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(54) **METHODS OF REDIRECTING OF IL-2 TO TARGET CELLS OF INTEREST**

(71) Applicant: **BRISTOL-MYERS SQUIBB COMPANY**, Princeton, NJ (US)

(72) Inventors: **Flavio SCHWARZ**, San Francisco, CA (US); **Xiaodi DENG**, San Mateo, CA (US); **Pavel STROP**, San Mateo, CA (US)

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(2) Date: **Feb. 13, 2023**

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C07K 14/715	(2006.01)
A61K 38/20	(2006.01)
A61P 35/00	(2006.01)

(52) **U.S. Cl.**

CPC ***C07K 16/2818*** (2013.01); ***C12N 15/86*** (2013.01); ***C07K 14/715*** (2013.01); *A61K 38/00* (2013.01); ***A61P 35/00*** (2018.01); *C12N 2740/15043* (2013.01); *C07K 2319/30* (2013.01); ***A61K 38/2013*** (2013.01)

(57) **ABSTRACT**

The present disclosure provides constructs comprising an anti-PDI antibody, or an alternative targeting moiety, fused to CD25 or an IL-2 binding fragment of CD25. Such constructs find use in treating human diseases, such as cancer.

Specification includes a Sequence Listing.

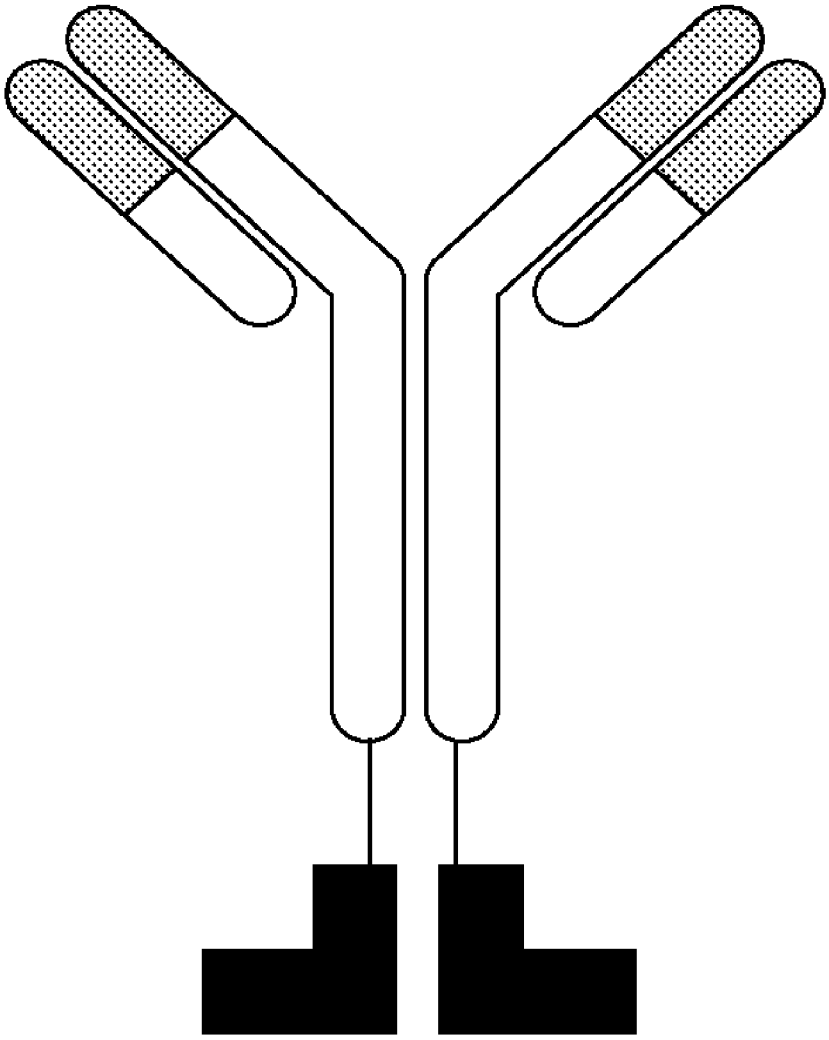


FIG. 1A

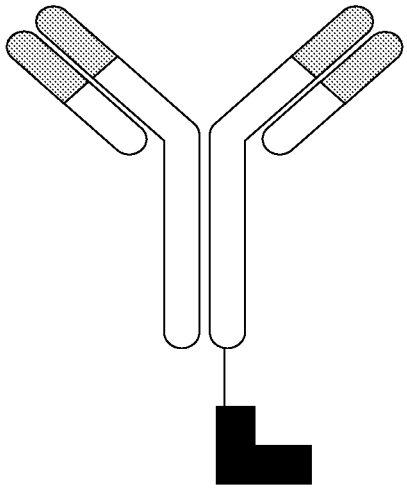


FIG. 1B

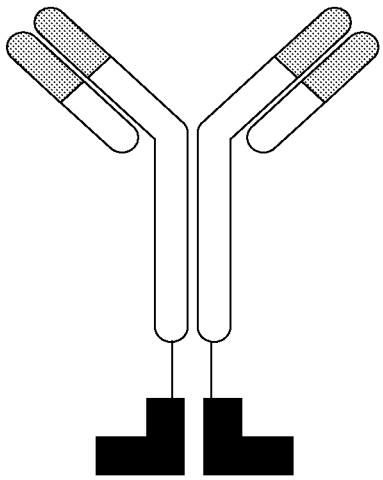


FIG. 2A

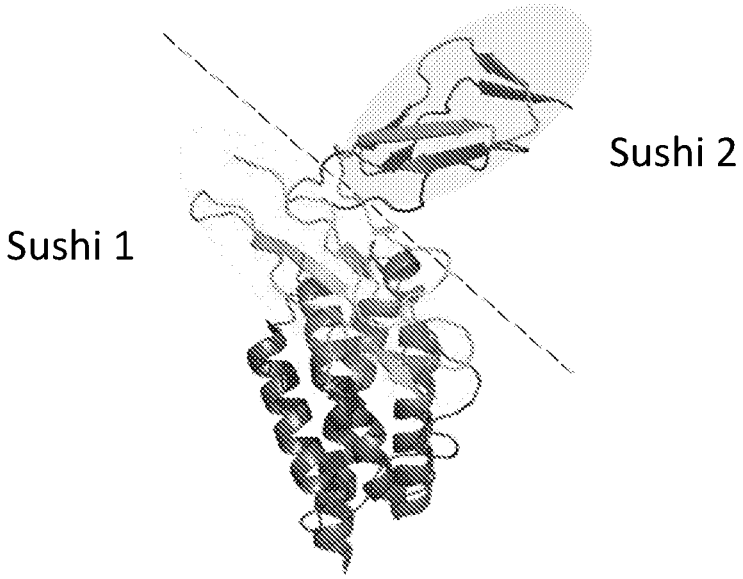


FIG. 2B

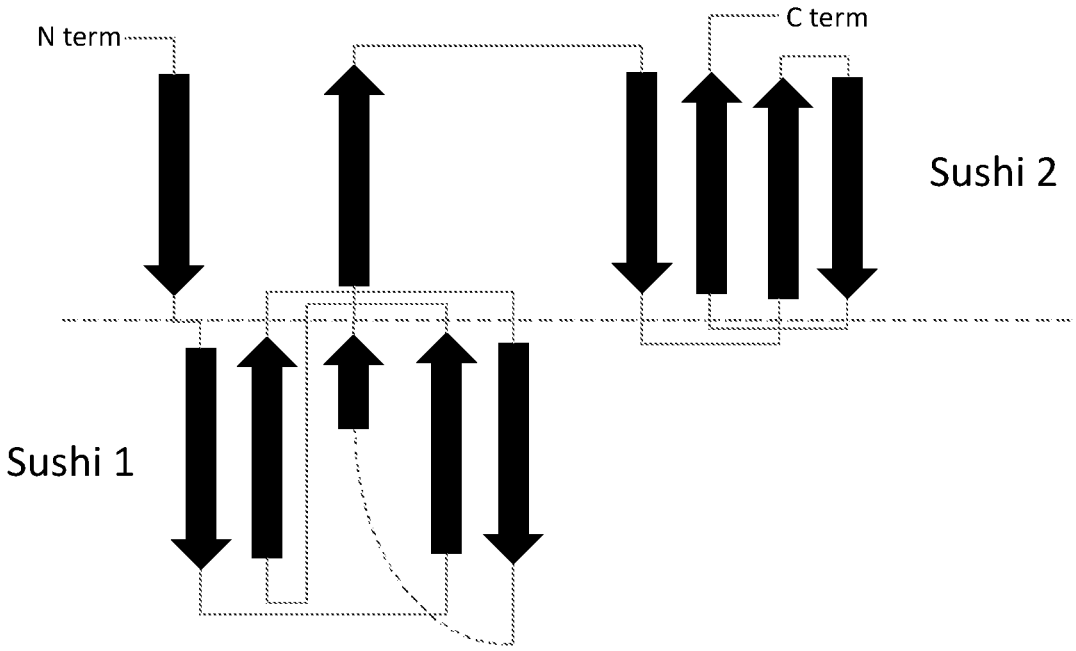


FIG. 2C

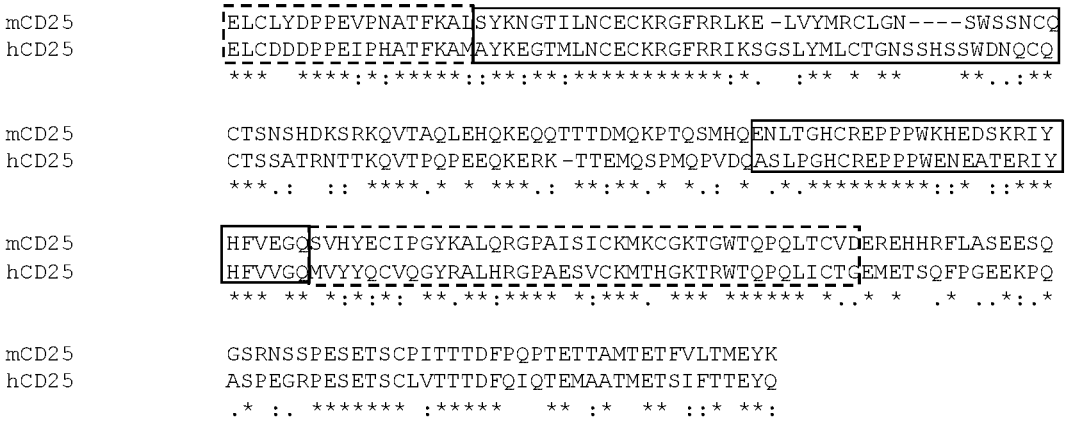

mCD25 ELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKE-LVYMRCLGN----SWSSNCQ
hCD25 ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQ
mCD25 CTSNSHDKSRKQVTAQLEHQKEQQTTTDMQKPTQSMHQENLTGHCREPPPWKHEDSKRIY
hCD25 CTSSATRNTTKQVTPQPEEQKERK-TTEMQSPMQPVDQASLPGHCREPPPWENEATERIY
mCD25 HFVEGQSVHYECIPGYKALQRGPAISICKMKCGKTGWTQPQLTCVDEREHHRFLASEESQ
hCD25 HFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQFPGEEKPQ
mCD25 GSRNSSPESETSCPITTTDFPQPTETTAMTETFVLTMEYK
hCD25 ASPEGRPESETSCLVTTTDFQIQTEMAATMETSIFTTEYQ

FIG. 3A

>mCD25.a (SEQ ID NO:2)(full-length ECD)
ELCLYDPPEVPNATFKAL*SYKNGTILNCECKRGFRRLKELVYMRCLGNSWSS*
*NCQ*CTSNSHDKSRKQVTAQLEHQKEQQTTTDMQKPTQSMHQ*ENLTGHCREPP*
*PWKHEDSKRIYHFVEGQ*SVHYECIPGYKALQRGPAISICKMKCGKTGWTQPQ
LTCVDEREHHRFLASEESQGSRNSSPESETSCPITTTDFPQPTETTAMTETF
VLTMEYK

>mCD25.b (SEQ ID NO:3)(1-198 SEQ ID NO:2)
ELCLYDPPEVPNATFKAL*SYKNGTILNCECKRGFRRLKELVYMRCLGNSWSS*
*NCQ*CTSNSHDKSRKQVTAQLEHQKEQQTTTDMQKPTQSMHQ*ENLTGHCREPP*
*PWKHEDSKRIYHFVEGQ*SVHYECIPGYKALQRGPAISICKMKCGKTGWTQPQ
LTCVDEREHHRFLASEESQGSRNSSPESETSCPITTTDFPQP

>mCD25.c (SEQ ID NO:4)(40-142 SEQ ID NO:2)
SYKNGTILNCECKRGFRRLKELVYMRCLGNSWSS
*NCQ*CTSNSHDKSRKQVTAQLEHQKEQQTTTDMQKPTQSMHQ*ENLTGHCREPP*
PWKHEDSKRIYHFVEGQ

FIG. 3B

>hCD25.a (SEQ ID NO:11)(full length ECD)
ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSH
SSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHC
REPPPWENEATERIYHFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRW
TQPQLICTGEMETSQFPGEEKPQASPEGRPESETSCLVTTTDFQIQTEMAAT
METSIFTTEYQ

>hCD25.b (SEQ ID NO:12)(1-202 SEQ ID NO:11)
ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSH
SSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHC
REPPPWENEATERIYHFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRW
TQPQLICTGEMETSQFPGEEKPQASPEGRPESETSCLVTTTDFQIQ

>hCD25.c (SEQ ID NO:13)(19-125 SEQ ID NO:11)
AYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSH
SSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHC
REPPPWENEATERIYHFVVGQ

>hCD25.d (SEQ ID NO:14)(1-165 SEQ ID NO:11)
ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSH
SSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHC
REPPPWENEATERIYHFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRW
TQPQLICTG

>hCD25.e (SEQ ID NO:15)(1-124 SEQ ID NO:11 w/C3A)
ELADDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSH
SSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHC
REPPPWENEATERIYHFVVG

>hCD25.f (SEQ ID NO:16)(23-64 SEQ ID NO:11)
GTMLNCECKRGFRRIKSGSLYMLCTGNSSH
SSWDNQCQCTSS

FIG. 4

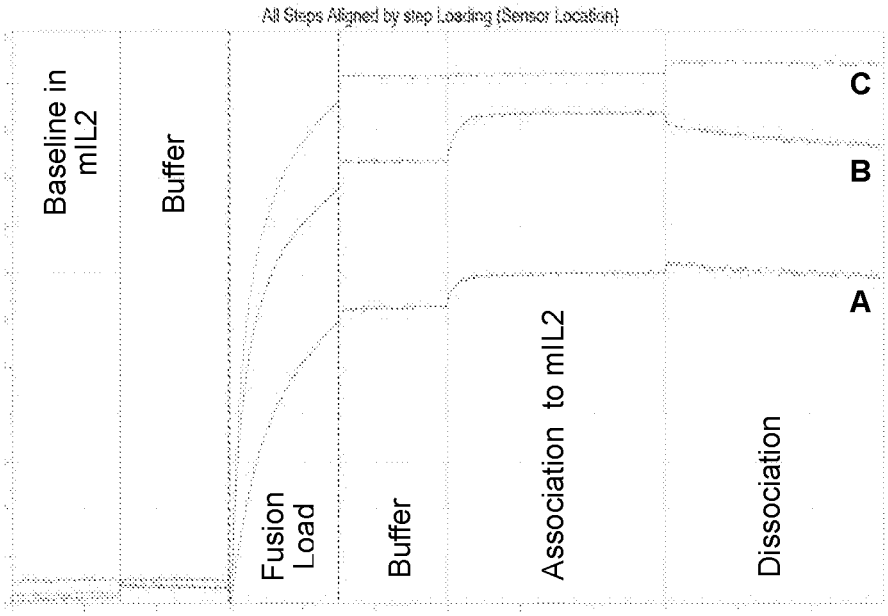

All Steps Aligned by step Loading (Sensor Location)
Baseline in mIL2
Buffer
Fusion Load
Buffer
Association to mIL2
Dissociation
C
B
A

FIG. 5A

4H2 mCD25 variant a (SEQ ID NO: 8)

QVQLKESGPGLVQPSQTLSLTCTVSGFSLTSYNVHWVRQPPGKGLEWMGGMR
YNEDTSYNSALKSRLSISRDTSKNQVFLKMNSLQTDDTGTYYCTRDAVYGGY
GGWFAYWGQGTLVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPE
PVTVTWNSGSLSSGVHTFPAVLESDLYTLSSSVTVPSSTWPSETVTCNVAHP
ASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCV
VVAISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWL
NGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLT
CMITDFFPEDITVEWQWNGQPAENYKNTQPIMKTDGSYFVYSKLNVQKSNWE
AGNTFTCSVLHEGLHNHHTEKSLSHSP<u>GGGGSGGGGSGGGGS</u>*ELCLYDPPEV*
PNATFKALSYKNGTILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSNSHD
KSRKQVTAQLEHQKEQQTTTDMQKPTQSMHQENLTGHCREPPPWKHEDSKRI
YHFVEGQSVHYECIPGYKALQRGPAISICKMKCGKTGWTQPQLTCVDEREHH
RFLASEESQGSRNSSPESETSCPITTTDFPQPTETTAMTETFVLTMEYK

FIG. 5B

4H2 mCD25 variant b (SEQ ID NO: 9)

QVQLKESGPGLVQPSQTLSLTCTVSGFSLTSYNVHWVRQPPGKGLEWMGGMR
YNEDTSYNSALKSRLSISRDTSKNQVFLKMNSLQTDDTGTYYCTRDAVYGGY
GGWFAYWGQGTLVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPE
PVTVTWNSGSLSSGVHTFPAVLESDLYTLSSSVTVPSSTWPSETVTCNVAHP
ASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCV
VVAISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWL
NGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLT
CMITDFFPEDITVEWQWNGQPAENYKNTQPIMKTDGSYFVYSKLNVQKSNWE
AGNTFTCSVLHEGLHNHHTEKSLSHSP<u>GGGGSGGGGSGGGGS</u>*ELCLYDPPEV*
PNATFKALSYKNGTILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSNSHD
KSRKQVTAQLEHQKEQQTTTDMQKPTQSMHQENLTGHCREPPPWKHEDSKRI
YHFVEGQSVHYECIPGYKALQRGPAISICKMKCGKTGWTQPQLTCVDEREHH
RFLASEESQGSRNSSPESETSCPITTTDFPQP

FIG. 6A

Nivolumab hCD25 variant a (SEQ ID NO: 28)

QVQLVESGGGVVQPGRSLRLDCKASGITFS**NSGMH**WVRQAPGKGLEWVA**VIW**
YDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCAT**NDDY**WG
QGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD
KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSLGGGGGSGGGGSGGGGS*ELCDDDPPEIPHAT*
FKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSAT
RNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERI
YHFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETS
QFPGEEKPQASPEGRPESETSCLVTTTDFQIQTEMAATMETSIFTTEYQ

FIG. 6B

Nivolumab hCD25 variant b (SEQ ID NO: 29)

