHTFPAVLQS 180
SGLYSLSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKRVK PKSCDKTHTC PPCPAPELLG 240
GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY 300
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD 360
ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTPP VLDSDGSFLL YSKLTVDKSR 420
WQQGNVFSVS VMHEALHNHY TQKSLSLSPG KGGGGSGGGG SGGGGGSLDT NYCFSSTEKN 480
CCVRQLYIDF RKDLGWKWIH EPKGYHANFC LGPCPYIWSL DTQYSKVLAL YNQHNPQASA 540
APCCVPQALE PLPIVYVYGR KPKVEQLSNM IVRSCKCS 578

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```

SEQ ID NO: 59      moltype = AA length = 510
FEATURE           Location/Qualifiers
source            1..510
                  mol_type = protein
                  organism = synthetic construct

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SEQUENCE: 59
MGVLLTQRTL LSLVLALLFP SMASMAMHVA QPAVVLASSR GIASFVCEYA SPGKATEVRV 60
TVLRQADSQV TEVCAATYMM GNELTFLDDS ICTGTSSGNQ VNLTIQGLRA MDTGLYICKV 120
ELMYPPPYL GIGNGTQIYV IDPEPCPDS QEPKSCDKTH TCPCCPAPEL LGGPSVFLFP 180
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPRE QYNSTYRVVS 240
VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS RDELTKNQVS 300
LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS 360
CSVMHEALHN HYTKSLSLS PGKGGGSGG GSGGGGGSAL DTNYCFSSTE KNCCVRQLYI 420
DFRKDLGWKW IHEPKGYHAN FCLGPCPYIW SLDTQYSKVL ALYNQHNPQA SAAPCCVPQA 480
LEPLPIVYV GRKPKVEQLS NMIVRSCKCS 510

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SEQ ID NO: 60      moltype = AA length = 484
FEATURE           Location/Qualifiers
source            1..484
                  mol_type = protein
                  organism = synthetic construct

```

```

SEQUENCE: 60
ALDTNYCFSS TEKNCCVRQL YIDFRKDLGW KWIHEPKGYH ANFCLGPCPY IWSLDTQYSK 60
VLALYNQHNP GASAAPCCVP QALEPLPIVY YVGRKPKVEQ LSNMIVRSCK CSEPKSCDKT 120
HTCPPCPAPE LLGGPSVFLF PKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE 180
VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP 240
REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS 300
FFLYSKLTV DKSRRQQGNV SCVSMEALH NHYTQKSLSL SPKGGGGSGG GGGSGGGGSA 360
MHVAQPAVVL ASSRGIASFV CEYASPGKAT EVRVTVLRQA DSQVTEVCAA TYMMGNELTF 420
LDDSICTGTS SGNQVNLTIQ GLRAMDTGLY ICKVELMYPP PYVLGIGNGT QIYVIDPEPC 480
PDS 484

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SEQ ID NO: 61      moltype = AA length = 651
FEATURE           Location/Qualifiers
source            1..651
                  mol_type = protein
                  organism = synthetic construct

```

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SEQUENCE: 61
EVQLVESGGG LVQPGSRSLR SCASGFTFD DYAMHWVRQA PGKLEWVSA ITWNSGHIDY 60
ADSVGRFTI SRDIAKNSLY LQMNSLRAED TAVYYCALVS YLSTASSLDY WGQGLTVTVS 120
SASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYPPEPVTV SWNSGALTSG VHTFPAVLQS 180
SGLYSLSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKAE PKSCDKTHTC PPCPAPELLG 240
GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY 300
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD 360
ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTPP VLDSDGSFLL YSKLTVDKSR 420
WQQGNVFSVS VMHEALHNHY TQKSLSLSPG KGGGGSGGGG SGGGGSQIAP PCTSEKHYEH 480
LGRCCNKCEP GKYMSSKCTT TSDSVCLPCG PDEYLDWNE EDKCLLHKVC DTGKALVAVV 540
AGNSTTPRR CACTAGYHWSQ DCECCRRNTE CAPGLGAQHP LQLNKDTVCK PCLAGYFSDA 600
FSSTDKCRPW TNCTFLGKRV EHHGTEKSDA VCSSSLPARK PNNEPHVYLP G 651

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SEQ ID NO: 62      moltype = AA length = 667
FEATURE           Location/Qualifiers
source            1..667
                  mol_type = protein
                  organism = synthetic construct

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SEQUENCE: 62

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LPAQVAFPTY APEPGSTCRL REYYDQTAQM CCSKCSPGQH AKVFCTKTS TVCDSCEDST 60
YTQLWNWVPE CLSCGSRCS DQVETQACTR EQNRICTRCP GWYCALSKQE GCRLCAPLRK 120
CRPGFGVARP GTETSDVVC PCAPGTFSNT TSSTDICRPH QICNVVAIPG NASMDAVCTS 180
TSPTSRMAPG AVHLPQPVST RSQHTQPTPE PSTAPSTSFL LPMGSPSPA E GSTGDEPKSC 240
DKHTCPCPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD 300
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK 360
GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLD 420
DGSFFLYSKL TVDKSRWQGG NVFSCSVME ALHNNHYTQKS LSLSPGKGGG GSGGGGSGGG 480
GSQIAPPCTS EKHYEHLGRC CNKCEPGKYM SSKCTTSDS VCLPCGPDEY LDSWNEEDKC 540
LLHKVCDTGK ALVAVVAGNS TTPRRCACTA GYHWSQDCEC CRRNTECAPG LGAQHPLQLN 600
KDTVCKPCLA GYPSDAFSS DKCRPWTNCT FLGKRVEHHG TEKSDAVCSS SLPARKPPNE 660
PHVYLP 667

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SEQ ID NO: 63      moltype = AA length = 675
FEATURE           Location/Qualifiers
source            1..675
                  mol_type = protein
                  organism = synthetic construct

```

```

SEQUENCE: 63
QIAPPCTSEK HYEHLGRCCN KCEPGKYMSS KCTTSDSVC LPCGPDEYLD SWNEEDKCLL 60
HKVCDTGKAL VAVVAGNSTT PRRCACTAGY HWSQDCECCR RNTECAPGLG AQHPLQLNKD 120
TVCKPCLAGY FSDAFSSTDK CRPWTNCTFL GKRVEHHGTE KSDAVCSSSL PARKPPNEPH 180
VYLPGGGGGS GGGGGGGGGS THTCPCPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV 240
VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNKEYKCKK 300
VSNKALPAPI EKTISKAKGQ PREPQVYTL PPREEMTKNQ VSLTCLVKGF YPSDIAVEWE 360
SNGQPENNYK TTPPVLDSDG SFPLYSKLTV DKSRWQGGNV FSCSVMEAL HNNHYTQKSL 420
LSPGKGGGGS GGGGGGGGGS LPAQVAFPTY APEPGSTCRL REYYDQTAQM CCSKCSPGQH 480
AKVFCTKTS TVCDSCEDST YTQLWNWVPE CLSCGSRCS DQVETQACTR EQNRICTRCP 540
GWYCALSKQE GCRLCAPLRK CRPGFGVARP GTETSDVVC PCAPGTFSNT TSSTDICRPH 600
QICNVVAIPG NASMDAVCTS TSPTSRMAPG AVHLPQPVST RSQHTQPTPE PSTAPSTSFL 660
LPMGSPSPA E GSTGD 667

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SEQ ID NO: 64      moltype = AA length = 583
FEATURE           Location/Qualifiers
source            1..583
                  mol_type = protein
                  organism = synthetic construct

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SEQUENCE: 64
MGVLLTQRTL LSLVLALFP SMASMAHVA QPAVVLASSR GIASFVCEYA SPGKATEVRV 60
TVLRQADSQV TEVCAATYMM GNELTFLLDD ICTGTSSGNQ VNLTIQGLRA MDTGLYICKV 120
ELMYPPPYL GIGNGTQIYV IDPEPCPDS QEPKSCDKTH TCPCCPAPEL LGGPSVFLFP 180
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPRE QYNSTYRVVS 240
VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKQPR EPQVYTLPPS RDELTKNQVS 300
LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQGNVFS 360
CSVMHEALHN HYTQKSLSL PGKGGGGSGG GSGGGGSGSI APPCTSEKHY EHLGRCCNKC 420
EPGKYMSSKC TTTSDSVCLP CGPDEYLD SWNEEDKCLLHK VCDTGKALVA VVAGNSTTPR 480
RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSDCKCR 540
PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPNEPHVY LPG 583

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SEQ ID NO: 65      moltype = AA length = 557
FEATURE           Location/Qualifiers
source            1..557
                  mol_type = protein
                  organism = synthetic construct