QVQLVESGGGVVQPGRSLRLDCKASGITFS**NSGMH**WVRQAPGKGLEWVA**VIW**
YDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCAT**NDDY**WG
QGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD
KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSLGGGGGSGGGGSGGGGS*ELCDDDPPEIPHAT*
FKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSAT
RNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERI
YHFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETS
QFPGEEKPQASPEGRPESETSCLVTTTDFQIQ

FIG. 6C

Nivolumab hCD25 variant d (SEQ ID NO: 30)

QVQLVESGGGVVQPGRSLRLDCKASGITFS**NSGMH**WVRQAPGKGLEWVA**VIW**
YDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCAT**NDDY**WG
QGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD
KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSLGGGGGSGGGGSGGGGS*ELCDDDPPEIPHAT*
FKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSAT
RNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERI
YHFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTG

FIG. 7A

Nivolumab hIgG1.3 hCD25 variant a (SEQ ID NO: 33)

QVQLVESGGGVVQPGRSLRLDCKASGITFS**NSGMH**WVRQAPGKGLEWVA**VIW**
YDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCAT**NDDY**WG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KRVEPKSCDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS*ELCDDDPPEIP*
HATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTS
SATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEAT
ERIYHFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEM
ETSQFPGEEKPQASPEGRPESETSCLVTTTDFQIQTEMAATMETSIFTTEYQ

FIG. 7B

Nivolumab hIgG1.3 hCD25 variant b (SEQ ID NO: 34)

QVQLVESGGGVVQPGRSLRLDCKASGITFS**NSGMH**WVRQAPGKGLEWVA**VIW**
YDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCAT**NDDY**WG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KRVEPKSCDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS*ELCDDDPPEIP*
HATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTS
SATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEAT
ERIYHFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEM
ETSQFPGEEKPQASPEGRPESETSCLVTTTDFQIQ

FIG. 7C

Nivolumab hIgG1.3 hCD25 variant d (SEQ ID NO: 35)

QVQLVESGGGVVQPGRSLRLDCKASGITFS**NSGMH**WVRQAPGKGLEWVA**VIW**
YDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCAT**NDDY**WG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KRVEPKSCDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS*ELCDDDPPEIP*
HATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTS
SATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEAT
ERIYHFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTG

FIG. 8A

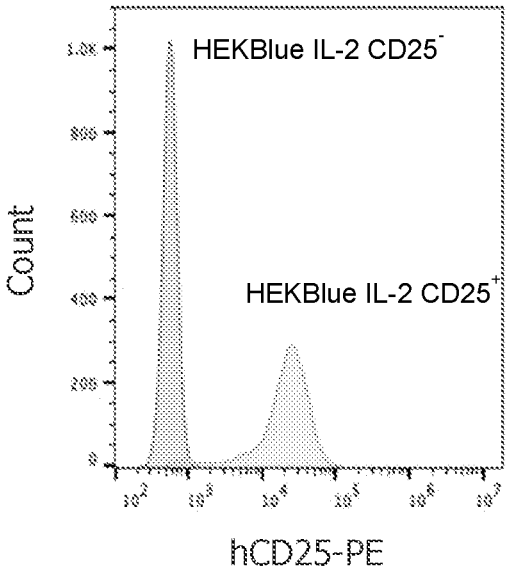

HEKBlue IL-2 CD25-
HEKBlue IL-2 CD25+
Count
hCD25-PE

FIG. 8B

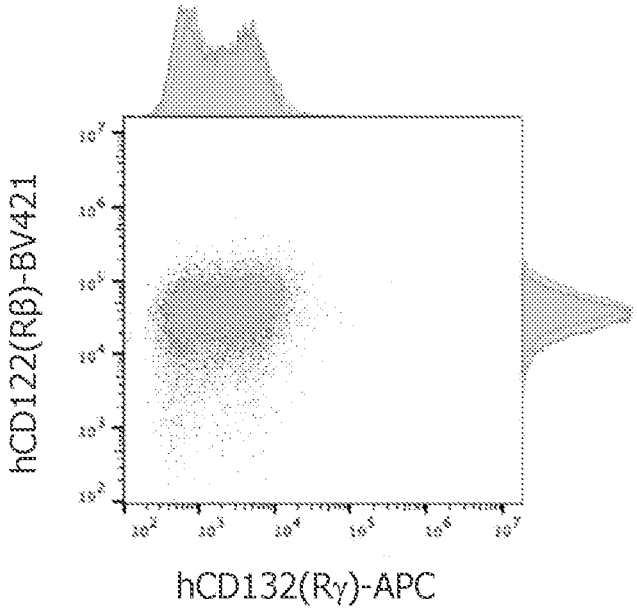

hCD122(Rβ)-BV421
hCD132(Rγ)-APC

FIG. 8C

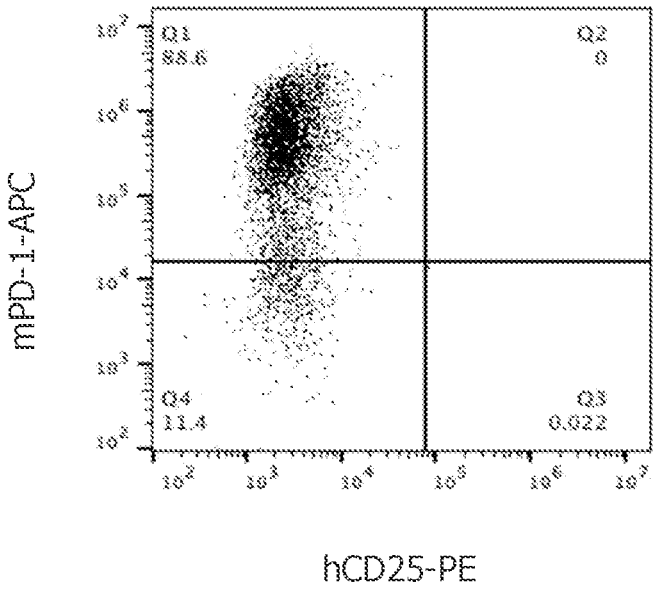

Q1
88.6
Q2
0
Q4
11.4
Q3
0.022
mPD-1-APC
hCD25-PE

FIG. 8D

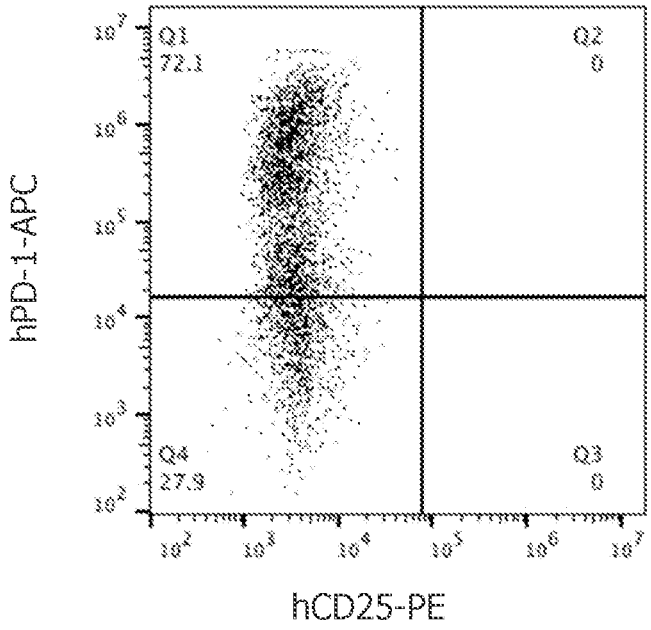

Q1
72.1
Q2
0
Q4
27.9
Q3
0
hPD-1-APC
hCD25-PE

FIG. 9

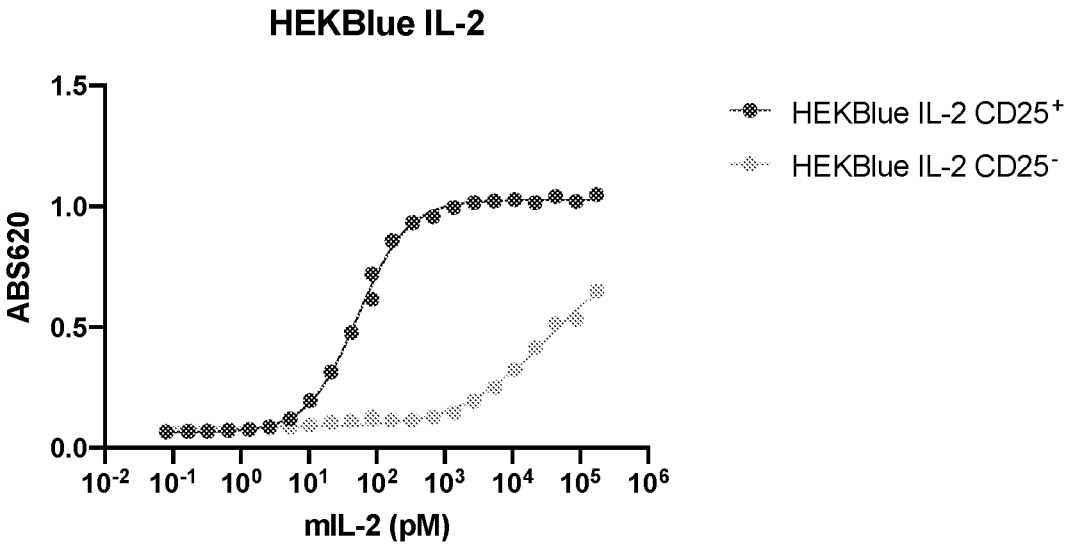

HEKBlue IL-2
ABS620
1.5
1.0
0.5
0.0
10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5} 10^{6}
mIL-2 (pM)
HEKBlue IL-2 CD25+
HEKBlue IL-2 CD25-

FIG. 10A

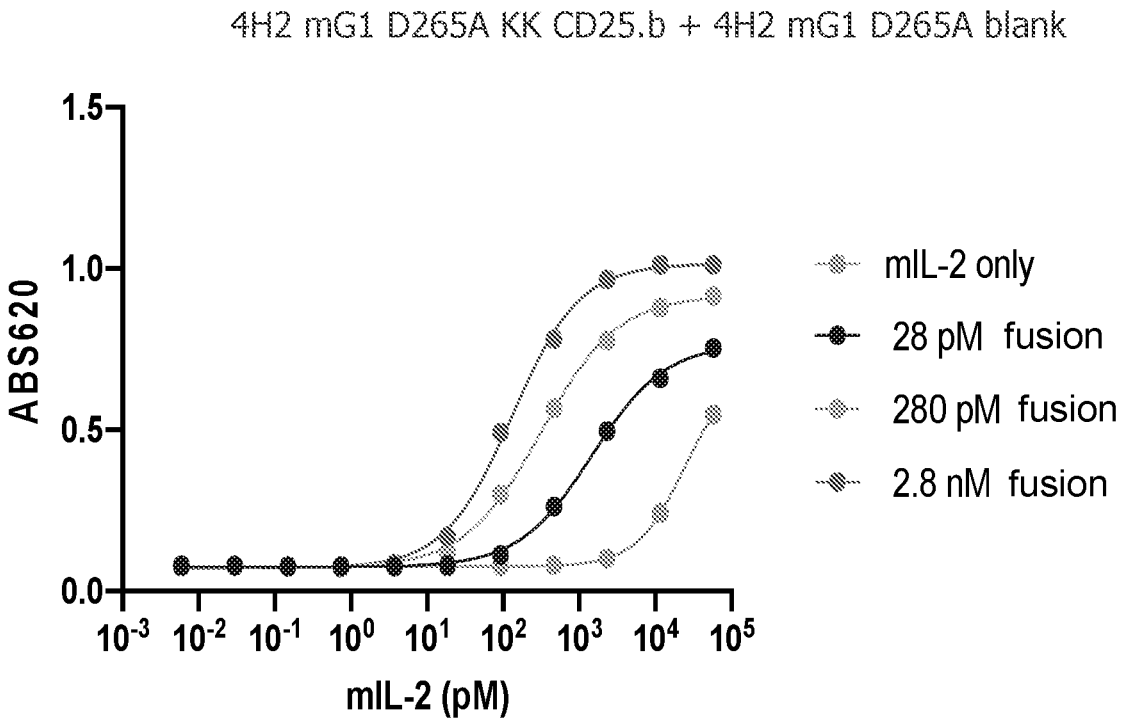


FIG. 10B

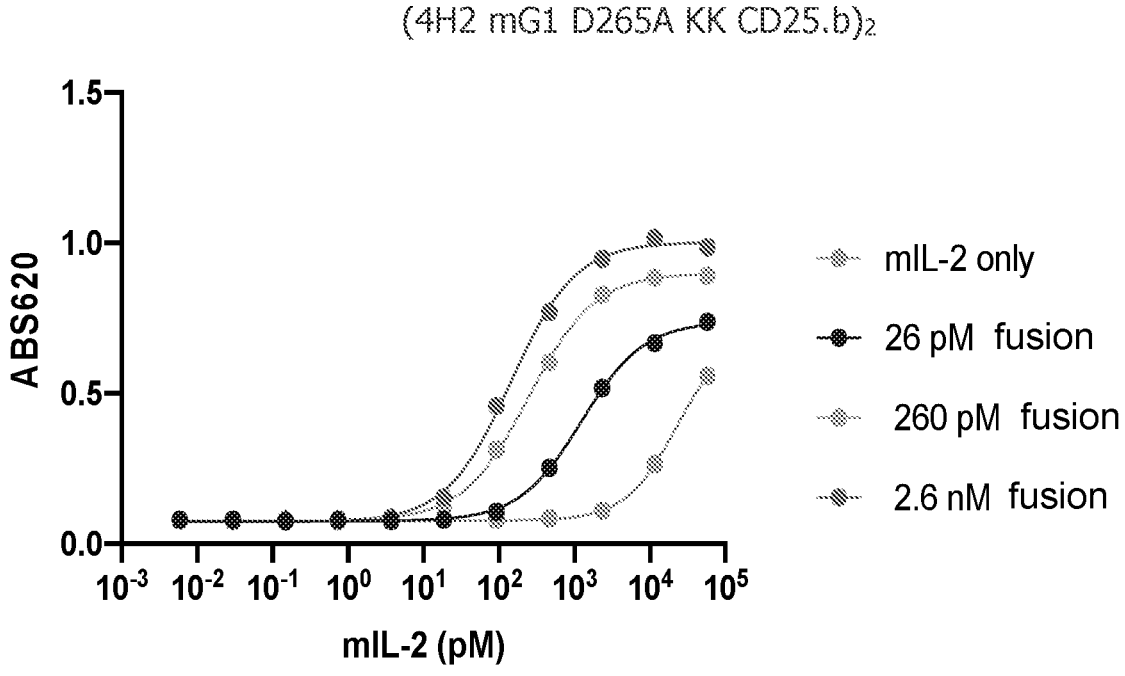


FIG. 10C

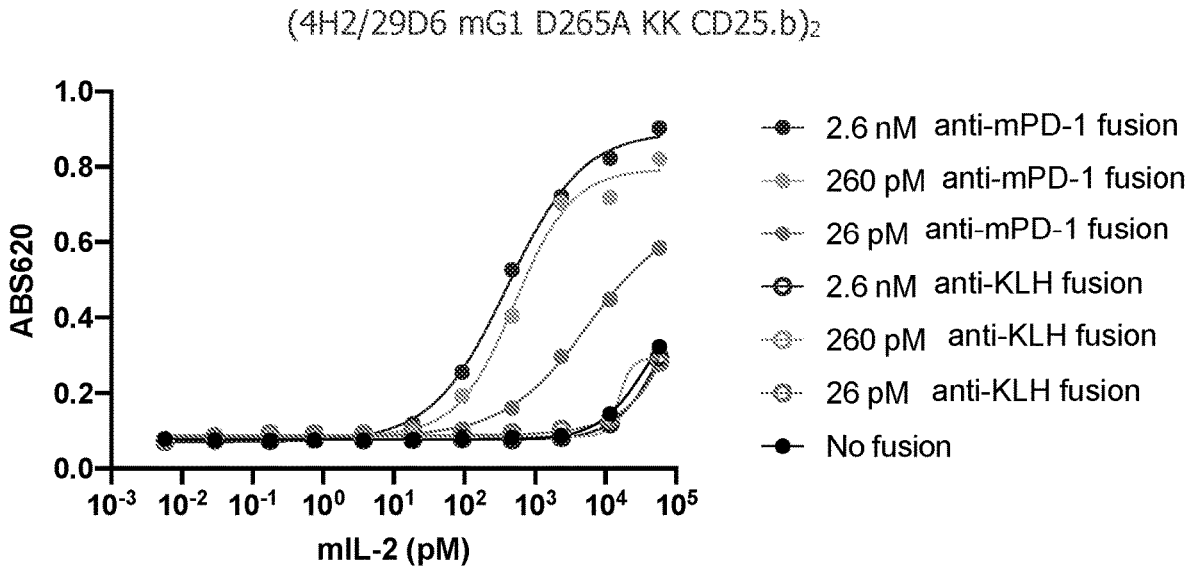

(4H2/29D6 mG1 D265A KK CD25.b)2
ABS620
1.0
0.8
0.6
0.4
0.2
0.0
10-3 10-2 10-1 100 101 102 103 104 105
mIL-2 (pM)
2.6 nM anti-mPD-1 fusion
260 pM anti-mPD-1 fusion
26 pM anti-mPD-1 fusion
2.6 nM anti-KLH fusion
260 pM anti-KLH fusion
26 pM anti-KLH fusion
No fusion

FIG. 11A

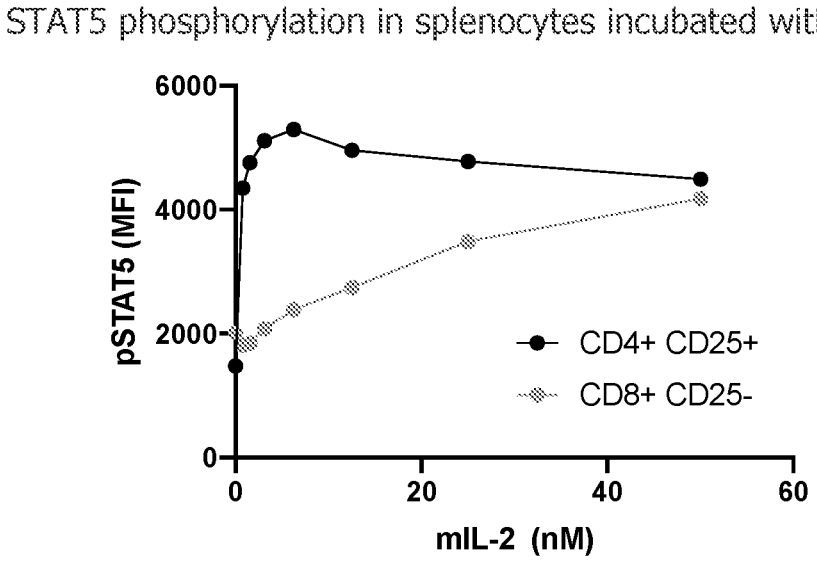


FIG. 11B

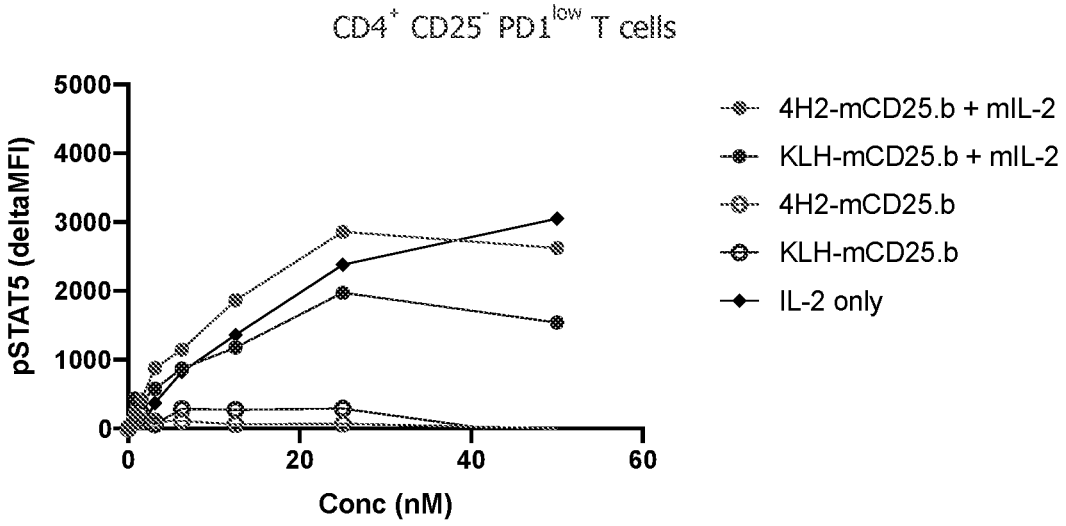


FIG. 11C

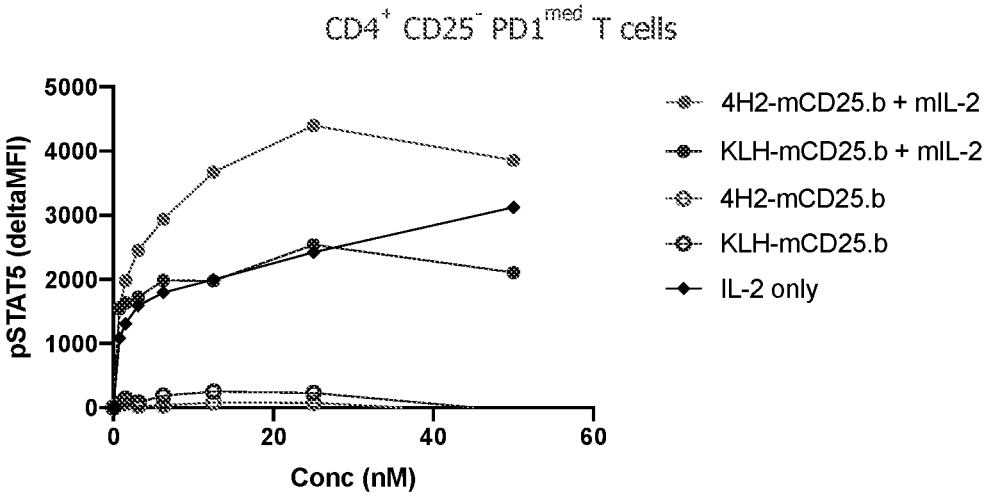

CD4+ CD25- PD1med T cells
pSTAT5 (deltaMFI)
5000
4000
3000
2000
1000
0
0
20
40
60
Conc (nM)
4H2-mCD25.b + mIL-2
KLH-mCD25.b + mIL-2
4H2-mCD25.b
KLH-mCD25.b
IL-2 only

FIG. 12A

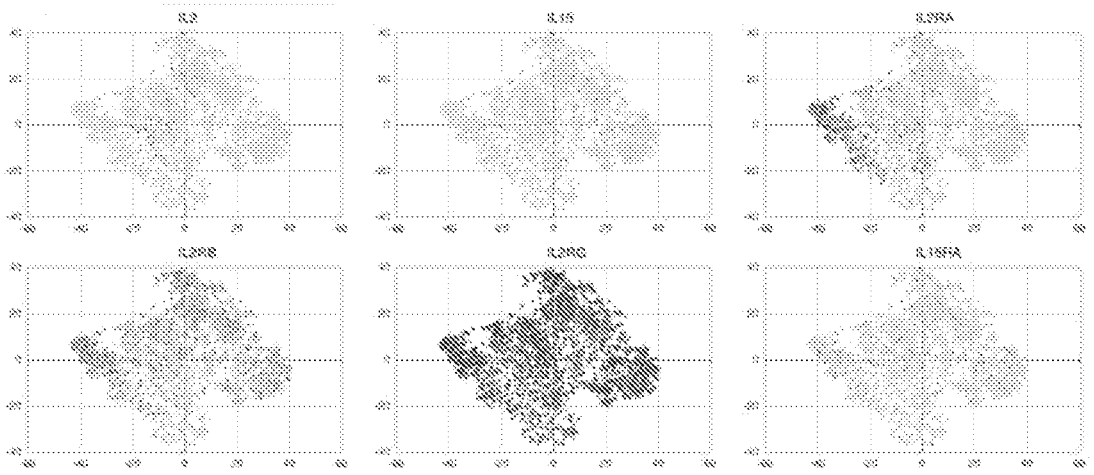


FIG. 12B

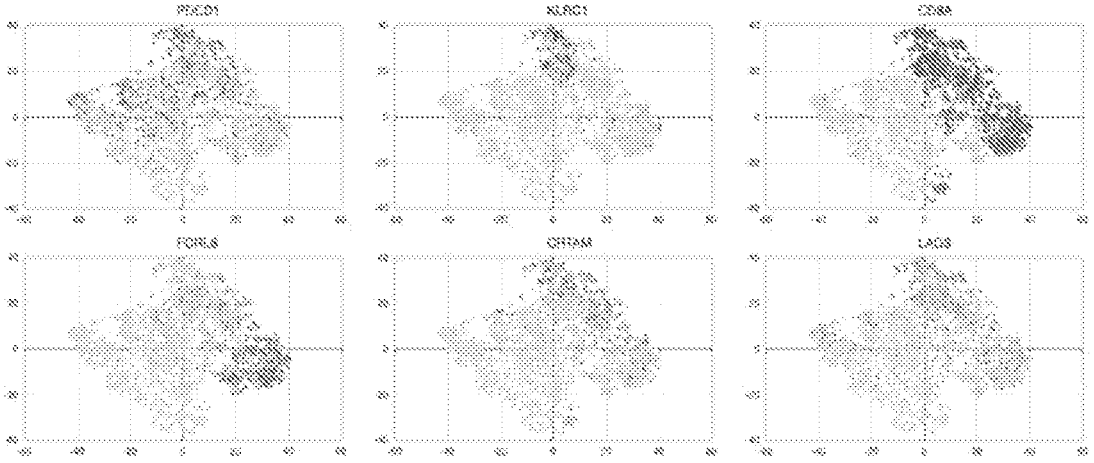
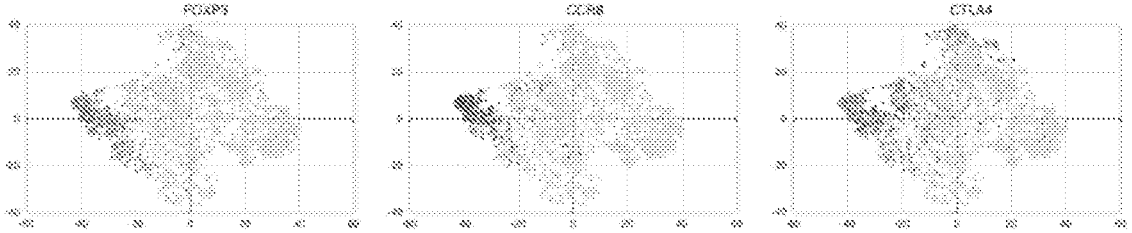


FIG. 12C



METHODS OF REDIRECTING OF IL-2 TO TARGET CELLS OF INTEREST

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 63/065,275 filed 13 Aug. 2020, the disclosure of which is incorporated herein by reference.

SEQUENCE LISTING

[0002] The Sequence Listing filed electronically herewith is also hereby incorporated by reference in its entirety (File Name: 20210809_SEQL_13390WOPCT_GB.txt; Date Created: 9 Aug. 2021; File Size: 142 KB).

BACKGROUND OF THE INVENTION

[0003] The immune system is capable of controlling tumor development and mediating tumor regression. Immune activating molecules, such as interleukin 2 (IL-2), can enhance anti-tumor immunity but can simultaneously lead to generalized immune activation and dose-limiting side effects. Aldesleukin (PROLEUKIN®), a slightly modified human IL-2 polypeptide, was first approved in the United States in 1998 for treatment of advanced and metastatic melanoma, but its use has been limited by toxicity issues, which are illustrated by the full-page black box warning on its prescribing information. As a cytokine, it also exhibits a short half-life (less than two hours) necessitating dosing multiple cycles of intravenous administration three times a day (TID) for five days in a row.

[0004] The need exists for improved methods of enhancing anti-tumor immune response that preferentially amplify naturally existing anti-tumor immune response against the tumor, without amplifying systemic effects leading to toxic side effects.

SUMMARY OF THE INVENTION

[0005] The present invention provides polypeptide constructs comprising a targeting moiety and a CD25 moiety. In various embodiments, the targeting moiety binds to PD-1, NKG2a, CD8a, FcRL6, CRTAM or LAG3, such as an antibody raised against one of these targets or an antigen binding fragment thereof. In one embodiment, the invention provides polypeptide constructs comprising a PD-1 binding moiety, such as an anti-PD-1 antibody or an antigen binding fragment thereof, and a CD25 moiety.

[0006] In some embodiments, the PD-1 binding moiety in the construct comprise an anti-PD-1 antibody or antigen fragment thereof, such as an anti-mouse PD-1 antibody (e.g. mAb 4H2) or an antigen fragment thereof, or an anti-human PD-1 antibody (e.g. nivolumab or pembrolizumab) or an antigen fragment thereof. In some embodiments the PD-1 moiety comprises the heavy and light chain sequences of anti-mouse mAb 4H2 (SEQ ID NOs: 5 and 6). In other embodiments the PD-1 moiety comprises the CDRs of nivolumab (SEQ ID NOs: 17-22), the heavy and light chain variable domain sequences of nivolumab (SEQ ID NOs: 23 and 24), or the heavy and light chain sequences of nivolumab (SEQ ID NOs: 25 and 27). In further embodiments the PD-1 moiety comprises the CDRs of pembrolizumab (SEQ ID NOs: 36-41), the heavy and light chain variable domain sequences of pembrolizumab (SEQ ID NOs: 42 and 43), or the heavy and light chain sequences of pembrolizumab (SEQ ID NOs: 44 and 46).

[0007] In some embodiments, the PD-1 binding moiety in the construct may comprise CD25 or an IL-2 binding fragment thereof, such as human CD25 or a human IL-2 (hIL-2) binding fragment thereof. Exemplary hIL-2 binding fragments of human CD25 include residues 22-240 (SEQ ID NO: 11) and residues 22-223 (SEQ ID NO: 12) and residues 22-186 (SEQ ID NO: 14) of full-length hCD25 (SEQ ID NO: 10).

[0008] In some embodiments the PD-1 binding moiety is nivolumab or an antigen binding fragment thereof, and the CD25 moiety is an IL-2 binding fragment of hCD25, such as hCD25 variant a (SEQ ID NO: 11), hCD25 variant b (SEQ ID NO: 12) or hCD25 variant d (SEQ ID NO: 14).

[0009] In some embodiments the CD25 moiety, such as hCD25 variant a, hCD25 variant b, or hCD25 variant d, is fused to the C-terminus of one of the heavy chains of an anti-PD1 antibody, such as nivolumab. In some embodiments the CD25 moiety, such as hCD25 variant a or hCD25 variant b, is fused to the C-termini of both of the heavy chains of an anti-PD1 antibody, such as nivolumab. In some embodiments the antibody heavy chain is linked to the CD25 moiety via a linker, such as $(G_4S)_3$ (SEQ ID NO: 7).

[0010] Exemplary mouse reagent constructs of the present invention comprise one CD25-4H2 heavy chain fusion polypeptide comprising the sequence of SEQ ID NO: 8 or 9, one 4H2 heavy chain comprising the sequence of SEQ ID NO: 5, and two 4H2 light chains comprising the sequence of SEQ ID NO: 6. Other exemplary constructs of the present invention comprise two CD25-4H2 heavy chain fusion polypeptides comprising the sequence of SEQ ID NO: 8 and two 4H2 light chains comprising the sequence of SEQ ID NO: 6; or alternatively two CD25-4H2 heavy chain fusion polypeptides comprising the sequence of SEQ ID NO: 9 and two 4H2 light chains comprising the sequence of SEQ ID NO: 6.

[0011] Exemplary human therapeutic constructs of the present invention comprise one CD25-nivolumab heavy chain fusion polypeptide comprising the sequence of SEQ ID NO: 28, one nivolumab heavy chain comprising the sequence of SEQ ID NO: 25 or 26, and two nivolumab light chains comprising the sequence of SEQ ID NO: 27; or alternatively one CD25-nivolumab heavy chain fusion polypeptide comprising the sequence of SEQ ID NO: 29, one nivolumab heavy chain comprising the sequence of SEQ ID NO: 25 or 26, and two nivolumab light chains comprising the sequence of SEQ ID NO: 27; or alternatively one CD25-nivolumab heavy chain fusion polypeptide comprising the sequence of SEQ ID NO: 30, one nivolumab heavy chain comprising the sequence of SEQ ID NO: 25 or 26, and two nivolumab light chains comprising the sequence of SEQ ID NO: 27.

[0012] Other exemplary constructs of the present invention comprise two CD25-nivolumab heavy chain fusion polypeptides comprising the sequence of SEQ ID NO: 28 and two nivolumab light chains comprising the sequence of SEQ ID NO: 27; or alternatively two CD25-nivolumab heavy chain fusion polypeptides comprising the sequence of SEQ ID NO: 29 and two nivolumab light chains comprising the sequence of SEQ ID NO: 27; or alternatively two CD25-nivolumab heavy chain fusion polypeptides comprising the sequence of SEQ ID NO: 30 and two nivolumab light chains comprising the sequence of SEQ ID NO: 27.

[0013] Additional exemplary therapeutic constructs of the present invention comprise one CD25-pembrolizumab heavy chain fusion polypeptide comprising the sequence of SEQ ID NO: 47, one pembrolizumab heavy chain comprising the sequence of SEQ ID NO: 44 or 45, and two pembrolizumab light chains comprising the sequence of SEQ ID NO: 46; or alternatively one CD25-pembrolizumab heavy chain fusion polypeptide comprising the sequence of SEQ ID NO: 48, one pembrolizumab heavy chain comprising the sequence of SEQ ID NO: 44 or 45, and two pembrolizumab light chains comprising the sequence of SEQ ID NO: 46; or alternatively one CD25-pembrolizumab heavy chain fusion polypeptide comprising the sequence of SEQ ID NO: 49, one pembrolizumab heavy chain comprising the sequence of SEQ ID NO: 44 or 45, and two pembrolizumab light chains comprising the sequence of SEQ ID NO: 46.

[0014] Other exemplary constructs of the present invention comprise two CD25-pembrolizumab heavy chain fusion polypeptides comprising the sequence of SEQ ID NO: 47 and two pembrolizumab light chains comprising the sequence of SEQ ID NO: 46; or alternatively two CD25-pembrolizumab heavy chain fusion polypeptides comprising the sequence of SEQ ID NO: 48 and two pembrolizumab light chains comprising the sequence of SEQ ID NO: 46; or alternatively two CD25-pembrolizumab heavy chain fusion polypeptides comprising the sequence of SEQ ID NO: 49 and two pembrolizumab light chains comprising the sequence of SEQ ID NO: 46.

[0015] In some embodiments comprising one heavy chain CD25 fusion polypeptide and one heavy chain lacking CD25, the heavy chains are modified by the knob-into-holes approach to promote formation of antibody constructs comprising one of each heavy chain sequence.

[0016] The invention also provides nucleic acids encoding the targeting moiety-CD25 moiety polypeptide construct, such as anti-PD-1 CD25 fusion construct, of the present invention, as well as expression vectors comprising these nucleic acids, host cells comprising the vectors, and method of producing the anti-PD-1 CD25 fusion constructs of the present invention by growing the host cells under conditions that allow their production. In some embodiments comprising a targeting moiety that is an antibody, such as an anti-PD-1 antibody, or antigen binding fragment thereof, the heavy and light chain sequences of the antibody are encoded in the same nucleic acid molecule, whereas in other embodiments the heavy and light chains are encoded by separate nucleic acid molecules.

[0017] The invention also provides pharmaceutical compositions of the polypeptide constructs of the present invention for use in treating human disease, such as cancer, which compositions comprise salt, buffer and other pharmaceutically acceptable excipients.

[0018] The invention further provides compositions of these therapeutic constructs for use in treating human disease, such as cancer, and methods of treating such diseases using the constructs. In various embodiments, the invention provides constructs for, and methods of, treating NSCLC, liver cancer, breast cancer, colorectal cancer (CRC), metastatic melanoma, colon cancer, and/or melanoma. In selected embodiments, the methods of treating cancer comprise constructs for, and methods of, treating NSCLC, liver cancer, and/or breast cancer. In a specific embodiment, the methods of treating cancer comprise constructs for, and methods of, treating NSCLC.

[0019] In some embodiments, the polypeptide constructs or anti-PD-1 CD25 fusion constructs of the present invention are administered without administration of IL-2 or any IL-2 derived therapeutic agent. In other embodiments, the polypeptide constructs or anti-PD-1 CD25 fusion constructs of the present invention are administered in combination therapy with human IL-2, or a therapeutically effective derivative thereof, such as aldesleukin (non-glycosylated Al A C125S human IL-2). In further embodiments, the anti-PD-1 CD25 fusion constructs of the present invention are pre-mixed with IL-2 or an IL-2 derived therapeutic agent and the mixture is administered to the subject.

[0020] The invention further provides methods of treatment of diseases, such as cancers, in which tumor samples from human patients are screened for their level of IL-2 and a therapeutic construct of the present invention is administered only to patients whose samples show a required minimum level of IL-2.

[0021] In other embodiments, the invention further provides methods of treatment of diseases, such as cancers, in which tumor infiltrating lymphocytes (TIL) from human patients are screened for the level of PD-1 expression, and a therapeutic construct of the present invention is administered only to patients whose samples show a required minimum threshold level of PD-1 expression in TIL.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] FIGS. **1**A and **1**B are schematic illustrations of two embodiments of the construct of the present invention. FIG. **1**A shows an anti-PD1 antibody with a CD25 moiety fused to the C-terminus of one heavy chain, whereas FIG. **1**B shows an anti-PD1 antibody with a CD25 moiety fused to the C-terminus of both heavy chains. Heavy and light chain variable domains are shown in gray, constant domains are in white, and CD25 moieties are in black.