```

```

SEQUENCE: 65
QIAPPCTSEK HYEHLGRCCN KCEPGKYMSS KCTTSDSVC LPCGPDEYLD SWNEEDKCLL 60
HKVCDTGKAL VAVVAGNSTT PRRCACTAGY HWSQDCECCR RNTECAPGLG AQHPLQLNKD 120
TVCKPCLAGY FSDAFSSTDK CRPWTNCTFL GKRVEHHGTE KSDAVCSSSL PARKPPNEPH 180
VYLPGEKPKSC DKHTCPCPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED 240
PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA 300
PIEKTISKAK GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN 360
YKTPPVLD DGSFFLYSKL TVDKSRWQGG NVFSCSVME ALHNNHYTQKS LSLSPGKGGG 420
GSGGGGSGGG GSAMHVAQPA VVLASSRGIA SFVCEYASPG KATEVRVTVL RQADSQVTEV 480
CAATYMMGNE LTFLLDSICT GTSSGNQVNL TIQGLRAMDT GLYICKVELM YPPPYLGIG 540
NGTQIYVIDP EPCPDS 557

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SEQ ID NO: 66      moltype = AA length = 552
FEATURE           Location/Qualifiers
source            1..552
                  mol_type = protein
                  organism = synthetic construct

```

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SEQUENCE: 66
ALDTNYCFSS TEKNCCVRQL YIDFRKDLGW KWIHEPKGYH ANFCLGPCPY IWSLDTQYSK 60
VLALYNQHNP GASAAPCCVP QALEPLPIVY YVGRKPKVEQ LSNMIVRSCK CSGGGGSGGG 120
GSGGGGSTHT CPGCPAPELL GGPSVFLFPP PKPKDTLMIS TPEVTCVVVD VSHEDPEVK 180
NWWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT 240
ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTPP 300
PVLDSDGSGG SAMHVAQPA VVLASSRGIA SFVCEYASPG KATEVRVTVL RQADSQVTEV 360
GSGGGGSGG PCTSEKHYE HLGRCCKNCE PGKYMSSKCT TSDSVCLPC GPDEYLD SWN 420

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EEDKLLHKV	CDTGKALVAV	VAGNSTTPRR	CACTAGYHWS	QDCECCRRNT	ECAPGLGAQH	480
PLQLNKDTVC	KPCLAGYFSD	AFSSTDKCRP	WTNCTFLGKR	VEHHGTEKSD	AVCSSSLPAR	540
KPPNEPHVYL	PG					552

SEQ ID NO: 67 moltype = AA length = 552
 FEATURE Location/Qualifiers
 source 1..552
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 67

QIAPPCTSEK	HYEHLGRCCN	KCEPGKYMSS	KCTTTSDSVC	LPCGPDEYLD	SWNEEDKCLL	60
HKVCDTGKAL	VAVVAGNSTT	PRRCACTAGY	HWSQDCECCR	RNTECAPGLG	AQHPLQLNKD	120
TVCKPCLAGY	FSDAFSSTDK	CRPWTNCTFL	GKRVEHHGTE	KSDAVCSSSL	PARKPPNEPH	180
VYLPGGGGGS	GGGGSGGGGS	THTCPPCPAP	ELLGGPSVFL	FPPKPKDTLM	ISRTPEVTCV	240
VVDVSHEDPE	VKFNWYVDGV	EVHNAKTKPR	EEQYNSTYRV	VSVLTVLHQD	WLNKKEYKCK	300
VSNKALPAPI	EKTISKAKGQ	PREPQVYTL	PSREEMTKNQ	VSLTCLVKGF	YPSDIAVEWE	360
SNGQPENNYK	TTPPVLDSDG	SFFLYSKLTV	DKSRWQQGNV	FSCSVMHEAL	HNHYTQKSLS	420
LSPGKGGGGS	GGGGSGGGGS	ALDTNYCFSS	TEKNCCVRQL	YIDFRKDLGW	KWIHEPKGYH	480
ANFCLGPCPY	IWSLDTQYSK	VLALYNQHNP	GASAAPCCVP	QALEPLPIVY	YVGRKPKVEQ	540
LSNMIVRSCK	CS					552

SEQ ID NO: 68 moltype = AA length = 729
 FEATURE Location/Qualifiers
 source 1..729
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 68

RIFAVFIFMT	YWHLLNAFTV	TVPKDLYVVE	YGSNMTIECK	FPVEKQLDLA	ALIVYWEMED	60
KNIIQFVHGE	EDLKVQHSSY	RQRARLLKDQ	LSLGNAALQI	TDVKLQDAGV	YRCMISYGGA	120
DYKRITVKVN	APYNKINQRI	LVVDPVTSEH	ELTCQAEGYP	KAETVIWSSD	HQVLSGKTTT	180
TNSKREEKLF	NVTSTLRINT	TTNEIFYCTF	RRLDPEENHT	AELVIPLEPL	AHPPNERTHL	240
VILGAILLCL	GVALTFIFRL	RKGRMMDVKK	CGIQDTNSKK	QSDTHLEETG	GGGSGGGGSG	300
GGGSTHTCCP	CPAPELLGGP	SVLFLPPKPK	DTLMSRTPE	VTCVVVDVSH	EDPEVKFNWY	360
VDGVEVHNAK	TKPREEQYNS	TYRVVSVLTV	LHQDWLNGKE	YKCKVSNKAL	PAPIEKTISK	420
AKGQPREPQV	YTLPPSREEM	TKNQVSLTCL	VKGFPSPDIA	VEWESNGQPE	NNYKTTTPVL	480
DSGGSFFLYS	KLTVDKSRWQ	QGNVVFSCSV	HEALHNHYTQ	KSLSLSPGKG	GGGSGGGGSG	540
GGGSIAPPC	TSEKHVYHLG	RCCNKCEPGK	YMSKCTTTS	DSVCLPCGPD	EYLDSWNEED	600
KCLLHKVCDT	GKALVAVVAG	NSTTPRRCAC	TAGYHWSQDC	ECCRRNTECA	PGLGAQHPLQ	660
LNKDTVCKPC	LAGYFSDAFS	STDKCRPWTN	CTFLGKRVEH	HGTEKSDAVC	SSSLPARKPP	720
NEPHVYLP						729

SEQ ID NO: 69 moltype = AA length = 729
 FEATURE Location/Qualifiers
 source 1..729
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 69

QIAPPCTSEK	HYEHLGRCCN	KCEPGKYMSS	KCTTTSDSVC	LPCGPDEYLD	SWNEEDKCLL	60
HKVCDTGKAL	VAVVAGNSTT	PRRCACTAGY	HWSQDCECCR	RNTECAPGLG	AQHPLQLNKD	120
TVCKPCLAGY	FSDAFSSTDK	CRPWTNCTFL	GKRVEHHGTE	KSDAVCSSSL	PARKPPNEPH	180
VYLPGGGGGS	GGGGSGGGGS	THTCPPCPAP	ELLGGPSVFL	FPPKPKDTLM	ISRTPEVTCV	240
VVDVSHEDPE	VKFNWYVDGV	EVHNAKTKPR	EEQYNSTYRV	VSVLTVLHQD	WLNKKEYKCK	300
VSNKALPAPI	EKTISKAKGQ	PREPQVYTL	PSREEMTKNQ	VSLTCLVKGF	YPSDIAVEWE	360
SNGQPENNYK	TTPPVLDSDG	SFFLYSKLTV	DKSRWQQGNV	FSCSVMHEAL	HNHYTQKSLS	420
LSPGKGGGGS	GGGGSGGGGS	RIFAVFIFMT	YWHLLNAFTV	TVPKDLYVVE	YGSNMTIECK	480
FPVEKQLDLA	ALIVYWEMED	KNIIQFVHGE	EDLKVQHSSY	RQRARLLKDQ	LSLGNAALQI	540
TDVKLQDAGV	YRCMISYGGA	DYKRITVKVN	APYNKINQRI	LVVDPVTSEH	ELTCQAEGYP	600
KAETVIWSSD	HQVLSGKTTT	TNSKREEKLF	NVTSTLRINT	TTNEIFYCTF	RRLDPEENHT	660
AELVIPLEPL	AHPPNERTHL	VILGAILLCL	GVALTFIFRL	RKGRMMDVKK	CGIQDTNSKK	720
QSDTHLEET						729

SEQ ID NO: 70 moltype = AA length = 214
 FEATURE Location/Qualifiers
 source 1..214
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 70