[0023] FIGS. **2**A, **2**B and **2**C are representations of the IL-2 binding domains of various mCD25 truncation constructs. FIG. **2**A provides a representation of a crystal structure of human CD25 with ribbon structures in the sushi 1 and sushi 2 domains (separated by a dashed line) and helices, corresponding roughly to residues 22-182 of SEQ ID NO: 1. Stauber et al. (2006) *Proc. Nat'l Acad. Sci.* (*USA*) 103: 2793; PDB 2ERJ. FIG. **2**B provides a two-dimensional topographic representation of the primary sequence of the sushi 1 and sushi 2 structural domains of CD25, with the sequence elements contributing to the sushi 2 domain above the dashed line and sequence elements contributing to sequence of the sushi 1 domain below the line. Ribbon structures are represented as arrows drawn N-terminal to C-terminal (as is conventional), and unstructured region of the sequence is represented by a curved dashed line. FIG. **2**C provides a lineup of mouse and human CD25 sushi domain sequences, SEQ ID NOs: 11 and 2, respectively. Structurally defined sushi 1 domain sequences are shown in solid boxes, and sushi 2 domain sequences are shown in dashed boxes.

[0024] FIGS. **3**A and **3**B provide sequences for various CD25 truncations of the present invention. FIG. **3**A shows mouse CD25 variants a, b and c. FIG. **3**B shows human CD25 variants a, b, c, d, e and f. In all cases sushi 2 domain residues are underlined, and structurally defined residues in

the sushi 1 domain residues are italicized. In hCD25 variant a in FIG. **3**B residues in human CD25 found in beta ribbons are in bold.

[0025] FIG. **4** provides surface plasmon resonance binding data for the three constructs illustrated in FIG. **3**A to mIL-2. See Example 1. SPR signal is provided (in nm), from left to right, as the sensor chip is flowed with mIL-2 for baseline; flowed with only buffer as a wash; flowed with a fusion construct of an anti-mPD1 antibody (4H2) to one of the three mCD25 truncations to load the surface; flowed with buffer; flowed with mIL-2 for association; and flowed with buffer only for dissociation. The abscissa is a timeline from 0 to 240 minutes, and the ordinate is a linear scale from 0 to 1.2 nm. The lower (A), middle (B) and upper (C) traces are for the mCD25 truncations from variants a, b and c from FIG. **3**A, respectively. Variant c, comprising only sushi 1 domain sequence, does not bind to mIL-2, whereas the variants a and b, which comprise both sushi 1 and sushi 2 domain sequences, and varying additional residues at the carboxy termini, do.

[0026] FIGS. **5**A and **5**B provide sequences for mCD25 anti-mPD1 mAb fusion constructs of the present invention. FIG. **5**A (SEQ ID NO: 8) provides the heavy chain of anti-mPD-1 mAb 4H2 (SEQ ID NO: 5) linked to mCD25 variant a (italic, SEQ ID NO: 2) by a $(G_4S)_3$ linker (double underlined, SEQ ID NO: 7). FIG. **5**B (SEQ ID NO: 9) provides the heavy chain of anti-mPD-1 mAb 4H2 (SEQ ID NO: 5) linked to mCD25 variant b (italic, SEQ ID NO: 3) by a $(G_4S)_3$ linker (double underlined, SEQ ID NO: 7).

[0027] FIGS. **6**A, **6**B and **6**C provide sequences for hCD25 anti-hPD1 (nivolumab) mAb fusion constructs of the present invention. FIG. **6**A (SEQ ID NO: 28) provides the heavy chain of anti-hPD-1 mAb nivolumab (SEQ ID NO: 26) linked to hCD25 variant a (italic, SEQ ID NO: 11) by a $(G_4S)_3$ linker (double underlined, SEQ ID NO: 7). FIG. **6**B (SEQ ID NO: 29) provides the heavy chain of anti-hPD-1 mAb nivolumab (SEQ ID NO: 26) linked to hCD25 variant b (italic, SEQ ID NO: 12) by a $(G_4S)_3$ linker (double underlined, SEQ ID NO: 7). FIG. **6**C (SEQ ID NO: 30) provides the heavy chain of anti-hPD-1 mAb nivolumab (SEQ ID NO: 26) linked to hCD25 variant d (italic, SEQ ID NO: 14) by a $(G_4S)_3$ linker (double underlined, SEQ ID NO: 7). The heavy chain variable domains in FIGS. **6**A-**6**C are underlined, and CDRs are bolded. Analogous pembrolizumab constructs are provided at SEQ ID NOs: 47, 48 and 49.

[0028] FIGS. **7**A, **7**B and **7**C are variants of the sequences of FIGS. **6**A, **6**B and **6**C, respectively, except that the nivolumab hIgG4 S228P heavy chain constant domain is replaced with the effectorless hIgG1.3. The nivolumab heavy chain with hIgG1.3 instead of hIgG4 S228P is provided at SEQ ID NOs: 31 and 32. The sequences provided at FIGS. **7**A, **7**B and **7**C are provided at SEQ ID NOs: 33, 34 and 35, respectively. The heavy chain variable domains in FIGS. **7**A-**7**C are underlined, and CDRs are bolded. Analogous pembrolizumab hIgG1.3 constructs are provided at SEQ ID NOs: 52, 53 and 54.

[0029] FIGS. **8**A-**8**D provide data characterizing cell lines engineered to illustrate the effects of the constructs of the present invention. See Example 2. FIG. **8**A shows sorting of HEK-Blue™ IL-2 cells, which express all three subunits of IL-2 receptor, after deletion of hCD25, showing a substantial population of $hCD25^-$ cells. FIG. **8**B shows sorting cells from the sort of FIG. **8**A confirming that they remain CD122 (IL-2Rβ) and CD132 (IL-2Rγ) positive. The CD25− HEK-Blue™ cells from FIG. **8**A were then transduced with mPD-1 or hPD-1 and sorted. FIGS. **8**C and 8D show that these cell populations are both hCD25− and mPD-1+ and hPD-1+, respectively. These cells find use in testing the anti-PD-1−hCD25 fusion constructs of the present invention, in which the anti-PD-1 moiety may be an anti-mPD-1 antibody (e.g. mAb 4H2) or an anti-hPD-1 antibody (e.g. nivolumab).

[0030] FIG. **9** shows a titration of mIL-2 binding to CD25+ (upper curve) and CD25− (lower curve) HEK-Blue™ IL-2 cells, confirming the importance of CD25 for IL-2 binding and signaling. See Example 3. Signaling data are reported as ABS 620 nM in an alkaline phosphatase activity assay based on differential expression of the SEAP (secreted embryonic alkaline phosphatase) reporter gene in the HEK-Blue™ reporter cell line.

[0031] FIGS. **10**A and **10**B show titrations of mIL-2 signaling in CD25−mPD-1+ HEK-Blue™ IL-2 cells in the presence of a hemi-mCD25 modified (4H2 mG1 D265A KK CD25.b+4H2 mG1 D265A blank) and a fully mCD25 modified ($(4H2\ mG1\ D265A\ KK\ CD25.b)_2$) anti-mPD-1 antibody (4H2), respectively. See Example 3. The hemi- and fully modified constructs showed similar ability to enhance mIL-2 signaling in a dose responsive manner. FIG. **10**C presents data essentially replicating those in FIG. **10**B, but also including control experiments with an anti-KLH mAb (mAb 29D6) fusion to CD25, demonstrating that the observed effects depend on PD-1 binding. For FIG. **10**A, mIL-2 only is lowest curve; 28 pM fusion is next higher curve; 280 pM fusion is next higher curve; 2.8 nM fusion is upper curve. For FIG. **10**B, mIL-2 only is lowest curve; 26 pM fusion is next higher curve; 260 pM fusion is next higher curve; 2.8 nM fusion is upper curve. For FIG. **10**C, 2.6 nM anti-mPD-1 fusion is the uppermost curve; 260 pM anti-mPD-1 fusion is the next lower curve; 26 pM anti-mPD-1 fusion is the next lower curve; the lower curve comprises data for 2.6 nM, 260 pM and 26 pM anti-KLH fusion and no fusion.

[0032] FIG. **11**A shows STAT5 phosphorylation as a function of mIL-2 for primary mouse $CD4^+CD25^+$ (upper curve) and $CD8^+CD25^-$ (lower curve) splenocytes, illustrating the dramatic deficiency of $CD25^-$ cells in IL-2 mediated signaling. See Example 4. CD4+ primary T cells and CD8+ primary T cells were gated for PD-1 expression, and then for low CD25 expression. FIGS. **11**B and **11**C show STAT5 signaling in these two cell preparations, CD8+CD25−$PD1^{low}$ and CD4+CD25−$PD1^{med}$ respectively, when treated with a mixture of mIL-2 and an anti-mPD-1−CD25 fusion construct of the present invention. For FIG. **11**B, 4H2-mCD25.b+mIL-2 is the upper curve at 25 nM; IL-2 only is the second highest curve at nM; KLH-mCD25.b+mIL-2 is the third highest curve at 25 nM; KLH-mCD25.b is the fourth highest (nearly baseline) curve at 25 nM; and 4H2-mCD25.b is the lowest curve (essentially at baseline throughout). For FIG. **11**C, 4H2-mCD25.b+mIL-2 is upper curve at 25 nM; KLH-mCD25.b+mIL-2 is the second highest curve at 25 nM; IL-2 only is third highest curve at 25 nM; KLH-mCD25.b is the fourth highest (nearly baseline) curve at 25 nM; and 4H2-mCD25.b is the lowest curve (essentially at baseline throughout).

[0033] FIGS. **12**A-**12**C show plots of single cell RNA sequencing data from tumor infiltrated lymphocytes (TIL). Data are presented for in 9,055 single T cells from 14

NSCLC patients. The dimensional reduction analysis (t-SNE) projections show sixteen main clusters, including seven for CD8+ T cells, seven for conventional CD4+ T cells and two for regulatory T cells. Each dot corresponds to a single cell, with darker color representing more intense staining. Gene Expression Omnibus Accession No: GSE99254; Guo et al. (2018) *Nat. Med.* 24:978. FIG. **12**A shows expression of IL-2, IL-15, IL2RA, IL2RB, IL2RG and IL15RA, as indicated. FIG. **12**B shows expression of PDCD1, KLRC1, CD8A, FCRL8, CRTAM and LAG3, as indicated. FIG. **12**C shows expression of FOXP3, CCR8 and CTLA-4, as indicated. See Example 5. Comparison of FIG. **12**A with FIG. **12**B shows that cells that express PDCD1, KLRC1, CD8A, FCRL8, CRTAM and LAG3 tend to also express IL2RB and IL2RG. Comparison of FIG. **12**A with FIG. **12**C shows that cells that express PDCD1, KLRC1, CD8A, FCRL8, CRTAM and LAG3 tend not to express T_{reg} markers FOXP3, CCR8 and CTLA-4.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0034] In order that the present disclosure may be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

[0035] "Administering" refers to the physical introduction of a composition comprising a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Preferred routes of administration for antibodies of the invention include intravenous, intraperitoneal, intramuscular, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intraperitoneal, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as in vivo electroporation. Alternatively, an antibody of the invention can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods. Administration may be performed by one or more individual, including but not limited to, a doctor, a nurse, another healthcare provider, or the patient himself or herself.

[0036] An "antibody" (Ab) shall include, without limitation, a glycoprotein immunoglobulin which binds specifically to an antigen and comprises at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, or an antigen-binding portion thereof. Each H chain comprises a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_{H1}, C_{H2} and C_{H3}. Each light chain comprises a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen.

[0037] As used herein, and in accord with conventional interpretation, an antibody that is described as comprising "a" heavy chain and/or "a" light chain refers to antibodies that comprise "at least one" of the recited heavy and/or light chains, and thus will encompass antibodies having two or more heavy and/or light chains. Specifically, antibodies so described will encompass conventional antibodies having two substantially identical heavy chains and two substantially identical light chains. Antibody chains may be substantially identical but not entirely identical if they differ due to post-translational modifications, such as C-terminal cleavage of lysine residues, alternative glycosylation patterns, etc.

[0038] Unless indicated otherwise or clear from the context, an antibody defined by its target specificity (e.g. an "anti-PD-1 antibody") refers to antibodies that can bind to its human target (e.g. human PD-1). Such antibodies may or may not bind to PD-1 from other species.

[0039] The immunoglobulin may derive from any of the commonly known isotypes, including but not limited to IgA, secretory IgA, IgG and IgM. The IgG isotype may be divided in subclasses in certain species: IgG1, IgG2, IgG3 and IgG4 in humans, and IgG1, IgG2a, IgG2b and IgG3 in mice. IgG antibodies may be referred to herein by the symbol gamma (γ) or simply "G," e.g. IgG1 may be expressed as "γ1" or as "G1," as will be clear from the context. "Isotype" refers to the antibody class (e.g., IgM or IgG1) that is encoded by the heavy chain constant region genes. "Antibody" includes, by way of example, both naturally occurring and non-naturally occurring antibodies; monoclonal and polyclonal antibodies; chimeric and humanized antibodies; human or nonhuman antibodies; wholly synthetic antibodies; and single chain antibodies. Unless otherwise indicated, or clear from the context, antibodies disclosed herein are human IgG1 antibodies.

[0040] An "isolated antibody" refers to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that binds specifically to PD-1 is substantially free of antibodies that bind specifically to antigens other than PD-1). An isolated antibody that binds specifically to PD-1 may, however, cross-react with other antigens, such as PD-1 molecules from different species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals. By comparison, an "isolated" nucleic acid refers to a nucleic acid composition of matter that is markedly different, i.e., has a distinctive chemical identity, nature and utility, from nucleic acids as they exist in nature. For example, an isolated DNA, unlike native DNA, is a free-standing portion of a native DNA and not an integral part of a larger structural complex, the chromosome, found in nature. Further, an isolated DNA, unlike native DNA, can be used as a PCR primer or a hybridization probe for, among other things, measuring gene expression and detecting bio-

marker genes or mutations for diagnosing disease or predicting the efficacy of a therapeutic. An isolated nucleic acid may also be purified so as to be substantially free of other cellular components or other contaminants, e.g., other cellular nucleic acids or proteins, using standard techniques well known in the art.

[0041] The term "monoclonal antibody" ("mAb") refers to a preparation of antibody molecules of single molecular composition, i.e., antibody molecules whose primary sequences are essentially identical, and which exhibits a single binding specificity and affinity for a particular epitope. Monoclonal antibodies may be produced by hybridoma, recombinant, transgenic or other techniques known to those skilled in the art.

[0042] The term "afucosylated," as used herein, refers to individual antibody heavy chains in which the N-linked glycan contains no fucose residues. The term "nonfucosylated" as used herein, refers to a preparation of antibodies containing antibodies with afucosylated heavy chains, and unless otherwise indicated over 95% afucosylated heavy chains. Such preparations of antibodies may be used as therapeutic compositions.

[0043] A "human" antibody (HuMAb) refers to an antibody having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. The terms "human" antibodies and "fully human" antibodies and are used synonymously.

[0044] An "antibody fragment" refers to a portion of a whole antibody, generally including the "antigen-binding portion" ("antigen-binding fragment") of an intact antibody which retains the ability to bind specifically to the antigen bound by the intact antibody, or the Fc region of an antibody which retains FcR binding capability. Exemplary antibody fragments include Fab fragments and single chain variable domain (scFv) fragments.

[0045] "Antibody-dependent cell-mediated cytotoxicity" ("ADCC") refers to an in vitro or in vivo cell-mediated reaction in which nonspecific cytotoxic cells that express FcRs (e.g., natural killer (NK) cells, macrophages, neutrophils and eosinophils) recognize antibody bound to a surface antigen on a target cell and subsequently cause lysis of the target cell. In principle, any effector cell with an activating FcR can be triggered to mediate ADCC.

[0046] "Cancer" refers a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth divide and grow results in the formation of malignant tumors or cells that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream.

[0047] A "cell surface receptor" refers to molecules and complexes of molecules capable of receiving a signal and transmitting such a signal across the plasma membrane of a cell.

[0048] An "effector cell" refers to a cell of the immune system that expresses one or more FcRs and mediates one or more effector functions. Preferably, the cell expresses at least one type of an activating Fc receptor, such as, for example, human FcγRIII, and performs ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMCs), NK cells, monocytes, macrophages, neutrophils and eosinophils.

[0049] "Effector function" refers to the interaction of an antibody Fc region with an Fc receptor or ligand, or a biochemical event that results therefrom. Exemplary "effector functions" include Clq binding, complement dependent cytotoxicity (CDC), Fc receptor binding, FcγR-mediated effector functions such as ADCC and antibody dependent cell-mediated phagocytosis (ADCP), and down-regulation of a cell surface receptor (e.g., the B cell receptor; BCR). Such effector functions generally require the Fc region to be combined with a binding domain (e.g., an antibody variable domain).

[0050] An "Fc receptor" or "FcR" is a receptor that binds to the Fc region of an immunoglobulin. FcRs that bind to an IgG antibody comprise receptors of the FcγR family, including allelic variants and alternatively spliced forms of these receptors. The FcγR family consists of three activating (FcγRI, FcγRIII, and FcγRIV in mice; FcγRIA, FcγRIIA, and FcγRIIIA in humans) receptors and one inhibitory (FcγRIIB) receptor. Various properties of human FcγRs are summarized in Table 1. The majority of innate effector cell types co-express one or more activating FcγR and the inhibitory FcγRIIB, whereas natural killer (NK) cells selectively express one activating Fc receptor (FcγRIII in mice and FcγRIIIA in humans) but not the inhibitory FcγRIIB in mice and humans.

[0051] An "Fc region" (fragment crystallizable region) or "Fc domain" or "Fc" refers to the C-terminal region of the heavy chain of an antibody that mediates the binding of the immunoglobulin to host tissues or factors, including binding to Fc receptors located on various cells of the immune system (e.g., effector cells) or to the first component (C1q) of the classical complement system. Thus, the Fc region is a polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. In IgG, IgA and IgD antibody isotypes, the Fc region is composed of two identical protein fragments, derived from the second (C_{H2}) and third (C_{H3}) constant domains of the antibody's two heavy chains; IgM and IgE Fc regions contain three heavy chain constant domains (C_H domains 2-4) in each polypeptide chain. For IgG, the Fc region comprises immunoglobulin domains Cγ2 and Cγ3 and the hinge between Cγ1 and Cγ2. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position C226 or P230 to the carboxy-terminus of the heavy chain, wherein the numbering is according to the EU index as in Kabat. The C_{H2} domain of a human IgG Fc region extends from about amino acid 231 to about amino acid 340, whereas the C_{H3} domain is positioned on C-terminal side of a C_{H2} domain in an Fc region, i.e., it extends from about amino acid 341 to

about amino acid 447 of an IgG. As used herein, the Fc region may be a native sequence Fc or a variant Fc. Fc may also refer to this region in isolation or in the context of an Fc-comprising protein polypeptide such as a "binding protein comprising an Fc region," also referred to as an "Fe fusion protein" (e.g., an antibody or immunoadhesin).

TABLE 1

Properties of Human FcγRs

Fcγ	Allelic variants	Affinity for human IgG	Isotype preference	Cellular distribution
FcγRI	None described	High (K_D~10 nM)	IgG1 = 3 > 4 >> 2	Monocytes, macrophages, activated neutrophils, dendritic cells?
FcγRIIA	H131	Low to medium	IgG1 > 3 > 2 > 4	Neutrophils, monocytes, macrophages, eosinophils, dendritic cells, platelets
	R131	Low	IgG1 > 3 > 4 > 2	
FcγRIIIA	V158	Medium	IgG1 = 3 >> 4 > 2	NK cells, monocytes, macrophages, mast cells, eosinophils, dendritic cells?
	F158	Low	IgG1 = 3 >> 4 > 2	
FcγRIIB	I232	Low	IgG1 = 3 = 4 > 2	B cells, monocytes, macrophages, dendritic cells, mast cells
	T232	Low	IgG1 = 3 = 4 > 2	

[0052] An "immune response" refers to a biological response within a vertebrate against foreign agents, which response protects the organism against these agents and diseases caused by them. The immune response is mediated by the action of a cell of the immune system (for example, a T lymphocyte, B lymphocyte, natural killer (NK) cell, macrophage, eosinophil, mast cell, dendritic cell or neutrophil) and soluble macromolecules produced by any of these cells or the liver (including antibodies, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from the vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[0053] An "immunomodulator" or "immunoregulator" refers to a component of a signaling pathway that may be involved in modulating, regulating, or modifying an immune response. "Modulating," "regulating," or "modifying" an immune response refers to any alteration in a cell of the immune system or in the activity of such cell. Such modulation includes stimulation or suppression of the immune system which may be manifested by an increase or decrease in the number of various cell types, an increase or decrease in the activity of these cells, or any other changes which can occur within the immune system. Both inhibitory and stimulatory immunomodulators have been identified, some of which may have enhanced function in a tumor microenvironment. In preferred embodiments of the disclosed invention, the immunomodulator is located on the surface of a T cell. An "immunomodulatory target" or "immunoregulatory target" is an immunomodulator that is targeted for binding by, and whose activity is altered by the binding of, a substance, agent, moiety, compound or molecule. Immunomodulatory targets include, for example, receptors on the surface of a cell ("immunomodulatory receptors") and receptor ligands ("immunomodulatory ligands").

[0054] "Immunotherapy" refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response.

[0055] "PD-1 Moiety," as used herein, refers to the PD-1 binding component of the bispecific construct of the present invention. Unless otherwise indicated, or clear from the context, PD-1 as used herein refers to human PD-1 (hPD-1), and anti-PD-1 antibody refers to an anti-hPD-1 antibody. The PD-1 binding component may be the antigen binding site of an anti-PD-1 antibody, such as anti-mPD-1 mAb 4H2, or anti-hPD-1 mAb nivolumab or pembrolizumab. Anti-mPD-1 mAb 4H2 is described at Li et al. (2009) *Clin. Cancer Res.* 15: 1623. Nivolumab is described, e.g., in U.S. Pat. Nos. 8,008,449 and 8,779,105, and also in WO 2013/173223. Pembrolizumab is described, e.g., in U.S. Pat. No. 8,354,509. Sequences for these antibodies are also provided in the Sequence Listing.

[0056] "CD25 Moiety," as used herein, refers to an IL-2-binding polypeptide that comprises some or all of the sequence of CD25 (IL-2Rα), such as mouse CD25 (mCD25) or human CD25 (hCD25). Unless otherwise indicated, or clear from the context, CD25 as used herein refers to human CD25. CD25 is the alpha subunit of the IL-2 receptor (IL-2R), along with CD122 (IL-2Rβ) and CD132 (IL-2Rγ). A CD25 Moiety will typically comprise a full-length CD25 sequence or a truncation that retains IL-2 binding activity. Exemplary mouse and human CD25 truncations include those provided at SEQ ID NOs: 2 and 3, and SEQ ID NOs: 11, 12 and 14, respectively.

[0057] A "polypeptide construct," as used herein with reference to the compositions of matter of the present invention, refers to a bispecific construct comprising a targeting moiety, such as PD-1 binding moiety, and a CD25 moiety. Such constructs may comprise one or more than one of each of the moieties, such as two PD-1 moieties and one CD25 moiety or two PD-1 moieties and two CD25 moieties. Such polypeptide constructs may synonymously be referred to as anti-PD-1 CD25 fusion constructs. Such polypeptide constructs may comprise one or more polypeptide chains, including two or more polypeptide chains comprising different sequences (e.g. antibody heavy and light chains), such as antibodies comprising one or more antibody light chains and one or more fusion constructs comprising an antibody heavy chain fused to a CD25 moiety, such as an antibody comprising two light chains and two heavy chain-CD25 fusion polypeptides.

[0058] "Hemi-CD25 modified," as used herein, refers to a bivalent antibody comprising two heavy chains in which only one of the two heavy chains further comprises a CD25 moiety. It is as opposed to a "fully CD25 modified" construct, in which both heavy chains are modified to further comprise a CD25 moiety. In hemi-CD25 modified embodiments, the CH3 domains of the hIgG4 antibodies nivolumab and pembrolizumab may be modified using the "knob-into-hole" method of Ridgway et al. (1996) *Protein Eng.* 9:617, as applied to hIgG4 variants in Spiess et al. (2013) *J. Biol. Chem.* 288:26583, to generate two separate heavy chain constant domain sequences that preferentially assemble into heterodimers, favoring the formation of hemi-CD25 modi-

fied antibodies rather than unmodified or fully CD25 modified species. Analogous knob-into-hole modifications may be made in hIgG1 variants of nivolumab and pembrolizumab, such as hIgG1.3 variants, as described. Ridgway et al. (1996) *Protein Eng.* 9:617; Merchant et al. (1998) *Nat. Biotechnol.* 16:677.

[0059] "Potentiating an endogenous immune response" means increasing the effectiveness or potency of an existing immune response in a subject. This increase in effectiveness and potency may be achieved, for example, by overcoming mechanisms that suppress the endogenous host immune response or by stimulating mechanisms that enhance the endogenous host immune response.

[0060] A "protein" refers to a chain comprising at least two consecutively linked amino acid residues, with no upper limit on the length of the chain. One or more amino acid residues in the protein may contain a modification such as, but not limited to, glycosylation, phosphorylation or disulfide bond formation. The term "protein" is used interchangeable herein with "polypeptide."

[0061] A "subject" includes any human or non-human animal. The term "non-human animal" includes, but is not limited to, vertebrates such as nonhuman primates, sheep, dogs, rabbits, rodents such as mice, rats and guinea pigs, avian species such as chickens, amphibians, and reptiles. In preferred embodiments, the subject is a mammal such as a nonhuman primate, sheep, dog, cat, rabbit, ferret or rodent. In more preferred embodiments of any aspect of the disclosed invention, the subject is a human. The terms, "subject" and "patient" are used interchangeably herein.

[0062] "Targeting moiety," as used herein, refers to the component of the fusion constructs of the present invention that binds to a surface marker on a desired target cell, such as anti-tumor CD8+ effector T cells, and promotes delivery of IL-2 to such target cells by providing CD25 to enhance IL-2 receptor activity. One such targeting moiety is PD-1. Alternative targeting moieties include, for example, NKG2a, CD8a, FcRL6, CRTAM and LAG3. Targeting moieties will typically comprise an antibody, or antigen binding portion thereof, that specifically binds to the alternative target, provided that any antigen binding portion an also be fused to CD25 or an active fragment thereof. Unless clear from the context, all methods and constructs of the present invention reciting anti-PD-1 antibodies also provide alternative embodiments using an alternative targeting moiety in place of anti-PD-1.

[0063] A "therapeutically effective amount" or "therapeutically effective dosage" of a drug or therapeutic agent, such as an Fc fusion protein of the invention, is any amount of the drug that, when used alone or in combination with another therapeutic agent, promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. A therapeutically effective amount or dosage of a drug includes a "prophylactically effective amount" or a "prophylactically effective dosage", which is any amount of the drug that, when administered alone or in combination with another therapeutic agent to a subject at risk of developing a disease or of suffering a recurrence of disease, inhibits the development or recurrence of the disease. The ability of a therapeutic agent to promote disease regression or inhibit the development or recurrence of the disease can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in in vitro assays.

[0064] By way of example, an anti-cancer agent promotes cancer regression in a subject. In preferred embodiments, a therapeutically effective amount of the drug promotes cancer regression to the point of eliminating the cancer. "Promoting cancer regression" means that administering an effective amount of the drug, alone or in combination with an anti-neoplastic agent, results in a reduction in tumor growth or size, necrosis of the tumor, a decrease in severity of at least one disease symptom, an increase in frequency and duration of disease symptom-free periods, a prevention of impairment or disability due to the disease affliction, or otherwise amelioration of disease symptoms in the patient. In addition, the terms "effective" and "effectiveness" with regard to a treatment includes both pharmacological effectiveness and physiological safety. Pharmacological effectiveness refers to the ability of the drug to promote cancer regression in the patient. Physiological safety refers to the level of toxicity, or other adverse physiological effects at the cellular, organ and/or organism level (adverse effects) resulting from administration of the drug.

[0065] By way of example for the treatment of tumors, a therapeutically effective amount or dosage of the drug preferably inhibits cell growth or tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. In the most preferred embodiments, a therapeutically effective amount or dosage of the drug completely inhibits cell growth or tumor growth, i.e., preferably inhibits cell growth or tumor growth by 100%. The ability of a compound to inhibit tumor growth can be evaluated in an animal model system, such as the CT26 colon adenocarcinoma, MC38 colon adenocarcinoma and Sa1N fibrosarcoma mouse tumor models described herein, which are predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit cell growth, such inhibition can be measured in vitro by assays known to the skilled practitioner. In other preferred embodiments of the invention, tumor regression may be observed and continue for a period of at least about 20 days, more preferably at least about 40 days, or even more preferably at least about 60 days.

[0066] "Treatment" or "therapy" of a subject refers to any type of intervention or process performed on, or administering an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or prevent the onset, progression, development, severity or recurrence of a symptom, complication, condition or biochemical indicia associated with a disease.

Anti-PD-1 CD25 Fusion Constructs for the Treatment of Cancer

[0067] Cytokines like IL-2 are potent activators of immune responses, and find use in treatment of cancers where they enhance anti-tumor immune response. In one aspect, the present invention provides anti-PD-1 CD25 polypeptide fusion constructs for use in treating diseases, such as cancer. Such constructs comprise a PD-1 binding moiety, such as an anti-PD-1 antibody or antigen binding fragment thereof, fused to a CD25 moiety, or IL-2 binding fragment thereof. Such constructs bind to endogenous IL-2

through the CD25 (IL-2Rα) moiety and redirect it to PD-1 expressing cells, such as NK cells and CD8+ effector T cells (T_{eff}) expressing CD122 (IL-2Rβ) and CD132 (IL-2Rγ) but not CD25.

[0068] In the absence of the anti-PD-1 CD25 fusion constructs of the present invention, immunosuppressive regulatory T cells (T_{reg})express all three IL-2R subunits (α, β and γ) and bind to IL-2 with high affinity (K_d~10 pm), whereas NK cells and T_{eff} express only the β and γ subunits and bind with intermediate affinity (K_d~1 nM). Spolski et al. (2018) *Nat. Rev. Immunol.* 18:648. This balance of IL-2 affinities ensures that T_{reg} will outcompete NK cells and T_{eff} for IL-2 stimulation when IL-2 levels are low, thus maintaining an immunosuppressed resting state. But at high levels of IL-2, NK cells and T_{eff} will bind IL-2, driving their growth and expansion an active immune response. By supplying the missing IL-2Rα subunit to PD-1+ T_{eff}, the anti-PD-1 CD25 fusion constructs of the present invention complete the high affinity trimeric IL-2 receptor complex and redirect IL-2 binding away from immunosuppressive T_{eff} and toward anti-tumor T_{eff}. Such redirection of IL-2 promotes anti-tumor responses without systemic administration of potentially toxic exogenous IL-2, while limit the stimulatory effects of IL-2 to PD-1+ cell populations.

[0069] In one embodiment, the PD-1 moiety is and anti-PD-1 antibody and the CD25 moiety is full length extracellular domain of CD25 (referred to herein as full-length CD25) or an IL-2 binding truncation of that sequence. Schematic illustrations of constructs with CD25 bound to the C terminus of one antibody heavy chain, and to the C terminus of both antibody heavy chains, are provided at FIGS. **1**A and **1**B, respectively. The tertiary, secondary and primary structures of CD25 are schematically illustrated in FIGS. **2**A, **2**B and **2**C, with sushi 2 domains above the line and sushi 1 domains (at the N- and C-termini) below the line in FIGS. **2**A and **2**B.

[0070] The CD25 moiety of the fusion constructs of the present invention may comprise the full extracellular domain of CD25, or a fragment thereof that retain IL-2Rα activity. Such activity is measured by the ability to enhance the binding of IL-2 to cells expressing IL-2Rβ and IL-2Rγ. The sequences of various CD25-related sequences are described at Table 2, and provided in the Sequence Listing (see Table 5). Sequences for CD25 fragments in Table 2 are defined by residue numbering in the full length CD25 sequences provided at SEQ ID NOs: 10 and 1 for human and mouse CD25, respectively.

TABLE 2

CD25 Variant Sequences

	Human	Mouse
full-length CD25	Acc. No. P01589.1 SEQ ID NO: 10 (1-272)	Acc. No. P01590.1 SEQ ID NO: 1 (1-268)
full-length mature	22-272	22-268
variant a	22-240	22-236
(full-length ECD)	SEQ ID NO: 11	SEQ ID NO: 2
variant b	22-223	22-219
	SEQ ID NO: 12	SEQ ID NO: 3
variant c	40-146	40-142
(sushi 1)	SEQ ID NO: 13	SEQ ID NO: 4
sushi 2	22-39 and 147-186	22-39 and 143-182
minimal core	44-85	44-81
variant d	22-186 SEQ ID NO: 14	
variant e	22-145 SEQ ID NO: 15	
variant f	44-85 SEQ ID NO: 16	

[0071] Exemplary mouse CD25 truncations are provided at FIG. **3**A, and human counterparts are provided at FIG. **3**B. Such truncations may be fused to targeting moieties, such as antibodies to selected targets, such as PD-1. FIG. **4** provides SPR binding data using the mouse CD25 truncations of FIG. **3**A, demonstrating that variant a (the full length mCD25 extracellular domain (ECD); SEQ ID NO: 2) binds to mIL-2, variant b (SEQ ID NO: 3) retains full binding affinity (K_d=14 nM), but variant c (sushi 1 domain; SEQ ID NO: 4) does not bind.

[0072] Exemplary mouse fusion proteins, comprising the heavy chain of anti-mPD1 mAb 4H2 fused to mCD25 variants a and b of the invention by a $(G_4S)_3$ linker, are provided at FIGS. **5**A and **5**B. Analogous human constructs comprising anti-hPD-1 mAb nivolumab heavy chain sequence fused to two variants of hCD25 by a $(G_4S)_3$ linker are provided at FIGS. **6**A, **6**B and **6**C, and nivolumab variants comprising the effectorless hIgG1.3 constant domain are provided at FIGS. **7**A, **7**B and **7**C.

[0073] Cell lines were constructed to test the constructs of the present invention. The starting point was a commercial HEK-Blue IL-2 reporter cell line expressing alkaline phosphatase in response to IL-2 stimulation, enabling convenient colorimetric readout. The cell line was modified to delete the hCD25 gene, and then transduced to express either mPD-1 or hPD-1. See FIGS. **8**A-**8**D. The resulting $CD25^-CD122^+$ $CD132^+PD\text{-}1^+$ cells recapitulate the receptor expression pattern of the CD8+ Teff cells to be targeted in patients in that they express PD-1 but not CD25.

[0074] Comparison of the effects of IL-2 on CD25+ and CD25− cell lines demonstrated how important CD25 is to efficient IL-2 binding and signaling. See FIG. **9**. FIGS. **10**A and **10**B, however, demonstrate that anti-PD-1 CD25 fusion constructs of the present invention, whether with CD25 on one antibody heavy chain or both, substantially restore IL-2 binding and signaling in a dose responsive manner. These effects were entirely dependent on PD-1 binding, as expected. See FIG. **10**C.

[0075] These constructs were then tested on primary mouse splenocytes, which were sorted into $CD8^+CD25^-$ and $CD4^+CD25^+$ fractions. These fractions were exposed to varying levels of mIL-2 and phospho-STAT5 was measured. Results are provided at Table 3. As with the reporter cell line, the absence of CD25 reduces sensitivity to IL-2 by orders of magnitude.

TABLE 3

Percent Phospho-STAT5 Cells After IL-2 Stimulation

[IL-2]:	0	0.35 nM	3.5 nM	35 nM
$CD8^+$ $CD25^-$	0.9%	1.5%	12%	63%
$CD4^+$ $CD25^+$	5.3%	90%	96%	94%

[0076] Similar results are provided graphically at FIG. **11**A, where $CD25^-$ cells are drastically less sensitive to IL-2. $CD8^+CD25^-$ and $CD4^+CD25^-$ mouse splenocytes were then sorted for PD-1 expression, to generate one pool of $CD8^+CD25^-PD\text{-}1^{low}$ T cells and another of $CD4^+CD25^-$ $PD\text{-}1^{med}$ T cells. Both pools were titrated with mIL-2 in the presence or absence of a mixture of mAb 4H2-mCD25 fusion construct and mIL-2. Results are provided at FIGS. **11**B and 11C. The results show higher IL-2 mediated signaling in cells with higher PD-1 expression, confirming the ability of an anti-PD-1–CD25 fusion construct of the present invention to enhance IL-2 signaling preferentially in cells expressing PD-1 at higher levels.

[0077] Taken together these results in mouse models suggest that the anti-PD-1 CD25 fusion constructs of the present invention can be used to supplement the CD25 missing from $PD\text{-}1^+CD25^-$ cells, like T_{eff} in human TIL, and induce a more robust anti-tumor response driven by endogenous IL-2 without the need for systemic administration of a toxic IL-2 construct.

Alternative Targeting Moieties and Disease Indications

[0078] The methods and constructs of the present invention are not limited to constructs comprising anti-PD-1 antibody binding domains. CD25 fusion construct comprising antibodies to other surface markers specific for anti-tumor CD8+ T, CD4+ T and NK cells may be used. Such surface markers will ideally be found on $CD8^+$ effector T cells (T_{eff}) expressing CD122 (IL-2Rβ) and CD132 (IL-2Rγ), but not CD25, such that the CD25 fusion construct of the present invention can enhance IL-2 signaling. The ideal surface marker would not be found on T_{regs}. Exemplary alternative cell surface markers for use in the present invention include NKG2a, CD8a, FcRL6, CRTAM and LAG3.

[0079] FIGS. **12**A-**12**C show gene expression data in human NSCLC samples. FIG. **12**A identifies populations of cells expressing IL2RB and IL2RG, the genes encoding the beta and gamma subunits (IL-2Rβ and IL-2Rγ) of the IL-2 receptor. Expression of these subunits is critical for treatment with the fusion constructs of the present invention, which deliver the missing IL-2Rα (CD25) subunit to complete the high affinity IL-2 receptor complex on target cells. Cells with low expression of IL2RA (encoding IL-2Rα) would be most likely to benefit from IL-2R supplementation by the methods and constructs of the present invention.