DIQMTQSPSS	LSASVGDRTV	ITCRASQDVN	TAVAWYQQKP	GKAPKLLIYS	ASFLYSGVPS	60
RFSGSRSGTD	FTLTSSSLQP	EDFATYYCQQ	HYTTPPTFGQ	GTKVEIKRTV	AAPSVFIAPP	120
SDEQLKSGTA	SVVCLLNFFY	PREAKVQWKV	DNALQSGNSQ	ESVTEQDSKD	STYLSSTLT	180
LSKADYEKHK	VYACEVTHQG	LSSPVTKSFN	RGEC			214

SEQ ID NO: 71 moltype = AA length = 214
 FEATURE Location/Qualifiers
 source 1..214
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 71

DILLTQSPVI	LSVSPGERVS	FSCRASQSIG	TNIHWYQQRT	NGSPRLLIKY	ASESISGIPS	60
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RFGSGSGSTD FTLINSVES EDIADYYCQQ NNNWPTTFGA GTKLELKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSSTLT 180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGE 214

SEQ ID NO: 72      moltype = AA length = 213
FEATURE           Location/Qualifiers
source            1..213
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 72
QIVLSQSPAI LSASVGEKVT MTCRASSSVS YIHWFQQKPG SSPKPWIYAT SNLASGVVPR 60
FSGSGSGTSY SLTISRVEAE DAATYYCQQW TSNPPTFGGG TKLEIKRTVA APSVFIFPPS 120
SDEQLKSGTA SVVCLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSSTLT 180
SKADYEKHK VYACEVTHQG LSSPVTKSFN RGE 214

SEQ ID NO: 73      moltype = AA length = 214
FEATURE           Location/Qualifiers
source            1..214
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 73
DIQMTQSPSS LSASVGDRTV ITCSASQDIS NYLNWYQKPK GKAPKVLIIY TSSLHSGVPS 60
RFGSGSGSTD FTLTISSLQP EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSSTLT 180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGE 214

SEQ ID NO: 74      moltype = AA length = 215
FEATURE           Location/Qualifiers
source            1..215
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 74
EIVLTQSPGT LSASVGERAT LSCRASQSVG SSYLAWYQKPK PGQAPRLIIY GAFSRATGIP 60
DRFSGSGSGT DFTLTISRLE PEDFAVYICQ QYGSSPWTFG QGTKVEIKRT VAAPSVFIFP 120
PSDEQLKSGT ASVCLNNFY YPREAKVQWKV DNALQSGNSQ QESVTEQDSK DSTYLSSTLT 180
TLKADYEKHK KYVACEVTHG LSSPVTKSF NRGE 215

SEQ ID NO: 75      moltype = AA length = 213
FEATURE           Location/Qualifiers
source            1..213
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 75
DIQMTQSPST LSASVGDRTV ITCSASSSIS YMHWYQKPK KAPKLLIYTT SNLASGVPAR 60
FSGSGSGTEF TLTISLQPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS 120
SDEQLKSGTA SVVCLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSSTLT 180
SKADYEKHK VYACEVTHQG LSSPVTKSFN RGE 213

SEQ ID NO: 76      moltype = AA length = 210
FEATURE           Location/Qualifiers
source            1..210
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 76
QIVSTQSPAI MSASVGEKVT MTCASASSRS YMQWYQKPK TSPKRWIYDT SKLASGVPAR 60
FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGK LEIKRTVAAP SVFIFPPSDE 120
QLKSGTASVV CLNNFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKSDSTY SLSTLTLSK 180
ADYEKHKVYA CEVTHQGLSS PVTKSFN RGE 210

SEQ ID NO: 77      moltype = AA length = 216
FEATURE           Location/Qualifiers
source            1..216
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 77
SYELSQPRSV SVSPGQTAGF TCGGDNVGRK SVQWYQKPK QAPVLVIYAT SERPSGIPAR 60
FSGNSNGNTA TLTISGVEAG DEADYYCQVW DSTADHWVFG GGTRLTVLGR TVAAPSVFIF 120
PPSDEQLKSG TASVCLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYLSST 180
LTLSKADYEK HKVYACEVTH QGLSSPVTKS FNRGE 216

SEQ ID NO: 78      moltype = AA length = 214
FEATURE           Location/Qualifiers
source            1..214
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 78
DIQMTQSPSS LSASVGDRTV ITCRASQGIR NYLAWYQQLP GKAPKLLIYA ASTLQSGVPS 60
RFGSGSGSTD FTLTISSLQP EDVATYYCQR YNRAPYTFGQ GTKVEIKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSSTLT 180

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LSKADYEKHK VYACEVTHQG LSSPVTKSPN RGEC 214

SEQ ID NO: 79 moltype = AA length = 567
 FEATURE Location/Qualifiers
 source 1..567
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 79

MGRGLLRGLW	PLHIVLWTRI	ASTIPPHVQK	SVNNDMIVTD	NNGAVKFPQL	CKFCDVRFST	60
CDNQKSCMSN	CSITSICEKP	QEVCAVWRK	NDENITLET	CHDPKLPYHD	FILEDAASPK	120
CIMKEKKKPG	ETFFMCS	DECNDNIIFS	EEYNTSNPDL	LLVIFQVTGI	SLLPPLGVAI	180
SVIIIFYCYR	VNRQKLSST	WETGKTRKLM	EFSEHCAIIL	EDDRSDISST	CANNINHNT	240
LLPIELDTLV	GKGRFAEVYK	AKLKQNTSEQ	PETVAVKIFP	YEEYASWKTE	KDIFSDINLK	300
HENILQFLTA	EERKTELQKQ	YWLITAFHAK	GNLQEYLTRH	VISWEDLRKL	GSSLARGIAH	360
LHSDHTPCGR	PKMPIVHRDL	KSSNILVKND	LTCCLCDFGL	SLRLDPTLSV	DDLANSQQVG	420
TARYMAPEVL	ESRMNLENVE	SFKQTDVYSM	ALVLWEMTSR	CNAVGEVKDY	EPPFGSKVRE	480
HPCVESMKDN	VLRDRGRPEI	PSFWLNHQGI	QMVCELTTEC	WDHDPPEARL	AQCVARFSE	540
LEHLDRLSGR	SCSEEEKIPED	GSLNTTK				567

SEQ ID NO: 80 moltype = AA length = 592
 FEATURE Location/Qualifiers
 source 1..592
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 80

MGRGLLRGLW	PLHIVLWTRI	ASTIPPHVQK	SDVEMEAQKD	EIICPSCNRT	AHPLRHINND	60
MIVTDNNGAV	KFPQLCKPCD	VRPSTCDNQK	SCMSNCSITS	ICEKPQEVCV	AVWRKNDENI	120
TLETVCHDPK	LPYHDFILED	AASPKCIMKE	KKKPGETFFM	CSCSSDECND	NIIFSEEYNT	180
SNPDLLLIVF	QVTGISLLPP	LGVAISVII	FYCYRVNRQQ	KLSSWTWETGK	TRKLMEFSEH	240
CAILEDERS	DISSTCANNI	NHNTLLPIE	LDTLVGKGRF	AEVYKAKLKQ	NTSEQFETVA	300
VKIPPYEEYA	SWKTEKDIFS	DINLKHENIL	QFLTAEERKT	ELGKQYWLIT	APHAKGNLQE	360
YLTRHVISWE	DLRLKGSSLA	RGIAHLHSDH	TPCGRPKMPI	VHRDLKSSNI	LVKNDLTCCCL	420
CDFGLSLRLD	PTLSVDDLAN	SGQVGTARYM	APEVLESRMN	LENVESFKQT	DVYSMALVLW	480
EMTSRCNAVG	EVKDYEPFPG	SKVREHPCVE	SMKDNVLRDR	GRPEIPSPFWL	NHQGIQMVCE	540
TLTECWHDHP	EARLTAQCVA	ERFSELEHLD	RLSGRSCSEE	KIPEDGSLNT	TK	592

SEQ ID NO: 81 moltype = AA length = 529
 FEATURE Location/Qualifiers
 source 1..529
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 81