[0080] FIG. **12**C shows populations of cells expressing FOXP3, CCR8 and CTLA4, which are markers for immunosuppressive regulatory T cells (T_{regs}). The methods of the present invention are intended to enhance IL-2 signaling in anti-tumor T_{eff} cells, to tip the balance between IL-2 signaling from T_{regs} to T_{eff}. Consequently, alternative targeting moieties of the present invention should not be expressed on T_{regs}.

[0081] Consequently, alternative targeting moieties of the present invention would ideally bind selectively to $IL2RB^+$ $IL2RG^+IL2RA^-FOXP3^-CCR8^-CTLA4^-$ T cells. FIG. **12**B shows the expression pattern for selected alternative targeting moieties of the present invention that meet these selection criteria in the tested NSCLC samples. Preferred targets include PD-1, NKG2a, CD8a, FcRL6, CRTAM and LAG3. As seen in FIGS. **12**A, **12**B and **12**C, the genes encoding these surface markers are selectively expressed on NSCLC cells that express the beta and gamma subunits of IL-2 receptor, lack expression of the alpha subunit, and that are not T_{regs}.

[0082] Human PD-1 (programmed cell death protein 1) is encoded by the gene PDCD1 (NCBI Gene ID No: 5133), and is also known as PD1, PD-1, CD279, SLEB2, hPD-1, hPD-1, and hSLE1. Protein and nucleic acid sequences for the precursor protein are found, e.g., at GenBank Accession Nos: NP_005009.2 and NM_005018.3, respectively. In one embodiment, the constructs of the present invention comprise a targeting moiety that specifically binds to PD-1, such as an anti-PD-1 antibody. An exemplary anti-PD-1 antibody is OPDIVO®/nivolumab (BMS-936558) or an antibody that comprises the CDRs or variable regions of one of antibodies 17D8, 2D3, 4H1, 5C4, 7D3, 5F4 and 4A11 described in WO 2006/121168. In certain embodiments, an anti-PD-1 antibody is MK-3475 (KEYTRUDA®/pembrolizumab/formerly lambrolizumab) described in WO 2012/145493; AMP-514/MEDI-0680 described in WO 2012/145493; and CT-011 (pidilizumab; previously CT-AcTibody or BAT; see, e.g., Rosenblatt et al. (2011) *J. Immunotherapy* 34:409). Further known PD-1 antibodies and other PD-1 inhibitors include those described in WO 2009/014708, WO 03/099196, WO 2009/114335, WO 2011/066389, WO 2011/161699, WO 2012/145493, U.S. Pat. Nos. 7,635,757 and 8,217,149, and U.S. Patent Publication No. 2009/0317368. Any of the anti-PD-1 antibodies disclosed in WO 2013/173223 may also be used. Additional anti-PD-1 antibodies may be raised by conventional methods, including but not limited to humanized transgenic mice and phage display.

[0083] Human NKG2a is encoded by the gene KLRC1 (NCBI Gene ID No: 3821; killer cell lectin like receptor C1), and is also known as NKG2 and CD159A. Protein and nucleic acid sequences for the protein are found, e.g., at GenBank Accession Nos: NP_002250.2 and NM_002259.5, respectively. In one embodiment, the constructs of the present invention comprise a targeting moiety that specifically binds to NKG2a, such as an anti-NKG2a antibody. An exemplary anti-NKG2a antibody is BMS-986315. See WO 2020/102501. Another exemplary anti-NKG2a antibody is monalizumab (IPH2201), for which the heavy and light chain sequences are publicly available at pINN publication WHO Drug Information (2015) Vol. 29:2.

[0084] Human CD8a (CD8 alpha chain) is encoded by the gene CD8A (NCBI Gene ID No: 925), and is also known as CD8, p32 and Leu2. Protein and nucleic acid sequences for the precursor protein are found, e.g., at GenBank Accession Nos: NP_001759.3 and NM_001768.7, respectively. In one embodiment, the constructs of the present invention comprise a targeting moiety that specifically binds to CD8a, such as an anti-CD8a antibody. Exemplary anti-CD8a antibodies are provided as mAbs OKT8 and 51.1 (FIGS. 25-28) in U.S. Pat. No. 10,428,155; and also at FIG. 16 of WO 2020/060924. Additional anti-CD8 mAbs are provided at WO 2019/023148 and at U.S. Pat. No. 10,072,080.

[0085] Human FcRL6 (Fc receptor like 6) is encoded by the gene FCRL6 (NCBI Gene ID No: 343413), and is also known as FcRH6. Protein and nucleic acid sequences for the precursor protein are found, e.g., at GenBank Accession Nos: NP_001004310.2 and NM_001004310.3, respectively. In one embodiment, the constructs of the present invention comprise a targeting moiety that specifically binds to FcRL6, such as an anti-FcRL6 antibody. Exemplary anti-FcRL6 antibodies 1D8 and 7B7 are described at Shreeder et

al. (2010) *J. Immunol.* 185:23 and Shreeder et al. (2008) *Eur. J. Immunol.* 38:3159. See also WO 2019/094743.

[0086] Human CRTAM (cytotoxic and regulatory T cell molecule) is encoded by the gene CRTAM (NCBI Gene ID No: 56253), and is also known as CD355. Protein and nucleic acid sequences for the precursor protein are found, e.g., at GenBank Accession Nos: NP_062550.2 and NM_019604.4, respectively. In one embodiment, the constructs of the present invention comprise a targeting moiety that specifically binds to CRTAM, such as an anti-CRTAM antibody. An exemplary anti-CRTAM is 5A11 at WO 2019/086878. See also WO 2009/029883.

[0087] Human LAG3 (lymphocyte activation gene 3) is encoded by the gene LAG3 (NCBI Gene ID No: 3902), and is also known as CD223. Protein and nucleic acid sequences for the precursor protein are found, e.g., at GenBank Accession Nos: NP_002277.4 and NM_002286.6, respectively. In one embodiment, the constructs of the present invention comprise a targeting moiety that specifically binds to LAG3, such as an anti-LAG3 antibody. Examples of anti-LAG3 antibodies include antibodies comprising the CDRs or variable regions of antibodies 25F7, 26H10, 25E3, 8B7, 11F2 or 17E5, which are described in U.S. Patent Publication No. US 2011/0150892 and WO 2014/008218. In one embodiment, an anti-LAG-3 antibody is relatlimab (BMS-986016). Other art recognized anti-LAG-3 antibodies that can be used include IMP731 described in US 2011/007023. IMP701, referred to as LAG525 in humanized form, as described and claimed in nucleic acid form in U.S. Pat. No. 10,711,060, may also be used. Agonist mAb IMP761 (mAb 13E2) may also be used. WO 2017/037203. Additional anti-LAG3 antibodies may be raised by conventional methods, including but not limited to humanized transgenic mice and phage display.

[0088] The same targets identified for use as targeting moieties in the methods and constructs of the present invention using NCSLC samples (Guo et al. (2018) *Nat. Med.* 24:978) are also preferentially expressed in the desired T cell populations in other cancers. Datasets were analyzed for the following cancers: breast cancer (Savas et al. (2018) *Nat. Med.* 24:986); melanoma (Li et al. (2019) *Cell* 176:775; Sadi-Feldman et al. (2018) *Cell* 175:998); metastatic melanoma (Tirosh et al. (2016) *Science* 352:189); colon cancer (Zhang et al. (2020) *Cell* 181:442); liver cancer (Zheng et al. (2017) *Cell* 169:1342); colorectal cancer (Zhang et al. (2018) *Nature* 564:268). As a consequence, the methods of the present invention using PD-1, NKG2a, CD8a, FcRL6, CRTAM and LAG3 as targeting moieties may find particular applicability in treating NSCLC, liver cancer, breast cancer, colorectal cancer (CRC), metastatic melanoma, colon cancer, and melanoma. In selected embodiments, methods and constructs of the present invention are used in treating NSCLC, liver cancer, breast cancer, such as specifically NSCLC.

Tumor-Targeted Antigen Binding

[0089] In various embodiments, the anti-PD-1 CD25 fusion construct of the present invention is modified to selectively block antigen binding in tissues and environments where antigen binding would be detrimental, but allow antigen binding where it would be beneficial. In one embodiment, a blocking peptide "mask" is generated that specifically binds to the antigen binding surface of the anti-PD-1 antibody and interferes with antigen binding, which mask is linked to each of the binding arms of the antibody by a peptidase cleavable linker. See Int'l Pat. App. Pub. No. WO 17/011580 to CytomX. Such constructs are useful for treatment of cancers in which protease levels are greatly increased in the tumor microenvironment compared with non-tumor tissues. Selective cleavage of the cleavable linker in the tumor microenvironment allows disassociation of the masking/blocking peptide, enabling antigen binding selectively in the tumor, rather than in peripheral tissues in which antigen binding might cause unwanted side effects.

[0090] Alternatively, in a related embodiment, a bivalent binding compound ("masking ligand") comprising two antigen binding domains is developed that binds to both antigen binding surfaces of the (bivalent) antibody and interfere with antigen binding, in which the two binding domains masks are linked to each other (but not the antibody) by a cleavable linker, for example cleavable by a peptidase. See, e.g., Int'l Pat. App. Pub. No. WO 2010/077643 to Tegopharm Corp. Masking ligands may comprise, or be derived from, the antigen to which the antibody is intended to bind, or may be independently generated. Such masking ligands are useful for treatment of cancers in which protease levels are greatly increased in the tumor microenvironment compared with non-tumor tissues. Selective cleavage of the cleavable linker in the tumor microenvironment allows disassociation of the two binding domains from each other, reducing the avidity for the antigen-binding surfaces of the antibody. The resulting dissociation of the masking ligand from the antibody enables antigen binding selectively in the tumor, rather than in peripheral tissues in which antigen binding might cause unwanted side effects.

[0091] In yet further embodiments, the anti-PD-1 CD25 fusion construct of the present invention comprises an antibody that preferentially binds to PD-1 at the pH of the tumor microenvironment (e.g. pH 6.0-6.5) rather than the pH of the periphery (e.g. pH 7.0-7.5). Int'l Pat. App. Pub. No. WO 20/214748; WO 20/092155.

Nucleic Acid Molecules Encoding Anti-PD-1 CD25 Fusion Constructs of the Invention

[0092] Another aspect of the present disclosure pertains to isolated nucleic acid molecules that encode any of the anti-PD-1 CD25 fusion constructs of the present invention, including the heavy and/or light chains of the anti-PD-1 antibody portion of the fusion constructs. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. The nucleic acid can be, for example, DNA or RNA, and may or may not contain intronic sequences. In certain embodiments, the DNA is genomic DNA, cDNA, or synthetic DNA, i.e., DNA synthesized in a laboratory, e.g., by the polymerase chain reaction or by chemical synthesis. In some embodiments the heavy and light chain sequences are encoded in the same nucleic acid, whereas in other constructs the heavy and light chains are encoded by separate nucleic acids.

Reduced Fucosylation, Nonfucosylation and Hypofucosylation

[0093] The interaction of anti-PD-1 CD25 fusion constructs of the present invention with FcγRs can also be enhanced by modifying the glycan moiety attached to each Fc fragment at the N297 residue. In particular, the absence of core fucose residues strongly enhances ADCC via

improved binding of IgG to activating FcγRIIIA without altering antigen binding or CDC. Natsume et al. (2009) *Drug Des. Devel. Ther.* 3:7. There is convincing evidence that afucosylated tumor-specific antibodies translate into enhanced therapeutic activity in mouse models in vivo. Nimmerjahn & Ravetch (2005) Science 310:1510; Mossner et al. (2010) *Blood* 115:4393.

[0094] Modification of antibody glycosylation can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Antibodies with reduced or eliminated fucosylation, which exhibit enhanced ADCC, are particularly useful in the methods of the present invention. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of this disclosure to thereby produce an antibody with altered glycosylation. For example, the cell lines Ms704, Ms705, and Ms709 lack the fucosyltransferase gene, FUT8 (α-(1,6) fucosyltransferase (see U.S. Pat. App. Publication No. 20040110704; Yamane-Ohnuki et al. (2004) *Biotechnol. Bioeng.* 87: 614), such that antibodies expressed in these cell lines lack fucose on their carbohydrates. As another example, EP 1176195 also describes a cell line with a functionally disrupted FUT8 gene as well as cell lines that have little or no activity for adding fucose to the N-acetylglucosamine that binds to the Fc region of the antibody, for example, the rat myeloma cell line YB2/0 (ATCC CRL 1662). PCT Publication WO 03/035835 describes a variant CHO cell line, Lec13, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell. See also Shields et al. (2002) *J. Biol. Chem.* 277:26733. Antibodies with a modified glycosylation profile can also be produced in chicken eggs, as described in PCT Publication No. WO 2006/089231. Alternatively, antibodies with a modified glycosylation profile can be produced in plant cells, such as Lemna. See e.g. U.S. Publication No. 2012/0276086. PCT Publication No. WO 99/54342 describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (e.g., beta(1,4)-N-acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNAc structures which results in increased ADCC activity of the antibodies. See also Umaña et al. (1999) *Nat. Biotech.* 17:176. Alternatively, the fucose residues of the antibody may be cleaved off using a fucosidase enzyme. For example, the enzyme alpha-L-fucosidase removes fucosyl residues from antibodies. Tarentino et al. (1975) *Biochem.* 14:5516. Antibodies with reduced fucosylation may also be produced in cells harboring a recombinant gene encoding an enzyme that uses GDP-6-deoxy-D-lyxo-4-hexylose as a substrate, such as GDP-6-deoxy-D-lyxo-4-hexylose reductase (RMD), as described at U.S. Pat. No. 8,642,292. Alternatively, cells may be grown in medium containing fucose analogs that block the addition of fucose residues to the N-linked glycan or a glycoprotein, such as antibody, produced by cells grown in the medium. U.S. Pat. No. 8,163,551; WO 09/135181.

[0095] Because afucosylated antibodies exhibit greatly enhanced ADCC compared with fucosylated antibodies, antibody preparations need not be completely free of fucosylated heavy chains to be useful in the methods of the present invention. Residual levels of fucosylated heavy chains will not significantly interfere with the ADCC activity of a preparation substantially of afucosylated heavy chains. Antibodies produced in conventional CHO cells, which are fully competent to add core fucose to N-glycans, may nevertheless comprise from a few percent up to 15% afucosylated antibodies. Afucosylated antibodies may exhibit ten-fold higher affinity for CD16, and up to 30- to 100-fold enhancement of ADCC activity, so even a small increase in the proportion of afucosylated antibodies may drastically increase the ADCC activity of a preparation. Any preparation comprising more afucosylated antibodies than would be produced in normal CHO cells in culture may exhibit some level of enhanced ADCC. Such antibody preparations are referred to herein as preparations having reduced fucosylation. Depending on the original level of afucosylation obtained from normal CHO cells, reduced fucosylation preparations may comprise as little as 50%, 30%, 20%, 10% and even 5% afucosylated antibodies. Reduced fucosylation is functionally defined as preparations exhibiting two-fold or greater enhancement of ADCC compared with antibodies prepared in normal CHO cells, and not with reference to any fixed percentage of afucosylated species.

[0096] In other embodiments the level of nonfucosylation is structurally defined. As used herein, nonfucosylated antibody preparations are antibody preparations comprising greater than 95% afucosylated antibody heavy chains, including 100%. Hypofucosylated antibody preparations are antibody preparations comprising less than or equal to 95% heavy chains lacking fucose, e.g. antibody preparations in which between 80 and 95% of heavy chains lack fucose, such as between 85 and 95%, and between 90 and 95%. Unless otherwise indicated, hypofucosylated refers to antibody preparations in which 80 to 95% of heavy chains lack fucose, nonfucosylated refers to antibody preparations in which over 95% of heavy chains lack fucose, and "hypofucosylated or nonfucosylated" refers to antibody preparations in which 80% or more of heavy chains lack fucose.

[0097] In some embodiments, hypofucosylated or nonfucosylated antibodies are produced in cells lacking an enzyme essential to fucosylation, such as alpha1,6-fucosyltransferase encoded by FUT8 (e.g. U.S. Pat. No. 7,214,775), or in cells in which an exogenous enzyme partially depletes the pool of metabolic precursors for fucosylation (e.g. U.S. Pat. No. 8,642,292), or in cells cultured in the presence of a small molecule inhibitor of an enzyme involved in fucosylation (e.g. WO 09/135181).

[0098] The level of fucosylation in an antibody preparation may be determined by any method known in the art, including but not limited to gel electrophoresis, liquid chromatography, and mass spectrometry. Unless otherwise indicated, for the purposes of the present invention, the level of fucosylation in an antibody preparation is determined by hydrophilic interaction chromatography (or hydrophilic interaction liquid chromatography, HILIC). To determine the level of fucosylation of an antibody preparation, samples are denatured treated with PNGase F to cleave N-linked glycans, which are then analyzed for fucose content. LC/MS of full-length antibody chains is an alternative method to detect the level of fucosylation of an antibody preparation, but mass spectroscopy is inherently less quantitative.

Pharmaceutical Compositions

[0099] Anti-PD-1 CD25 fusion constructs of the present invention may be constituted in a composition, e.g., a pharmaceutical composition, containing the binding protein,

for example an antibody or a fragment thereof, and a pharmaceutically acceptable carrier. As used herein, a "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, subcutaneous, intramuscular, parenteral, spinal or epidermal administration (e.g., by injection or infusion). A pharmaceutical composition of the invention may include one or more pharmaceutically acceptable salts, anti-oxidant, aqueous and non-aqueous carriers, and/or adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents.

[0100] Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being unduly toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts. One of ordinary skill in the art would be able to determine appropriate dosages based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected. A composition of the present invention can be administered via one or more routes of administration using one or more of a variety of methods well known in the art.

Therapeutic Uses and Methods of the Invention

[0101] This disclosure provides methods for cancer immunotherapy, e.g. potentiating an endogenous immune response in a subject afflicted with a cancer so as to thereby treat the subject, which method comprises administering to the subject a therapeutically effective amount of any of the anti-PD-1 CD25 fusion constructs described herein. In preferred embodiments of the present immunotherapeutic methods, the subject is a human.

[0102] Examples of other cancers that may be treated using the immunotherapeutic methods of the disclosure include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, breast cancer, lung cancer, cutaneous or intraocular malignant melanoma, renal cancer, uterine cancer, ovarian cancer, colorectal cancer, colon cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, a hematological malignancy, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, environmentally induced cancers including those induced by asbestos, metastatic cancers, and any combinations of said cancers. In preferred embodiments, the cancer is selected from MEL, RCC, squamous NSCLC, non-squamous NSCLC, CRC, CRPC, squamous cell carcinoma of the head and neck, and carcinomas of the esophagus, ovary, gastrointestinal tract and breast. The present methods are also applicable to treatment of metastatic cancers.