MGRGLLRGLW	PLHIVLWTRI	ASTIPPHVQK	SVNNDMIVTD	NNGAVKFPQL	CKFCDVRFST	60
CDNQKSCMSN	CSITSICEKP	QEVCAVWRK	NDENITLET	CHDPKLPYHD	FILEDAASPK	120
CIMKEKKKPG	ETFFMCS	DECNDNIIFS	EEYNTSNPDL	LLVIFQVTGI	SLLPPLGVAI	180
SVIIIFYCYR	VNRQKLSST	WETGKTRKLM	EFSEHCAIIL	EDDRSDISST	CANNINHNT	240
LLPIELDTLV	GKGRFAEVYK	AKLKQNTSEQ	PETVAVKIFP	YEEYASWKTE	KDIFSDINLK	300
HENILQFLTA	EERKTELQKQ	YWLITAFHAK	GNLQEYLTRH	VISWEDLRKL	GSSLARGIAH	360
LHSDHTPCGR	PKMPIVHRDL	KSSNILVKND	LTCCLCDFGL	SLRLDPTLSV	DDLANSQQVG	420
TARYMAPEVL	ESRMNLENVE	SFKQTDVYSM	ALVLWEMTSR	CNAVGEVKDY	EPPFGSKVRE	480
HPCVESMKDN	VLRDRGRPEI	PSFWLNHQGI	QMVCELTTEC	WDHDPPEARL		529

SEQ ID NO: 82 moltype = AA length = 554
 FEATURE Location/Qualifiers
 source 1..554
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 82

MGRGLLRGLW	PLHIVLWTRI	ASTIPPHVQK	SDVEMEAQKD	EIICPSCNRT	AHPLRHINND	60
MIVTDNNGAV	KFPQLCKPCD	VRPSTCDNQK	SCMSNCSITS	ICEKPQEVCV	AVWRKNDENI	120
TLETVCHDPK	LPYHDFILED	AASPKCIMKE	KKKPGETFFM	CSCSSDECND	NIIFSEEYNT	180
SNPDLLLIVF	QVTGISLLPP	LGVAISVII	FYCYRVNRQQ	KLSSWTWETGK	TRKLMEFSEH	240
CAILEDERS	DISSTCANNI	NHNTLLPIE	LDTLVGKGRF	AEVYKAKLKQ	NTSEQFETVA	300
VKIPPYEEYA	SWKTEKDIFS	DINLKHENIL	QFLTAEERKT	ELGKQYWLIT	APHAKGNLQE	360
YLTRHVISWE	DLRLKGSSLA	RGIAHLHSDH	TPCGRPKMPI	VHRDLKSSNI	LVKNDLTCCCL	420
CDFGLSLRLD	PTLSVDDLAN	SGQVGTARYM	APEVLESRMN	LENVESFKQT	DVYSMALVLW	480
EMTSRCNAVG	EVKDYEPFPG	SKVREHPCVE	SMKDNVLRDR	GRPEIPSPFWL	NHQGIQMVCE	540
TLTECWHDHP	EARL					554

SEQ ID NO: 83 moltype = AA length = 199
 FEATURE Location/Qualifiers
 source 1..199
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 83

MGRGLLRGLW	PLHIVLWTRI	ASTIPPHVQK	SVNNDMIVTD	NNGAVKFPQL	CKFCDVRFST	60
CDNQKSCMSN	CSITSICEKP	QEVCAVWRK	NDENITLET	CHDPKLPYHD	FILEDAASPK	120
CIMKEKKKPG	ETFFMCS	DECNDNIIFS	EEYNTSNPDL	LLVIFQVTGI	SLLPPLGVAI	180
SVIIIFYCYR	VNRQKLS					199

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SEQ ID NO: 84 moltype = AA length = 224
FEATURE Location/Qualifiers
source 1..224
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 84
MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND 60
MIVTDNNGAV KFPQLCKPCD VRFSTCDNQK SCMSNCSITS ICEKPQEVCV AVWRKNDENI 120
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT 180
SNPDLLLVIF QVTGISLLPP LGVAISVIII FYCYRVNRQQ KLSS 224

SEQ ID NO: 85 moltype = AA length = 166
FEATURE Location/Qualifiers
source 1..166
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 85
MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST 60
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK 120
CIMKEKKKPG ETFFMCS CSS DECNDNIIFS EYNTSNPDL LLVIFQ 166

SEQ ID NO: 86 moltype = AA length = 191
FEATURE Location/Qualifiers
source 1..191
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 86
MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND 60
MIVTDNNGAV KFPQLCKPCD VRFSTCDNQK SCMSNCSITS ICEKPQEVCV AVWRKNDENI 120
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT 180
SNPDLLLVIF Q 191

SEQ ID NO: 87 moltype = AA length = 137
FEATURE Location/Qualifiers
source 1..137
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 87
TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS ITSICEKPQE 60
VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI MKEKKKPGET FFMCS CSSDE 120
CNDNIIFSEE YNTSNPD 137

SEQ ID NO: 88 moltype = AA length = 162
FEATURE Location/Qualifiers
source 1..162
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 88
TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF PQLCKFCFCDVR 60
FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL ETVCHDPKLP YHDFILEDAA 120
SPKCIMKEKK KPGETFFMCS CSSDECNDNI IFSEEYNTSN PD 162

SEQ ID NO: 89 moltype = AA length = 112
FEATURE Location/Qualifiers
source 1..112
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 89
PQLCKFCFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL ETVCHDPKLP 60
YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI IFSEEYNTSN PD 112

SEQ ID NO: 90 moltype = AA length = 507
FEATURE Location/Qualifiers
source 1..507
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 90
MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST 60
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK 120
CIMKEKKKPG ETFFMCS CSS DECNDNIIFS EYNTSNPDL LLVIFQVTGI SLLPPLGVAI 180
SVIIFCYCYR VNRQQKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT 240
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVRIPP YEEYASWKE KDIFSDINLK 300
HENILQFLTA EERKTELKQK YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH 360
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQQVG 420
TARYMAPEVL ESRMNLNVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE 480
LEHLDRLSGR SCSEKIPED GSLNTTK 507

SEQ ID NO: 91 moltype = AA length = 550

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FEATURE	Location/Qualifiers	
source	1..550	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 91		
MGRGLLRGLW	PLHIVLWTRI	ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST 60
CDNQKSCMSN	CSITSICEKP	QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK 120
CIMKEKKKPG	ETFFMCSCSS	DECNDNIIFS EYNTSNPDL LLVIFQVTGI SLLPPLGVAI 180
SVIIIFYCYR	VNRQOKLSST	WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT 240
LLPIELDTLV	GKGRFAEVYK	AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK 300
HENILQFLTA	EERKTELKQK	YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH 360
LHSDHTPCGR	PKMPIVHRDL	KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQQVG 420
TARYMAPEVL	ESRMNLENVE	SPKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE 480
HPCVESMKDA	SGIQMV CETL	TECWDHDEPA RLTAQCVAER FSELEHLRL SGRSCSEKI 540
PEDGSLNTTK		550
SEQ ID NO: 92		
	moltype = AA length = 288	
FEATURE	Location/Qualifiers	
source	1..288	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 92		
MQIQAPWPV	VWAVLQLGWR	PGWFLDSPDR PWNPTFSPA LLVVTEGDNA TFTCSFSNTS 60
ESFVLNRYM	SPSNQTDKLA	APPEDRSQPG QDCRFVTLQ PNGRDFHMSV VRARRNDSGT 120
YLCGAISLAP	KAQIKESLRA	ELRVTERRAE VPTAHPSPSP RPAGQFQTLV VGVVGGLLGS 180
LVLLVWVLAV	ICSRAARGTI	GARRTGQPLK EDPASVPVFS VDYGELDFQW REKTPPEPPV 240
CVPEQTEYAT	IVFPSPGMGTS	SPARRGSADG PRSAQPLRPE DGHCSWPL 288
SEQ ID NO: 93		
	moltype = AA length = 150	
FEATURE	Location/Qualifiers	
source	1..150	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 93		
PGWFLDSPDR	PWNPTFSPA	LLVVTEGDNA TFTCSFSNTS ESFVLNRYM SPSNQTDKLA 60
APPEDRSQPG	QDCRFVTLQ	PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA 120
ELRVTERRAE	VPTAHPSPSP	RPAGQFQTLV
SEQ ID NO: 94		
	moltype = AA length = 142	
FEATURE	Location/Qualifiers	
source	1..142	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 94		
DSPDRPWNPP	TFSPALLVVT	EGDNATFTCS FSNTSESVFL NWRMSPSNQ TDKLAAPFED 60
RSQPGQDCRF	RVTQLPNGRD	FHMSVVRARR NDSGTYLCGA ISLAPKAQIK ESLRAELRV 120
ERRAEVPTAH	PSPSPRPAGQ	FQ 142
SEQ ID NO: 95		
	moltype = AA length = 616	
FEATURE	Location/Qualifiers	
source	1..616	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 95		
MAPRARRRRR	LFALLLLCAL	LARLQVALQI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC 60
TTTSDSVCLP	CGPDEYLD SW	NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCTACTAGYHW 120
SQDCECCRRN	TECAPGLGAQ	HPLQLNKDITV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK 180
RVEHHGTEKS	DAVCSSSLPA	RKPPNEPHVY LPGLIILLLF ASVALVAAII FGVCYRKKGK 240
ALTANLWHWI	NEACGRSLSGD	KESSGSDSCVS THTANFGQQG ACEGVLLLTLE EEKTFPEDMC 300
YPDQGGVCQG	TCVGGGPIYA	GEDARMLSLV SKTEIEEDSF RQMPTEDEYM DRPSQPTDQL 360
LFLTEPGSKS	TPPFSEPLEV	GENDSLQSCF TGTQSTVGSE SCNCTEPLCR TDWTPMSSSEN 420
YLQKEVDSGH	CPHWAASPS	NWADVCTGCR NPPGEDCEPL VGSPPKRGPLP QCAYMGLPLP 480
EEEA SRTEAR	DQPEDGADGR	LPSSARAGAG SGSSPGGQSP ASGNVTGNSN STFISSGQVM 540
NFKGDIIVVY	VSQTSQEGAA	AAAEPMGRPV QEETLARDDS FAGNGPRFPD PCGGPEGLRE 600
PEKASRPVQE	QGGAKA	616
SEQ ID NO: 96		
	moltype = AA length = 185	
FEATURE	Location/Qualifiers	
source	1..185	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 96		
QIAPPCTSEK	HYEHLGRCCN	KCEPGKYMSS KCTTTSDSVC LPCGPDEYLD SWNEEDKCLL 60
HKVCDTGKAL	VAVVAGNSTT	PRRCACTAGY HWSQDCECCR RNTECAPGLG AQHPLQLNKD 120
TVCKPCLAGY	FSDAFSSTDK	CRPWTNCTFL GKRVEHHGTE KSDAVCSSSL PARKPPNEPH 180
VYLP		185
SEQ ID NO: 97		
	moltype = AA length = 213	
FEATURE	Location/Qualifiers	