[0103] Other cancers include hematologic malignancies including, for example, multiple myeloma, B-cell lymphoma, Hodgkin lymphoma/primary mediastinal B-cell lymphoma, non-Hodgkin's lymphomas, acute myeloid lymphoma, chronic myelogenous leukemia, chronic lymphoid leukemia, follicular lymphoma, diffuse large B-cell lymphoma, Burkitt's lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, mantle cell lymphoma, acute lymphoblastic leukemia, mycosis fungoides, anaplastic large cell lymphoma, T-cell lymphoma, and precursor T-lymphoblastic lymphoma, and any combinations of said cancers.

Combination Therapy

[0104] In certain embodiments of these methods for treating a cancer patient, the anti-PD-1 CD25 fusion construct of the present invention is administered to the subject as monotherapy, whereas in other embodiments, stimulation or blockade of immunomodulatory targets may be effectively combined with standard cancer treatments, including chemotherapeutic regimes, radiation, surgery, hormone deprivation and angiogenesis inhibitors.

[0105] Anti-PD-1 CD25 fusion constructs of the present invention may also be used in combination with other immunomodulatory agents, such as antibodies against other immunomodulatory receptors or their ligands. Several other co-stimulatory and inhibitory receptors and ligands that regulate T cell responses have been identified. Examples of stimulatory receptors include Inducible T cell Co-Stimulator (ICOS), CD137 (4-1BB), CD134 (OX40), CD27, Glucocorticoid-Induced TNFR-Related protein (GITR), and Herpes-Virus Entry Mediator (HVEM), whereas examples of inhibitory receptors include Programmed Death-1 (PD-1), B and T Lymphocyte Attenuator (BTLA), T cell Immunoglobulin and Mucin domain-3 (TIM-3), Lymphocyte Activation Gene-3 (LAG-3), adenosine A2a receptor (A2aR), Killer cell Lectin-like Receptor G1 (KLRG-1), Natural Killer Cell Receptor 2B4 (CD244), CD160, T cell Immunoreceptor with Ig and ITIM domains (TIGIT), and the receptor for V-domain Ig Suppressor of T cell Activation (VISTA). Mellman et al. (2011) *Nature* 480:480; Pardoll (2012) *Nat. Rev. Cancer* 12: 252; Baitsch et al. (2012) *PloS One* 7:e30852. These receptors and their ligands provide targets for therapeutics designed to stimulate, or prevent the suppression, of an immune response so as to thereby attack tumor cells. Weber (2010) *Semin. Oncol.* 37:430; Flies et al. (2011) *Yale J. Biol. Med.* 84:409; Mellman et al. (2011) *Nature* 480:480; Pardoll (2012) *Nat. Rev. Cancer* 12:252. Stimulatory receptors or receptor ligands are targeted by agonist agents, whereas inhibitory receptors or receptor ligands are targeted by blocking agents. Among the most promising approaches to enhancing immunotherapeutic anti-tumor activity is the blockade of so-called "immune

checkpoints," which refer to the plethora of inhibitory signaling pathways that regulate the immune system and are crucial for maintaining self-tolerance and modulating the duration and amplitude of physiological immune responses in peripheral tissues in order to minimize collateral tissue damage. See e.g. Weber (2010) *Semin. Oncol.* 37:430; Pardoll (2012) *Nat. Rev. Cancer* 12:252. Because many of the immune checkpoints are initiated by ligand-receptor interactions, they can be readily blocked by antibodies or modulated by recombinant forms of ligands or receptors.

[0106] The present invention is further illustrated by the following examples, which should not be construed as limiting. The contents of all figures and all references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

EXAMPLE 1

Binding of Truncated Anti-PD-1–mCD25 Variant Fusion Proteins to mIL-2

[0107] Surface plasmon resonance spectroscopy (SPR) was used to measure binding of selected truncated mCD25 variants to mIL-2 when present in a fusion construct with anti-mPD-1 mAb 4H2. Truncations of mCD25 are presented at FIG. **3**A.

[0108] Unless otherwise indicated, binding kinetics were determined with a BIACORE® SPR surface plasmon resonance spectrometer (Biacore AB, Uppsala, Sweden). The mouse IL-2 binding affinity was determined for mPD1–mCD25 variants of the present invention using a Biacore™ T200 instrument. The assay temperature was 37° C. and the running buffer was HEPES buffered saline pH 7.4 supplemented with 0.05% (v/v) Tween-20 and 1 g/L BSA. Purified mPD1–mCD25 variants were captured on a Biacore™ CM4 chip with immobilized anti-mouse IgG polyclonal capture antibody. Mouse IL-2 was injected as analyte in a six-membered, three-fold dilution series with 250 nM top concentration and a duplicate injection at 83 nM. Between cycles, the capture surface was regenerated for three minutes with 10 mM Glycine pH 1.7. Double-referenced sensorgrams were fitted to a 1:1 Langmuir binding model with mass transport to determine equilibrium dissociation constants (K_D), as well as association (k_a) and dissociation (k_d) rate constants where appropriate. Both the full-length construct and CD25.b bind mIL-2 with a K_D of 14 nM.

[0109] Binding analyses were also performed with an Octet HTX. Briefly, mPD1–mCD25 variants of the present invention were produced and captured on anti-mouse Fc tips. Mouse IL-2 incubated as analyte at 0.6 μM concentration at 25° C. HEPES buffered saline pH 7.4 containing 150 mM NaCl, 0.05% Tween and 0.5% BSA was used for these experiments. Data are provided as sensorgrams at FIG. **4**. Full length mCD25 ECD, variant a, binds to mIL-2, as does variant b, but variant c, comprising only the sushi 1 domain, does not.

[0110] Additional modified hCD25 variants d, e and f were also prepared, with sequences as provided at FIG. **3**B and at SEQ ID NOs: 14, 15 and 16, respectively. Octet binding experiments demonstrated that like variant c, variants e and f bound poorly to hCD25. SPR experiments were performed to determine the binding parameters for variant a, variant b and variant d, with results provided at Table 4. All variants tested bound with K_D of 12 to 14 nM. Taken as a whole these results, consistent with the mouse data provided at FIG. **4**, demonstrate that all sushi 2 domain residues and all structurally defined sushi 1 domain residues are necessary, and sufficient, for a construct that binds to hCD25, with human variant d as the minimal essential construct among those tested.

TABLE 4

Summary of the Sequence Listing

Antibody	Antigen	ka (1/Ms)	kd (1/s)	K_D (M)
GS_hCD25.a	hIL2-Miltenyi	5.2E+06	7.5E−02	1.4E−08
GS_hCD25.b	hIL2-Miltenyi	1.1E+07	Fast	1.2E−08
GS_hCD25.d	hIL2-Miltenyi	5.4E+06	6.3E−02	1.2E−08

EXAMPLE 2

Generation of hCD25$^-$hCD122$^+$hCD132$^+$mPD-1$^+$ and hCD25$^-$hCD122$^+$hCD132$^+$hPD-1$^+$ HEK-Blue™ IL-2 Reporter Cell Lines

[0111] Reporter cell lines were constructed to test the anti-PD-1–CD25 constructs of the present invention. HEK-Blue™ IL-2 cells were modified to delete hCD25, and to add either mPD-1 or hPD-1, as follows. Briefly, cell lines were derived from HEK-Blue™ IL-2 reporter cells engineered to generate and chromogenic alkaline phosphatase signal reflecting hIL-2 signaling. InvivoGen, San Diego, Calif., USA. The cells are engineered to express hCD25 (IL-2Rα), hCD122 (IL-2Rβ) and hCD132 (IL-2Rγ), which are the three subunits of the IL-2 receptor, as well as hJAK3, hSTAT5, and a STAT5-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. Human CD25 was deleted from the HEK-Blue™ IL-2 reporter cells as follows. A plasmid encoding for guide RNAs targeting human CD25 gene, Cas9 enzyme and GFP was transfected into HEK-Blue™ IL-2 cells. After 24 hours, cells were sorted based on GFP expression, and GFP positive cells were cultured. CD25-positive and CD25-negative cells were sorted using a Sony MA900 cell sorter.

[0112] Deletion of the hCD25 gene was confirmed by FACS. See FIG. **8**A. This hCD25$^-$hCD122$^+$hCD132$^+$ reporter cell line was used in Example 3 (infra). The CD25 deleted cells were then transduced with vectors driving expression of mPD-1 or hPD-1, as follows. DNA sequences of human or mouse PD1 were cloned downstream to a promoter in a lentiviral vector. Lentiviral particles were produced using standard protocol. CD25-positive and CD25-negative HEK Blue IL-2 cells were transduced with human or mouse PD1 constructs. PD-1 expression was confirmed by FACS. See FIGS. **8**C and **8**D. The resulting CD25–PD-1+ reporter cell lines find use in evaluating the anti-PD-1–CD25 fusion constructs of the present invention.

EXAMPLE 3

IL-2 Stimulation of Reporter Cell Lines

[0113] The HEK-Blue™ IL-2 reporter cell line and the hCD25$^-$ HEK-Blue™ IL-2 reporter cell line generated in Example 2 were titrated with mouse IL-2. Results are provided at FIG. **9**.

[0114] The hCD25^{-} HEK-Blue™ IL-2 reporter cell line was then titrated with mIL-2 in the presence or absence of varying amounts of hemi-CD25 modified or fully CD25 modified mAb 4H2 fusion constructs. Results are provided at FIGS. **10**A and **10**B, respectively. Both constructs partially restored IL-2 signaling to CD25+ levels in a dose-dependent fashion. The mIL-2 titration with the fully CD25 modified 4H2 construct was repeated with an analogous fully CD25 modified anti-KLH antibody (29D6) construct. Results are provided at FIG. **10**C.

EXAMPLE 4

Selective Stimulation of PD-1+ Primary T Cells

[0115] Mouse splenocytes were sorted into CD4+CD25+ and CD8+CD25− pools. The CD4+CD25+ and CD8+CD25− were titrated with mIL-2, and STAT5 phosphorylation was measured by flow cytometry. Results are provided at FIG. **11**A. As with the HEK-Blue™ IL-2 reporter cell lines, lack of CD25 dramatically reduces IL-2 response.

[0116] In other experiments, CD4+ and CD8+ mouse splenocyte pools were stained at the same time for PD-1 and CD25 expression. CD25-negative cells were separated into two PD-1 expressing fractions ($PD1^{low}$ and $PD1^{medium}$). Cells were incubated with a titration of mIL-2 in the presence and absence of fully CD25 modified 4H2 or fully CD25 modified anti-KLH mAb constructs, both alone and as mixtures with mIL-2. CD25 constructs with mouse IL-2 were pre-mixed at equal molar ratio for 30 minutes, and then incubated with the mouse cells for 40 minutes. Cells were then fixed, permeabilized and stained with anti-CD4, anti-CD8, anti-CD25, anti-PD1 and anti-phospho-STAT5 antibodies. Results are provided at FIGS. **11**B and **11**C.

EXAMPLE 5

Targets

[0117] Cell surface markers for use in targeting moieties in the methods and fusion constructs of the present invention were selected tumor samples for genes selectively expressed on T_{eff}, rather than T_{regs}, and specifically on T_{eff}, that also express the beta and gamma subunits of IL-2 receptor but not the alpha subunits. The constructs of the present invention deliver the missing alpha subunit to these T_{eff}, completing the trimeric (high affinity) IL-2 receptor complex, but will not bind to T_{regs}.

[0118] Single cell RNA sequencing data from tumor infiltrated lymphocytes (TIL) from NSCLC patients (Guo et al. (2018) *Nat. Med.* 24:978) were queried for expression of candidate target genes, T_{reg} markers FOXP3, CCR8 and CTLA-4, as well as IL2RA, IL2RB, IL2RG. Results, provided at FIGS. **12**A-**12**C, demonstrate that PDCD1, KLRC1, CD8A, FCRL8, CRTAM and LAG3 are not expressed on T_{regs}, and are expressed on T_{eff} that also express IL2RB and IL2RG but not IL2RA.

[0119] Analogous analyses (not shown) were performed on single cell gene expression data from T cells from other tumor types, specifically liver cancer, breast cancer, colorectal cancer (CRC), metastatic melanoma, colon cancer, and melanoma. Savas et al. (2018) *Nat. Med.* 24:986); Li et al. (2019) *Cell* 176:775; Sadi-Feldman et al. (2018) *Cell* 175: 998; Tirosh et al. (2016) *Science* 352:189; Zhang et al. (2020) *Cell* 181:442; Zheng et al. (2017) *Cell* 169:1342; Zhang et al. (2018) *Nature* 564:268. Results confirmed that the same targets found in NSCLC samples (PDCD1, KLRC1, CD8A, FCRL8, CRTAM and LAG3) would find use in treating all these cancers in addition to NSCLC.

TABLE 5

Summary of the Sequence Listing

SEQ ID NO.	Description
1	mCD25
2	mCD25 variant a
3	mCD25 variant b
4	mCD25 variant c
5	4H2 heavy chain
6	4H2 light chain
7	$(G_4S)_3$ linker
8	4H2-mCD25 variant a
9	4H2-mCD25 variant b
10	hCD25
11	hCD25 variant a
12	hCD25 variant b
13	hCD25 variant c
14	hCD25 variant d
15	hCD25 variant e
16	hCD25 variant f
17	nivolumab CDRH1
18	nivolumab CDRH2
19	nivolumab CDRH3
20	nivolumab CDRL1
21	nivolumab CDRL2
22	nivolumab CDRL3
23	nivolumab heavy chain variable region
24	nivolumab light chain variable region
25	nivolumab heavy chain w/o C-terminal K
26	nivolumab heavy chain
27	nivolumab light chain
28	nivolumab-hCD25 variant a
29	nivolumab-hCD25 variant b
30	nivolumab-hCD25 variant d
31	nivolumab heavy chain IgG1.3 w/o C-terminal K
32	nivolumab heavy chain IgG1.3
33	nivolumab IgG1.3 hCD25 variant a
34	nivolumab IgG1.3 hCD25 variant b
35	nivolumab IgG1.3 hCD25 variant d
36	pembrolizumab CDRH1
37	pembrolizumab CDRH2
38	pembrolizumab CDRH3
39	pembrolizumab CDRL1
40	pembrolizumab CDRL2
41	pembrolizumab CDRL3
42	pembrolizumab heavy chain variable region
43	pembrolizumab light chain variable region
44	pembrolizumab heavy chain w/o C-terminal K
45	pembrolizumab heavy chain
46	pembrolizumab light chain
47	pembrolizumab-hCD25 variant a
48	pembrolizumab-hCD25 variant b
49	pembrolizumab-hCD25 variant d
50	pembrolizumab heavy chain IgG1.3 w/o C-terminal K
51	pembrolizumab heavy chain IgG1.3
52	pembrolizumab IgG1.3 hCD25 variant a
53	pembrolizumab IgG1.3 hCD25 variant b
54	pembrolizumab IgG1.3 hCD25 variant d

[0120] With regard to antibody sequences, the Sequence Listing provides the sequences of the mature variable regions of the heavy and light chains, i.e. the sequences do not include signal peptides. Any signal sequence suitable for use in the production cell line being used may be used in production of the antibodies of the present invention. Heavy chain amino acid sequences may be provided without a C-terminal lysine residue, but in some embodiments such residue is encoded in the nucleic acid construct for the antibody.

EQUIVALENTS

[0121] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments disclosed herein. Such equivalents are intended to be encompassed by the following claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 54

<210> SEQ ID NO 1
<211> LENGTH: 268
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 1

```
Met Glu Pro Arg Leu Leu Met Leu Gly Phe Leu Ser Leu Thr Ile Val
1               5                  10                  15

Pro Ser Cys Arg Ala Glu Leu Cys Leu Tyr Asp Pro Pro Glu Val Pro
            20                  25                  30

Asn Ala Thr Phe Lys Ala Leu Ser Tyr Lys Asn Gly Thr Ile Leu Asn
        35                  40                  45

Cys Glu Cys Lys Arg Gly Phe Arg Arg Leu Lys Glu Leu Val Tyr Met
    50                  55                  60

Arg Cys Leu Gly Asn Ser Trp Ser Ser Asn Cys Gln Cys Thr Ser Asn
65                  70                  75                  80

Ser His Asp Lys Ser Arg Lys Gln Val Thr Ala Gln Leu Glu His Gln
                85                  90                  95

Lys Glu Gln Gln Thr Thr Thr Asp Met Gln Lys Pro Thr Gln Ser Met
            100                 105                 110

His Gln Glu Asn Leu Thr Gly His Cys Arg Glu Pro Pro Pro Trp Lys
        115                 120                 125

His Glu Asp Ser Lys Arg Ile Tyr His Phe Val Glu Gly Gln Ser Val
    130                 135                 140

His Tyr Glu Cys Ile Pro Gly Tyr Lys Ala Leu Gln Arg Gly Pro Ala
145                 150                 155                 160

Ile Ser Ile Cys Lys Met Lys Cys Gly Lys Thr Gly Trp Thr Gln Pro
                165                 170                 175

Gln Leu Thr Cys Val Asp Glu Arg Glu His His Arg Phe Leu Ala Ser
            180                 185                 190

Glu Glu Ser Gln Gly Ser Arg Asn Ser Ser Pro Glu Ser Glu Thr Ser
        195                 200                 205

Cys Pro Ile Thr Thr Thr Asp Phe Pro Gln Pro Thr Glu Thr Thr Ala
    210                 215                 220

Met Thr Glu Thr Phe Val Leu Thr Met Glu Tyr Lys Val Ala Val Ala
225                 230                 235                 240

Ser Cys Leu Phe Leu Leu Ile Ser Ile Leu Leu Leu Ser Gly Leu Thr
                245                 250                 255

Trp Gln His Arg Trp Arg Lys Ser Arg Arg Thr Ile
            260                 265
```

<210> SEQ ID NO 2
<211> LENGTH: 215
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 2

```
Glu Leu Cys Leu Tyr Asp Pro Pro Glu Val Pro Asn Ala Thr Phe Lys
1               5                  10                  15
```