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source                1..213
                      mol_type = protein
                      organism = synthetic construct

SEQUENCE: 97
MAPRARRRRP LFAALLLCAL LARLQVALQI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC 60
TTTSDSVCLP CGPDEYLDLW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCTACTAGYHW 120
SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK 180
RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG 213

SEQ ID NO: 98         moltype = AA length = 401
FEATURE              Location/Qualifiers
source                1..401
                      mol_type = protein
                      organism = synthetic construct

SEQUENCE: 98
MNKLLCCALV FLDISIKWTT QETFPKPYLH YDEETSHQLL CDKCPPGTYL KQHCTAKWKT 60
VCAPCPDHYH TDSWHTSDEC LYCSPVCKEL QYVKQECNRT HNRVCECKEG RYLEIEFCLK 120
HRSCPPGFGV VQAGTPERNT VCKRCPDGFF SNETSSKAPC RKHTNCSVFG LLLTQKGNAT 180
HDNICSGNSE STQKCGIDVT LCEEAFRFA VPTKFTPNWL SVLVDNLPGT KVNAESVERI 240
KRQHSSQEQT FQLLKLWKHQ NKAQDIVKKI IQDIDLCENS VQRHIGHANL TFEQLRSLME 300
SLPGKKVGAE DIEKTIKACK PSDQILKLLS LWRIKNGDQD TLKGLMHALK HSKTYHFPKT 360
VTQSLKKTIR FLHSFTMYKL YQKLFLEMIG NQVQSVKISC L 401

SEQ ID NO: 99         moltype = AA length = 290
FEATURE              Location/Qualifiers
source                1..290
                      mol_type = protein
                      organism = synthetic construct

SEQUENCE: 99
MRIFAVFIFM TWHLLNAFT VTVPKDLVYV EYGSNMTIEC KFPVEKQLDL AALIVYWEME 60
DKNIIQFVHG EBDLVQVHSS YRQARLLKD QLSLGNALQ ITDVKLQDAG VYRCMISYGG 120
ADYKRITVKV NAPYNKINQR ILVVDPTVSE HELTCQAEQY PKAEVIWTSS DHQVLSGKTT 180
TTNSKREKEL FNVSTLRIN TTTNEIFYCT FRRLDPEENH TAEVLVPELP LAHPPNERTH 240
LVILGAILLC LGVALTFFIR LRKGRMMDVK KCGIQDTNSK QSDTHLEET 290

SEQ ID NO: 100        moltype = AA length = 209
FEATURE              Location/Qualifiers
source                1..209
                      mol_type = protein
                      organism = synthetic construct

SEQUENCE: 100
AFTVTVPKDL YVVEYGSNMT IECKFPVEKQ LDLAALIVYW EMEDKNIIQF VHGEEDLVKQ 60
HSSYRQARL LKQQLSLGNA ALQITDVKLQ DAGVYRCMIS YGGADYKRIT VKVNAPYINKI 120
NQRIILVDPV TSEHELTQQA EGYPKAEVIW TSSDHQVLSG KTTTNSKRE EKLNVSTL 180
RINTTTNEIF YCTFRRLDPE ENHTAELVI 209

SEQ ID NO: 101        moltype = AA length = 289
FEATURE              Location/Qualifiers
source                1..289
                      mol_type = protein
                      organism = synthetic construct

SEQUENCE: 101
RIFAVFIFMT YWHLNNAFT TVPKDLVVE YGSNMTIECK FPVEKQLDLA ALIVYWEMED 60
KNIIQFVHGE EDLVQVHSS YRQARLLKDQ LSLGNALQI TDVKLQDAGV YRCMISYGGA 120
DYKRITVKVN APYNKINQRI LVDVPTVSEH ELTCQAEQY PKAEVIWTSS DHQVLSGKTTT 180
TNSKREKELF NVTSTLRINT TTNEIFYCTF RRLDPEENHT AELVPELPL AHPPNERTHL 240
VILGAILLCL GVALTFIFRL RKGRMMDVKK CGIQDTNSK QSDTHLEET 289

SEQ ID NO: 102        moltype = AA length = 390
FEATURE              Location/Qualifiers
source                1..390
                      mol_type = protein
                      organism = synthetic construct

SEQUENCE: 102
MPPSGLRLLP LLLPLRLLV LTPGRPAAGL STCKTIDMEL VKRKRIEAIR GQILSKLRLS 60
SPPSQGEVPP VPLPEAVLAL YNSTRDRVAG ESAEPEPEPE ADYYAKEVTR VLMVENTNKI 120
YEKVKKSPHS IYMLFNTSEL REAVPEPVLL SRAELRLLRL KLKAEQHVLE YQKYSNDSWR 180
YLSNRLLAPS DTPEWLSFDV TGVVRQWLSH GGEVEGFRLS AHCSCDSKDN TLQVDINGFS 240
SSRRGDLATI HGMNRPFLLL MATPLERAQH LHSSRQRRAL DTNYCFSSTE KNCCVRQLYI 300
DFRKDLGWKW IHEPKGYHAN FCLGPCPYIW SLDTQYSKVL ALYNQHNPQA SAAPCCVPQA 360
LEPLPIVYVY GRKPKVEQLS NMIVRSCKCS 390

SEQ ID NO: 103        moltype = AA length = 112
FEATURE              Location/Qualifiers
source                1..112
                      mol_type = protein
                      organism = synthetic construct

SEQUENCE: 103
ALDTNYCFSS TEKNCCVRQL YIDFRKDLGW KWIHEPKGYH ANFCLGPCPY IWSLDTQYSK 60

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-continued

VLALYNQHNP GASAAPCCVP QALEPLPIVY YVGRKPKVEQ LSNMIVRSCK CS	112
SEQ ID NO: 104	moltype = AA length = 15
FEATURE	Location/Qualifiers
REGION	1..15
	note = Synthetic construct
source	1..15
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 104	
GGGGSGGGGS GGGGS	15
SEQ ID NO: 105	moltype = AA length = 7
FEATURE	Location/Qualifiers
REGION	1..7
	note = Synthetic construct
source	1..7
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 105	
EPKSCDK	7
SEQ ID NO: 106	moltype = AA length = 7
FEATURE	Location/Qualifiers
REGION	1..7
	note = Synthetic construct
source	1..7
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 106	
IEGRDMD	7
SEQ ID NO: 107	moltype = AA length = 4
FEATURE	Location/Qualifiers
REGION	1..4
	note = Synthetic construct
source	1..4
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 107	
KKAE	4
SEQ ID NO: 108	moltype = AA length = 4
FEATURE	Location/Qualifiers
REGION	1..4
	note = Synthetic construct
source	1..4
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 108	
KRVE	4
SEQ ID NO: 109	moltype = AA length = 4
FEATURE	Location/Qualifiers
REGION	1..4
	note = Synthetic construct
source	1..4
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 109	
KKVE	4
SEQ ID NO: 110	moltype = AA length = 8
FEATURE	Location/Qualifiers
REGION	1..8
	note = Synthetic construct
source	1..8
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 110	
QEPKSCDK	8
SEQ ID NO: 111	moltype = DNA length = 21
FEATURE	Location/Qualifiers
misc_feature	1..21
	note = Primer
source	1..21
	mol_type = other DNA
	organism = synthetic construct

-continued

SEQUENCE: 111
ctagtgcac ctgggaattc a 21

SEQ ID NO: 112 moltype = DNA length = 24
FEATURE Location/Qualifiers
misc_feature 1..24
note = Primer
source 1..24
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 112
catcattagc tgatctccag ctca 24

SEQ ID NO: 113 moltype = DNA length = 38
FEATURE Location/Qualifiers
misc_feature 1..38
note = Synthetic construct
source 1..38
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 113
catcagtgc agttacttct tcaccttcta cacagaga 38

SEQ ID NO: 114 moltype = DNA length = 1821
FEATURE Location/Qualifiers
source 1..1821
mol_type = other DNA
organism = Homo sapiens

SEQUENCE: 114

gagctcgagg	tgcagctggt	ggaatccggc	ggaggactgg	tgcagcctgg	cggatccctg	60
agactgtctt	gcgcgcgctc	cggcttcaac	atcaaggaca	cctacatcca	ctgggtgcga	120
caggcccttg	gcaagggact	ggaatgggtg	gcccggtatc	acccaccaa	cggctacacc	180
agatacgccg	actccgtgaa	ggcccggttc	accatctccg	cgcacacctc	caagaacacc	240
gcctacctgc	agatgaactc	cctgcgggcc	gaggacaccg	ccgtgtacta	ctgetccaga	300
tggggaggcg	acggcttcta	cgccatggac	tactggggcc	agggcacctt	ggtgacagtg	360
tcctctgcct	ccaccaaggg	ccctctgttg	ttccctctgg	ccccttccag	caagtccaca	420
tctggcggca	cgcgcgctct	gggctgcctg	gtgaaagact	acttccccga	gcccgtgacc	480
gtgtcttgga	actctggcgc	cctgacctcc	ggcgtgcaca	cctttccagc	cgtgctgcag	540
tcctccggcc	tgtactccct	gtcctccgtg	gtgacctgac	cctccagctc	cttgggcacc	600
cagacctaca	ctctcaacgt	gaaccacaag	ccctccaaca	ccaaggtgga	caagaagggtg	660
gaacccaagt	ctgcgcacaa	gaccacaccc	tgtccccctt	gccctgctcc	tgaactgctg	720
ggcgggacct	ccgtgttctc	gttcccccca	aagcccaagg	acacctgat	gatctcccg	780
acccccgaag	tgacctcgct	gggtggtgac	gtgtcccacg	aggacctga	agtgaagtgc	840
aattggtatg	tggacggcgt	ggaagtgcac	aacgcccaaga	ccaagcccag	agaggaacag	900
tacaactcca	cctaccgggt	ggtgtctgtg	ctgacctgac	tgcaccagga	ctggctgaac	960
ggcaaaagagt	acaagtgcac	ggtgtccaac	aaggccctgc	ctgcccccat	cgaaaagacc	1020
atctccaagg	ccaagggcca	gcctcgcgag	cctcaggtgt	acacactgcc	ccctagcccg	1080
gaagagatga	ccaagaacca	ggtgtccctg	acctgtctgg	tgaaggctt	ctaccctcc	1140
gatatcgccg	tggaaatgga	gtccaaacggc	cagcccgaga	acaactacaa	gaccaccccc	1200
cctgtgctgg	actccgacgg	ctcattcttc	ctgtactcca	agctgaccgt	ggacaagtcc	1260
cgggtggcagc	agggcaacgt	gttctcctgc	tcctgtatgc	acgaggccct	gcacaaccac	1320
tacaccagga	agtcctgttc	cctgagccct	ggaaaggcg	gaggcggctc	tgggtggtgga	1380
ggctctggag	gcggaggctc	taccatccct	ccacacgtgc	agaaatccgt	gaacaacgac	1440
atgatcgtga	ccgacaaaca	cggcgccgtg	aagttccccc	agctgtgcaa	gttctgcgac	1500
gtgcggttct	ctacctgcga	caaccagaaa	tcctgcatgt	ccaactgctc	catcacctcc	1560
atctgcgaga	agccccagga	agtgtgcgtg	gccgtgtggc	ggaagaacga	cgagaacatc	1620
accctggaaa	ccgtgtgcga	cgaccccaag	ctgccctacc	acgacttcat	cctggaagat	1680
gccgcctccc	ccaagtgcac	catgaaggaa	aagaagaagc	ccggcgagac	attcttcatg	1740
tgcagctgct	cctccgacga	gtgcaacgac	aacatcatct	tctccgaaga	gtacaacacc	1800
tccaaccccc	actgaggtac	c				1821

SEQ ID NO: 115 moltype = DNA length = 657
FEATURE Location/Qualifiers
source 1..657
mol_type = other DNA
organism = Homo sapiens

SEQUENCE: 115

gagctcgaca	tccagatgac	ccagtcctcc	tccagcctgt	ccgcctctgt	gggcgacaga	60
gtgaccatca	cctgtcgggc	ctctcaggac	gtgaacaccg	ccgtggccctg	gtatcagcag	120
aagcctggca	agggccccaa	gctgctgac	tactccgctt	ccttccgtga	ctccgggggtg	180
ccatcccggt	tctccggctc	tagatccggc	accgacttca	ccttgacctt	ctccagcctg	240
cagcccgagg	acttcgccac	ctactactgc	cagcagcact	acaccacccc	ccctaccttc	300
ggccagggca	ccaaggtgga	aatcaagcgg	accgtggccg	ctccctccgt	gttcattctc	360
ccaccctccg	acgagcagct	gaagtctggc	accgcctccg	tcgtgtgcct	gctgaacaac	420
ttctaccccc	gcgagggcca	ggtgcagtgg	aaggtggaca	acgcccgtga	gtccggcaac	480
tcccaggaa	ccgtgaccca	gcaggactcc	aaggacagca	cctactccct	gtcctccacc	540
ctgacctgtg	ccaagggcca	ctacgagaag	cacaaggtgt	acgctgcga	agtgacccac	600
cagggcctgt	ccagccccgt	gaccaagtcc	ttcaaccggg	gcgagtgcgt	aggtacc	657

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SEQ ID NO: 116      moltype = DNA length = 1818
FEATURE            Location/Qualifiers
source              1..1818
                    mol_type = other DNA
                    organism = Homo sapiens

SEQUENCE: 116
gagctccagg tgcagctgaa gcagtcggcg ccaggactgg tgcagccttc ccagtcctcg 60
tccatcacct gtaccgtgtc cggtctctcc ctgaccaact acggcggtgca ctgggtccga 120
cagtcctccag gcaaggggcct ggaatggctg ggagtgattt ggagcggcgg caacaccgac 180
tacaacaccc ccttcacctc ccggctgtcc atcaacaagg acaactccaa gtcccagggtg 240
ttcttcaaga tgaactccct gcagtcctaa gacaccggca tctactactg cgccagagcc 300
ctgacctact atgactacga gttcgcttac tggggacagg gcacctggtt caccgtgtct 360
gccgcctcta ccaaggggccc ctccgtgttt ccctggcccc cctccagcaa gtccacatct 420
ggcggcaccg ccgctctggg ctgcctggto aaggactact tcccagagcc cgtgaccgtg 480
tcttggaaact ctggcgccct gacctccggc gtgcacacct ttccagccgt gctgcagtc 540
tccggcctgt actccctgtc ctccgtgtgt accgtgacct ccagctctct gggcaccag 600
acctacatct gcaacgtgaa ccacaagccc tccaacacca aggtggacaa gcgggtggaa 660
cccagtcctt gcgacaaagc ccacacctgt ccccccctgc ctgccccctg actgctggga 720
ggcccttcct tgttctctgt ccccccaaag cccaaggaca cctgatgat ctcccggacc 780
ccgaaagtga gtcgctggt ggtggacgtg tcccacgagg accctgaagt gaagtccaat 840
tggtacgtgg acggcggtga agtgacacac gccaaagaca agcccagaga ggaacagtac 900
aaactccacct accgggtggt gtccgtgctg accgtgctgc accaggactg gctgaacggc 960
aaagagtaca agtgcaaggt ctccaacaag gccctgctct cccccatcga aaagaccatc 1020
tccaaggcca agggccagcc ccgcgagcct caggtgtaca ctctgcctcc cagccgggac 1080
gagctgacca agaaccaggt gtccctgacc tgtctgggtc agggcttcta cccctccgat 1140
atcgccgtgg aatgggagtc caacggccag cccgagaaca actacaagac caccctccct 1200
gtgctggagt ccgacggctc attcttctct tactccaagc tgacctgga caagtcccgg 1260
tggcagcagg gcaacgtggt ctccgtctcc gtgatgcac agggcctgca caaccactac 1320
accagaaagt cctgtctctt gaggccccgc aaggcgagg gcgatctgg tgggtgtggc 1380
tctggtggcg gaggctctac catccctcca cagtgacaga aatccgtgaa caacgacatg 1440
atcgtgaccg acaacaacgg cgccgtgaag ttccccagc tgtgcaagtt ctgcgacgtg 1500
cggttctcta cctgcgacaa ccagaaatcc tgcattgcca actgctccat cactccatc 1560
tgcgagaagc cccaggaagt gtgcgtcgcc gtctggcgga agaaccgaca gaacatcacc 1620
ctggaaacgg tgtgccacga ccccaagctg cctaccacg acttcatcct ggaagatgcc 1680
gccctcccca agtgcatcat gaaggaaaag aagaagcccg gcgagacttt ctctatgtgc 1740
agctgctcct ccgacgagtg caacgacaac atcatcttct ccgaagagta caacacctcc 1800
aaccgccgact gaggtaacc                                     1818

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SEQ ID NO: 117      moltype = DNA length = 657
FEATURE            Location/Qualifiers
source              1..657
                    mol_type = other DNA
                    organism = Homo sapiens

SEQUENCE: 117
gagctcgata tcttgetgac ccagtcctccc gtgatcctgt ccgtgtctec tggcgagcgg 60
gtgtccttct cctgcggggc ctcccagtc atcgccacca acatccactg gtatcagcag 120
cggaccaacg gctcccctcg gctgctgatt aagtacgcct ccgagttctat ctccggcatc 180
cctccccggg tctccggctc tggctccggc accgacttca cctgtccat caactccgtg 240
gaatccgagg atatcgccga ctactactgc cagcagaaca acaactggcc caccaccttc 300
ggcgctggca ccaagctgga actgaagcgg accgtggccc ctccctccgt gttcatcttc 360
ccaccctccg acgagcagct gaagtcggcg accgcctccg tctgtgtcct gctgaacaac 420
ttctaccccc gcgagggccaa ggtgcagtg aaagtggaac acgcccctgca gtccggcaac 480
tccaggaat ccgtcaccca gcaggactcc aaggacagca cctactcctt gtccctccac 540
ctgacctgtt ccaaggccga ctacgagaag cacaaggtgt acgctcgca agtgacccac 600
cagggcctgt ccagccccgt gaccaagtcc ttcaaccggg gcgagtgtct aggtacc 657

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SEQ ID NO: 118      moltype = DNA length = 1824
FEATURE            Location/Qualifiers
source              1..1824
                    mol_type = other DNA
                    organism = Homo sapiens

SEQUENCE: 118
gagctccagg tgcagctgca gcagcctggc gccgagctgg tcaagcctgg cgcttccgtg 60
aagatgtcct gcaaggcctc cggtacaccc ttaccagctt acaacatgca ctgggtcaag 120
cagacccccg cgagaggcct ggaatggatc ggcgccatct accccggcaa cggcgacacc 180
tcttacaacc agaagttcaa gggaaaggcc accctgaccg ccgacaagtc ctctccacc 240
gectacatgc agctgtcctc cctgacctcc gaggactccg ccgtgtacta ctgcgccgg 300
tccacctact acggcgccga ctggtacttc aacgtgtggg gcgctggcac caccgtgacc 360
gtgtctggcg cctctaccaaa gggccccctc gtgtttcccc tggccccctc cagcaagtc 420
acatctggcg gcaccggcgc tctgggctgc ctggtcaagg actacttccc cgagcccgtg 480
acagtgtcct ggaactctgg cgccctgacc agcggcgctg acaccttccc agcctgtgtg 540
cagtcctctg gctctgtact cctgtccagc gtctgaccc tgccctccag ctctctggg 600
accagacctt acatctgcaa cgtgaaccac aagccctcca acaccaaggt ggacaagaag 660
gccgagccca agtctctgca caagacccac acctgtcccc cctgccccgc cctgaaactg 720
ctgggaggcc cttctgtgtt cctgttcccc ccaaagccca aggacacctt gatgatctcc 780
cggacccccg aagtgacatg cgtgggtgtg gacgtgtccc acgaggaacc tgaagtgaag 840
ttcaatttgt acgtggacgg cgtgggaagt cacaacgcca agaccaagcc cagagaggaa 900
cagtacaact ccacctaccg ggtggtgtcc gtgctgaccg tgctgcacca ggactggctg 960
aacggcaag agtacaagtg caaggtgtcc aacaaggccc tgcctgcccc catcgaaaag 1020

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accatctcca aggccaaagg ccagcccccg gagcctcagg tgtacactct gctctcccag 1080
cgggagcagc tgaccaagaa ccaggtgtcc ctgacctgtc tgggtcaagg cttctacccc 1140
tccgatatcg ccgtggaatg ggagtccaac ggccagcccg agaacaacta caagaccacc 1200
ccccctgtgc tggactccga cggtcatctt ttctgtactt ccaagctgac cgtggacaag 1260
tcccgttggc agcagggcaa cgtgttctcc tgctccgtga tgcacgagge cctgcacaa 1320
cactacaccc agaagtcctt gtccctgagc cccggaaagg gcggaggcgg atctggtggt 1380
ggaggatcag gcggcggagg ctctaccatc cccccacacg tgcagaaatc cgtgaacaac 1440
gacatgatcg tgaccgacaa caacggcgcc gtgaagtctc cccagctgtg caagtctctg 1500
gacgtgcggg tctctacctg cgacaaccag aaatcctgca tgtccaactg ctccatcacc 1560
tccatctgcg agaagcccca ggaagtgtgc gtcccgctct ggccgaaaga cgacgagaac 1620
atcacccctg aaaccgtgtg ccacgacccc aagctgacct accacgactt catcctggaa 1680
gatgccgctt cccccaagtg catcatgaag gaaaaagaag agcccggcga gactttcttc 1740
atgtgctctt gctcctccga cgagtgcacg gacaacatca tcttctccga agagtacaac 1800
acctccaacc ccgactgagg tacc 1824

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SEQ ID NO: 119      moltype = DNA length = 654
FEATURE            Location/Qualifiers
source              1..654
                    mol_type = other DNA
                    organism = Homo sapiens

```

```

SEQUENCE: 119
gagctccaga tcgtgctgtc ccagtcctcc gccatcctgt ctgctagccc tggcgagaaa 60
gtgacaatga cctgcggggc ctccctctcc gtgtcctaca tccactggtt ccagcagaag 120
cccggctcca gccccaagcc ctggatctac gccacctcca acctggcctc cggcgtgcca 180
gtgcggttct ctggctccgg ctcgggcacc tctactctcc tgaccatctc ccgggtggaa 240
gccgaggacg ccgccacctc ctactgccag cagtggacct ccaaccccc cactttggc 300
ggaggaccca agctggaagt caagcggacc gtggcgcctc cctccgtgtt catcttccca 360
ccctccgacg agcagctgaa gtccggcacc gcctccgtcg tgtgcctgct gaacaacttc 420
taccctcgcg aggccaaagt gcagtggaag gtggacaacg ccttcagctc cggcaactcc 480
caggaatccg tcaccgagca ggactccaag gacagcacct acagcctgtc ctccacctcg 540
acctgttcca aggcgagcta cgagaagcac aaggtgtacg cctgcgaagt gacctccag 600
ggcctgtcca gccccgtgac caagtccctc aaccggggcg agtgctgagg tacc 654

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SEQ ID NO: 120      moltype = DNA length = 1830
FEATURE            Location/Qualifiers
source              1..1830
                    mol_type = other DNA
                    organism = Homo sapiens

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SEQUENCE: 120
gagctcgagg tgcagctggt ggaatccggc ggaggcctgg tccagcctgg cggatccctg 60
agactgtcct gtgcgcctcc ccgctacacc ttcaccaact acggcatgaa ctgggtccga 120
caggccctcg gcaaggggct ggaatgggtc ggatggatca acacctaac cggcgagccc 180
acctacgcgg ccgacttcaa gcggcggttc accttctccc tggacacctc caagtccacc 240
gcctacctcg agatgaaact cctgcggggc gaggacacgg ccgtgtacta ctgcgccaa 300
taccctccact actacggctc ctccactgg tacttcgacg tgtggggcca gggaacctg 360
gtcaccgtgt cctccgcctc taccaaaggg ccctccgtgt tccctctgge cccctccagc 420
aagtccacat ctggcgccac cgcgcctctg gggtgcctgg tcaaggacta ctcccccgag 480
ccgtgacagg tgtcctggaa ctctggcgcc ctgacctccg gcgtgcacac ctttccagcc 540
gtgctgcagt cctccggcct gtactccctg tctccgtcg tgaccgtgce ctccagctct 600
ctgggcaccc agacctacat ctgcaacgtg aaccacaagc cctccaacac caaggtggac 660
aagaagggtg aacccaagtc ctgcgacaag acccacacct gtccccctcg cctgccccct 720
gaactgctgg gaggccctag cgtgttctct tccccccaa agcccaagga caccctgatg 780
atctcccgga cccccaagt gacctcgctg gtggtggacg tgtcccacga ggacctgaa 840
gtgaagtcca attggtacct ggaaggcggt gaagtgcaca acgccaagac caagcccgaa 900
gaggaaacgt acaactccac ctaccgggtg gtgtccgtgc tgacctgtct gacccaggac 960
tggtgtaacg gcaaaagata caagtgcagg gtgtccaaca aggcctgccc tgcccccatc 1020
gaaaagacca tctccaaggg caagggccag ccccgcgagc ctacaggtgta cactctgccc 1080
cctagccggg aagagatgac caagaaccag gtgtccctga cctgtctggt caagggcttc 1140
taccctcccg atatcgccgt ggaatgggag tccaacggcc agcccgagaa caactacaag 1200
accaccccc ctgtgtctgga ctccgacggc tcattcttcc tgtactccaa gctgacctgt 1260
gacaagtcct ggtggcagca gggcaacgtg ttctcctgct ccgtgatgca cgaggccctg 1320
cacaaccact acaccagaa gtccctgtcc ctgagccag gcaaggcgg aggcggatct 1380
ggtggtggag gatcaggcgg cggaggctct accatcccc cacacgtgca gaaatccgtg 1440
aacaacgaca tgactcgtgac cgacaacaac ggcgcctgta agttccccca gctgtgcaag 1500
ttctgcgacg tgcggttctc tacctgcgac aaccagaaat cctgcatgct caactgctcc 1560
atcacctcca tctgcgagaa gccccaggaa gtgtgcgtcg ccgtctggcg gaagaacgac 1620
gagaacatca ccttggaac cgtgtgccac gaccccaagc tgccctacca cgacttcac 1680
ctggaagatg ccgctcccc caagtgcac atgaaggaaa agaagaagcc cggcgagact 1740
ttctcatgt gcagctgctc ctccgacgag tgcaacgaca acatcatctt ctccgaagag 1800
tacaacacct ccaaccccca ctgaggtacc 1830

```

```

SEQ ID NO: 121      moltype = DNA length = 657
FEATURE            Location/Qualifiers
source              1..657
                    mol_type = other DNA
                    organism = Homo sapiens

```

```

SEQUENCE: 121
gagctcgata tccagatgac ccagtcctcc tccagcctgt ccgcctctgt gggcgacaga 60
gtgaccatca cctgttccgc cagccaggac atctccaact acctgaactg gtatcagcag 120

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aagccccgga agggccctaa ggtgctgac tacttcacct cctccctgca ctccggcggtg 180
ccctccagat tctccggctc tggtccgggc accgacttta ccttgaccat ctccagcctg 240
cagcccgagg acttcgccac ctactactgc cagcagtaact ccaccgtgcc ctggaccttc 300
ggccagggca ccaagtgga aatcaagcgg accgtggccg ctccctccgt gttcatcttc 360
ccaccctccg acgagcagct gaagtccggc accgcctccg tcgtgtgcct gctgaacaac 420
ttctaccccc gcgaggccaa ggtgcagtgg aaggtggaca acgcctgca gtccggcaac 480
tcccaggaat ccgtcaccga gcaggactcc aaggacagca cctactccct gtccctccac 540
ctgaccctgt ccaaggccga ctacgagaag cacaaggtgt acgcctgcga agtgaccac 600
cagggcctgt ccagcccctg gaccaagtc ttcaaccggg gcgagtgtgt aggtacc 657

SEQ ID NO: 122      moltype = AA length = 40
FEATURE            Location/Qualifiers
REGION             1..40
                    note = Synthetic construct
source             1..40
                    mol_type = protein
                    organism = synthetic construct

SEQUENCE: 122
GGGSGGGGS GGGSGGGGS GGGSGGGGS GGGSGGGGS 40

SEQ ID NO: 123      moltype = AA length = 5
FEATURE            Location/Qualifiers
source             1..5
                    mol_type = protein
                    organism = synthetic construct
VARIANT            1..5
                    note = The entire sequence of amino acids 1-5 can be
                        repeated one to eight times.

SEQUENCE: 123
GGGS 5

SEQ ID NO: 124      moltype = AA length = 211
FEATURE            Location/Qualifiers
source             1..211
                    mol_type = protein
                    organism = synthetic construct

SEQUENCE: 124
QIVSTQSPAI MSASPGEKVT MTCSASSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR 60
FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE 120
QLKSGTASVV CLLNNFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKSTY SLSSLTLSK 180
ADYEKHKVYA CEVTHQGLSS PVTKSFNRGE C 211

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What is claimed is:

1. An isolated molecule comprising:
 - (a) a targeting moiety comprising an antibody or antibody fragment thereof, comprising amino acids 1-119 of SEQ ID NO: 2 and the amino acid sequence of SEQ ID NO: 71, which specifically binds Epidermal Growth Factor Receptor (EGFR); and
 - (b) an immunomodulatory moiety comprising an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor II (TGF-βRII) comprising the amino acid sequence of SEQ ID NO: 87.
2. The molecule of claim 1, wherein the targeting moiety comprises an antibody fragment selected from a Fab or scFv.
3. The molecule of claim 2, wherein the antibody fragment comprises a light chain domain or a variable heavy chain domain.
4. The molecule of claim 3, wherein the antibody fragment comprises a light chain domain comprising the amino acid sequence of SEQ ID NO: 71.
5. The molecule of claim 4, further comprising a polypeptide linker comprising the amino acid sequence of SEQ ID NO: 104.
6. The molecule of claim 5, wherein the light chain domain comprising the amino acid sequence of SEQ ID NO: 71 is fused to an N-terminus of the polypeptide linker comprising the amino acid sequence of SEQ ID NO: 104.
7. The molecule of claim 6, wherein the immunomodulatory moiety comprising the amino acid sequence of SEQ

ID NO: 87 is fused to a C-terminus of the polypeptide linker comprising the amino acid sequence of SEQ ID NO: 104.

8. The molecule of claim 3, wherein the variable heavy chain domain comprises amino acids 1-119 of SEQ ID NO: 2.

9. An isolated molecule comprising:

- (a) a targeting moiety comprising an antibody fragment which specifically binds Epidermal Growth Factor Receptor (EGFR) comprising a light chain domain comprising the amino acid sequence of SEQ ID NO: 71 and a variable heavy chain domain comprising amino acids 1-119 of SEQ ID NO: 2;

- (b) an immunomodulatory moiety comprising an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor II (TGF-βRII) comprising the amino acid sequence of SEQ ID NO: 87; and

- (c) a polypeptide linker comprising the amino acid sequence of SEQ ID NO: 104; wherein the light chain domain comprising the amino acid sequence of SEQ ID NO: 71 is fused to an N-terminus of the polypeptide linker comprising the amino acid of SEQ ID NO: 104, and

wherein the immunomodulatory moiety comprising the amino acid sequence of SEQ ID NO: 87 is fused to a C-terminus of the polypeptide linker comprising the amino acid sequence of SEQ ID NO: 104.

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