-continued

```
Ala Leu Ser Tyr Lys Asn Gly Thr Ile Leu Asn Cys Glu Cys Lys Arg
            20                  25                  30

Gly Phe Arg Arg Leu Lys Glu Leu Val Tyr Met Arg Cys Leu Gly Asn
        35                  40                  45

Ser Trp Ser Ser Asn Cys Gln Cys Thr Ser Asn Ser His Asp Lys Ser
    50                  55                  60

Arg Lys Gln Val Thr Ala Gln Leu Glu His Gln Lys Glu Gln Gln Thr
65                  70                  75                  80

Thr Thr Asp Met Gln Lys Pro Thr Gln Ser Met His Gln Glu Asn Leu
                85                  90                  95

Thr Gly His Cys Arg Glu Pro Pro Pro Trp Lys His Glu Asp Ser Lys
            100                 105                 110

Arg Ile Tyr His Phe Val Glu Gly Gln Ser Val His Tyr Glu Cys Ile
        115                 120                 125

Pro Gly Tyr Lys Ala Leu Gln Arg Gly Pro Ala Ile Ser Ile Cys Lys
    130                 135                 140

Met Lys Cys Gly Lys Thr Gly Trp Thr Gln Pro Gln Leu Thr Cys Val
145                 150                 155                 160

Asp Glu Arg Glu His His Arg Phe Leu Ala Ser Glu Glu Ser Gln Gly
                165                 170                 175

Ser Arg Asn Ser Ser Pro Glu Ser Glu Thr Ser Cys Pro Ile Thr Thr
            180                 185                 190

Thr Asp Phe Pro Gln Pro Thr Glu Thr Thr Ala Met Thr Glu Thr Phe
        195                 200                 205

Val Leu Thr Met Glu Tyr Lys
    210                 215


<210> SEQ ID NO 3
<211> LENGTH: 198
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 3

Glu Leu Cys Leu Tyr Asp Pro Pro Glu Val Pro Asn Ala Thr Phe Lys
1               5                   10                  15

Ala Leu Ser Tyr Lys Asn Gly Thr Ile Leu Asn Cys Glu Cys Lys Arg
            20                  25                  30

Gly Phe Arg Arg Leu Lys Glu Leu Val Tyr Met Arg Cys Leu Gly Asn
        35                  40                  45

Ser Trp Ser Ser Asn Cys Gln Cys Thr Ser Asn Ser His Asp Lys Ser
    50                  55                  60

Arg Lys Gln Val Thr Ala Gln Leu Glu His Gln Lys Glu Gln Gln Thr
65                  70                  75                  80

Thr Thr Asp Met Gln Lys Pro Thr Gln Ser Met His Gln Glu Asn Leu
                85                  90                  95

Thr Gly His Cys Arg Glu Pro Pro Pro Trp Lys His Glu Asp Ser Lys
            100                 105                 110

Arg Ile Tyr His Phe Val Glu Gly Gln Ser Val His Tyr Glu Cys Ile
        115                 120                 125

Pro Gly Tyr Lys Ala Leu Gln Arg Gly Pro Ala Ile Ser Ile Cys Lys
    130                 135                 140

Met Lys Cys Gly Lys Thr Gly Trp Thr Gln Pro Gln Leu Thr Cys Val
```

-continued

```
145                 150                 155                 160

Asp Glu Arg Glu His His Arg Phe Leu Ala Ser Glu Glu Ser Gln Gly
                165                 170                 175

Ser Arg Asn Ser Ser Pro Glu Ser Glu Thr Ser Cys Pro Ile Thr Thr
            180                 185                 190

Thr Asp Phe Pro Gln Pro
        195


<210> SEQ ID NO 4
<211> LENGTH: 103
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 4

Ser Tyr Lys Asn Gly Thr Ile Leu Asn Cys Glu Cys Lys Arg Gly Phe
1               5                   10                  15

Arg Arg Leu Lys Glu Leu Val Tyr Met Arg Cys Leu Gly Asn Ser Trp
            20                  25                  30

Ser Ser Asn Cys Gln Cys Thr Ser Asn Ser His Asp Lys Ser Arg Lys
        35                  40                  45

Gln Val Thr Ala Gln Leu Glu His Gln Lys Glu Gln Gln Thr Thr Thr
    50                  55                  60

Asp Met Gln Lys Pro Thr Gln Ser Met His Gln Glu Asn Leu Thr Gly
65                  70                  75                  80

His Cys Arg Glu Pro Pro Pro Trp Lys His Glu Asp Ser Lys Arg Ile
                85                  90                  95

Tyr His Phe Val Glu Gly Gln
            100


<210> SEQ ID NO 5
<211> LENGTH: 443
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 5

Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
1               5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr
            20                  25                  30

Asn Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Met Arg Tyr Asn Glu Asp Thr Ser Tyr Asn Ser Ala Leu Lys
    50                  55                  60

Ser Arg Leu Ser Ile Ser Arg Asp Thr Ser Lys Asn Gln Val Phe Leu
65                  70                  75                  80

Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Gly Thr Tyr Tyr Cys Thr
                85                  90                  95

Arg Asp Ala Val Tyr Gly Gly Tyr Gly Gly Trp Phe Ala Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser
        115                 120                 125

Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val
    130                 135                 140

Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val
```

-continued

```
145                 150                 155                 160

Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala
                165                 170                 175

Val Leu Glu Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro
            180                 185                 190

Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro
        195                 200                 205

Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly
    210                 215                 220

Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile
225                 230                 235                 240

Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys
                245                 250                 255

Val Thr Cys Val Val Val Ala Ile Ser Lys Asp Asp Pro Glu Val Gln
            260                 265                 270

Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln
        275                 280                 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu
    290                 295                 300

Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg
305                 310                 315                 320

Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                325                 330                 335

Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro
            340                 345                 350

Lys Lys Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile Thr
        355                 360                 365

Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln
    370                 375                 380

Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Lys Thr Asp Gly
385                 390                 395                 400

Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu
                405                 410                 415

Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn
            420                 425                 430

His His Thr Glu Lys Ser Leu Ser His Ser Pro
        435                 440


<210> SEQ ID NO 6
<211> LENGTH: 216
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 6

Asp Thr Val Leu Thr Gln Ser Pro Ala Leu Ala Val Ser Leu Gly Gln
1               5                   10                  15

Arg Val Thr Ile Ser Cys Lys Ala Ser Glu Thr Val Ser Ser Ser Met
            20                  25                  30

Tyr Ser Tyr Ile His Trp Tyr Gln Gln Lys Pro Gly Gln Gln Pro Lys
        35                  40                  45

Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Val Pro Ala Arg
    50                  55                  60
```

-continued

```
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asp Pro
65                  70                  75                  80

Val Glu Ala Asp Asp Val Ala Thr Tyr Phe Cys Gln Gln Ser Trp Asn
                85                  90                  95

Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp
            100                 105                 110

Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr
        115                 120                 125

Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys
    130                 135                 140

Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly
145                 150                 155                 160

Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser
                165                 170                 175

Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn
            180                 185                 190

Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val
        195                 200                 205

Lys Ser Phe Asn Arg Asn Glu Cys
    210                 215


<210> SEQ ID NO 7
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic linker

<400> SEQUENCE: 7

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1               5                   10                  15


<210> SEQ ID NO 8
<211> LENGTH: 673
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: mAb 4H2 fusion to mCD25 variant a

<400> SEQUENCE: 8

Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
1               5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr
            20                  25                  30

Asn Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Met Arg Tyr Asn Glu Asp Thr Ser Tyr Asn Ser Ala Leu Lys
    50                  55                  60

Ser Arg Leu Ser Ile Ser Arg Asp Thr Ser Lys Asn Gln Val Phe Leu
65                  70                  75                  80

Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Gly Thr Tyr Tyr Cys Thr
                85                  90                  95

Arg Asp Ala Val Tyr Gly Gly Tyr Gly Gly Trp Phe Ala Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser
        115                 120                 125
```

-continued

```
Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val
    130                 135                 140

Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val
145                 150                 155                 160

Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala
                165                 170                 175

Val Leu Glu Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro
            180                 185                 190

Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro
        195                 200                 205

Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly
    210                 215                 220

Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile
225                 230                 235                 240

Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys
                245                 250                 255

Val Thr Cys Val Val Val Ala Ile Ser Lys Asp Asp Pro Glu Val Gln
            260                 265                 270

Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln
        275                 280                 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu
    290                 295                 300

Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg
305                 310                 315                 320

Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                325                 330                 335

Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro
            340                 345                 350

Lys Lys Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile Thr
        355                 360                 365

Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln
    370                 375                 380

Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Lys Thr Asp Gly
385                 390                 395                 400

Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu
                405                 410                 415

Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn
            420                 425                 430

His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Gly Gly Gly Ser
        435                 440                 445

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Leu Cys Leu Tyr Asp
    450                 455                 460

Pro Pro Glu Val Pro Asn Ala Thr Phe Lys Ala Leu Ser Tyr Lys Asn
465                 470                 475                 480

Gly Thr Ile Leu Asn Cys Glu Cys Lys Arg Gly Phe Arg Arg Leu Lys
                485                 490                 495

Glu Leu Val Tyr Met Arg Cys Leu Gly Asn Ser Trp Ser Ser Asn Cys
            500                 505                 510

Gln Cys Thr Ser Asn Ser His Asp Lys Ser Arg Lys Gln Val Thr Ala
        515                 520                 525
```

-continued

```
Gln Leu Glu His Gln Lys Glu Gln Gln Thr Thr Thr Asp Met Gln Lys
    530                 535                 540

Pro Thr Gln Ser Met His Gln Glu Asn Leu Thr Gly His Cys Arg Glu
545                 550                 555                 560

Pro Pro Pro Trp Lys His Glu Asp Ser Lys Arg Ile Tyr His Phe Val
                565                 570                 575

Glu Gly Gln Ser Val His Tyr Glu Cys Ile Pro Gly Tyr Lys Ala Leu
            580                 585                 590

Gln Arg Gly Pro Ala Ile Ser Ile Cys Lys Met Lys Cys Gly Lys Thr
        595                 600                 605

Gly Trp Thr Gln Pro Gln Leu Thr Cys Val Asp Glu Arg Glu His His
    610                 615                 620

Arg Phe Leu Ala Ser Glu Glu Ser Gln Gly Ser Arg Asn Ser Ser Pro
625                 630                 635                 640

Glu Ser Glu Thr Ser Cys Pro Ile Thr Thr Thr Asp Phe Pro Gln Pro
                645                 650                 655

Thr Glu Thr Thr Ala Met Thr Glu Thr Phe Val Leu Thr Met Glu Tyr
            660                 665                 670

Lys
```

<210> SEQ ID NO 9
<211> LENGTH: 656
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: mAb 4H2 fusion to mCD25 variant b

<400> SEQUENCE: 9

```
Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
1               5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr
            20                  25                  30

Asn Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Met Arg Tyr Asn Glu Asp Thr Ser Tyr Asn Ser Ala Leu Lys
    50                  55                  60

Ser Arg Leu Ser Ile Ser Arg Asp Thr Ser Lys Asn Gln Val Phe Leu
65                  70                  75                  80

Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Gly Thr Tyr Tyr Cys Thr
                85                  90                  95

Arg Asp Ala Val Tyr Gly Gly Tyr Gly Gly Trp Phe Ala Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser
        115                 120                 125

Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val
    130                 135                 140

Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val
145                 150                 155                 160

Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala
                165                 170                 175

Val Leu Glu Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro
            180                 185                 190

Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro
```

-continued

```
        195                 200                 205

Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly
    210                 215                 220

Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile
225                 230                 235                 240

Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys
                245                 250                 255

Val Thr Cys Val Val Val Ala Ile Ser Lys Asp Asp Pro Glu Val Gln
            260                 265                 270

Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln
        275                 280                 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu
    290                 295                 300

Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg
305                 310                 315                 320

Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                325                 330                 335

Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro
            340                 345                 350

Lys Lys Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile Thr
        355                 360                 365

Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln
    370                 375                 380

Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Lys Thr Asp Gly
385                 390                 395                 400

Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu
                405                 410                 415

Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn
            420                 425                 430

His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Gly Gly Gly Ser
        435                 440                 445

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Leu Cys Leu Tyr Asp
    450                 455                 460

Pro Pro Glu Val Pro Asn Ala Thr Phe Lys Ala Leu Ser Tyr Lys Asn
465                 470                 475                 480

Gly Thr Ile Leu Asn Cys Glu Cys Lys Arg Gly Phe Arg Arg Leu Lys
                485                 490                 495

Glu Leu Val Tyr Met Arg Cys Leu Gly Asn Ser Trp Ser Ser Asn Cys
            500                 505                 510

Gln Cys Thr Ser Asn Ser His Asp Lys Ser Arg Lys Gln Val Thr Ala
        515                 520                 525

Gln Leu Glu His Gln Lys Glu Gln Gln Thr Thr Thr Asp Met Gln Lys
    530                 535                 540

Pro Thr Gln Ser Met His Gln Glu Asn Leu Thr Gly His Cys Arg Glu
545                 550                 555                 560

Pro Pro Pro Trp Lys His Glu Asp Ser Lys Arg Ile Tyr His Phe Val
                565                 570                 575

Glu Gly Gln Ser Val His Tyr Glu Cys Ile Pro Gly Tyr Lys Ala Leu
            580                 585                 590

Gln Arg Gly Pro Ala Ile Ser Ile Cys Lys Met Lys Cys Gly Lys Thr
        595                 600                 605
```

-continued

```
Gly Trp Thr Gln Pro Gln Leu Thr Cys Val Asp Glu Arg Glu His His
    610                 615                 620

Arg Phe Leu Ala Ser Glu Glu Ser Gln Gly Ser Arg Asn Ser Ser Pro
625                 630                 635                 640

Glu Ser Glu Thr Ser Cys Pro Ile Thr Thr Thr Asp Phe Pro Gln Pro
                645                 650                 655


<210> SEQ ID NO 10
<211> LENGTH: 272
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Met Asp Ser Tyr Leu Leu Met Trp Gly Leu Leu Thr Phe Ile Met Val
1               5                   10                  15

Pro Gly Cys Gln Ala Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile Pro
            20                  25                  30

His Ala Thr Phe Lys Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Asn
        35                  40                  45

Cys Glu Cys Lys Arg Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu Tyr
    50                  55                  60

Met Leu Cys Thr Gly Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys
65                  70                  75                  80

Gln Cys Thr Ser Ser Ala Thr Arg Asn Thr Thr Lys Gln Val Thr Pro
                85                  90                  95

Gln Pro Glu Glu Gln Lys Glu Arg Lys Thr Thr Glu Met Gln Ser Pro
            100                 105                 110

Met Gln Pro Val Asp Gln Ala Ser Leu Pro Gly His Cys Arg Glu Pro
        115                 120                 125

Pro Pro Trp Glu Asn Glu Ala Thr Glu Arg Ile Tyr His Phe Val Val
    130                 135                 140

Gly Gln Met Val Tyr Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu His
145                 150                 155                 160

Arg Gly Pro Ala Glu Ser Val Cys Lys Met Thr His Gly Lys Thr Arg
                165                 170                 175

Trp Thr Gln Pro Gln Leu Ile Cys Thr Gly Glu Met Glu Thr Ser Gln
            180                 185                 190

Phe Pro Gly Glu Glu Lys Pro Gln Ala Ser Pro Glu Gly Arg Pro Glu
        195                 200                 205

Ser Glu Thr Ser Cys Leu Val Thr Thr Thr Asp Phe Gln Ile Gln Thr
    210                 215                 220

Glu Met Ala Ala Thr Met Glu Thr Ser Ile Phe Thr Thr Glu Tyr Gln
225                 230                 235                 240

Val Ala Val Ala Gly Cys Val Phe Leu Leu Ile Ser Val Leu Leu Leu
                245                 250                 255

Ser Gly Leu Thr Trp Gln Arg Arg Gln Arg Lys Ser Arg Arg Thr Ile
            260                 265                 270


<210> SEQ ID NO 11
<211> LENGTH: 219
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11
```

-continued

```
Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile Pro His Ala Thr Phe Lys
1               5                   10                  15

Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg
            20                  25                  30

Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu Cys Thr Gly
        35                  40                  45

Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser
    50                  55                  60

Ala Thr Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu Gln
65                  70                  75                  80

Lys Glu Arg Lys Thr Thr Glu Met Gln Ser Pro Met Gln Pro Val Asp
                85                  90                  95

Gln Ala Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn
            100                 105                 110

Glu Ala Thr Glu Arg Ile Tyr His Phe Val Val Gly Gln Met Val Tyr
        115                 120                 125

Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu His Arg Gly Pro Ala Glu
    130                 135                 140

Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Trp Thr Gln Pro Gln
145                 150                 155                 160

Leu Ile Cys Thr Gly Glu Met Glu Thr Ser Gln Phe Pro Gly Glu Glu
                165                 170                 175

Lys Pro Gln Ala Ser Pro Glu Gly Arg Pro Glu Ser Glu Thr Ser Cys
            180                 185                 190

Leu Val Thr Thr Thr Asp Phe Gln Ile Gln Thr Glu Met Ala Ala Thr
        195                 200                 205

Met Glu Thr Ser Ile Phe Thr Thr Glu Tyr Gln
    210                 215


<210> SEQ ID NO 12
<211> LENGTH: 202
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile Pro His Ala Thr Phe Lys
1               5                   10                  15

Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg
            20                  25                  30

Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu Cys Thr Gly
        35                  40                  45

Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser
    50                  55                  60

Ala Thr Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu Gln
65                  70                  75                  80

Lys Glu Arg Lys Thr Thr Glu Met Gln Ser Pro Met Gln Pro Val Asp
                85                  90                  95

Gln Ala Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn
            100                 105                 110

Glu Ala Thr Glu Arg Ile Tyr His Phe Val Val Gly Gln Met Val Tyr
        115                 120                 125

Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu His Arg Gly Pro Ala Glu
```

-continued

```
    130                 135                 140

Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Trp Thr Gln Pro Gln
145                 150                 155                 160

Leu Ile Cys Thr Gly Glu Met Glu Thr Ser Gln Phe Pro Gly Glu Glu
                165                 170                 175

Lys Pro Gln Ala Ser Pro Glu Gly Arg Pro Glu Ser Glu Thr Ser Cys
            180                 185                 190

Leu Val Thr Thr Thr Asp Phe Gln Ile Gln
        195                 200


<210> SEQ ID NO 13
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Ala Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg Gly Phe
1               5                   10                  15

Arg Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu Cys Thr Gly Asn Ser
            20                  25                  30

Ser His Ser Ser Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser Ala Thr
        35                  40                  45

Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu Gln Lys Glu
    50                  55                  60

Arg Lys Thr Thr Glu Met Gln Ser Pro Met Gln Pro Val Asp Gln Ala
65                  70                  75                  80

Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn Glu Ala
                85                  90                  95

Thr Glu Arg Ile Tyr His Phe Val Val Gly Gln
            100                 105


<210> SEQ ID NO 14
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile Pro His Ala Thr Phe Lys
1               5                   10                  15

Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg
            20                  25                  30

Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu Cys Thr Gly
        35                  40                  45

Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser
    50                  55                  60

Ala Thr Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu Gln
65                  70                  75                  80

Lys Glu Arg Lys Thr Thr Glu Met Gln Ser Pro Met Gln Pro Val Asp
                85                  90                  95

Gln Ala Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn
            100                 105                 110

Glu Ala Thr Glu Arg Ile Tyr His Phe Val Val Gly Gln Met Val Tyr
        115                 120                 125

Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu His Arg Gly Pro Ala Glu
```

-continued

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    130                 135                 140

Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Trp Thr Gln Pro Gln
145                 150                 155                 160

Leu Ile Cys Thr Gly
                165


<210> SEQ ID NO 15
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: human CD25 ECD residues 1 - 124 with C3A change

<400> SEQUENCE: 15

Glu Leu Ala Asp Asp Asp Pro Pro Glu Ile Pro His Ala Thr Phe Lys
1               5                   10                  15

Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg
            20                  25                  30

Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu Cys Thr Gly
        35                  40                  45

Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser
    50                  55                  60

Ala Thr Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu Gln
65                  70                  75                  80

Lys Glu Arg Lys Thr Thr Glu Met Gln Ser Pro Met Gln Pro Val Asp
                85                  90                  95

Gln Ala Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn
            100                 105                 110

Glu Ala Thr Glu Arg Ile Tyr His Phe Val Val Gly
        115                 120


<210> SEQ ID NO 16
<211> LENGTH: 42
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Gly Thr Met Leu Asn Cys Glu Cys Lys Arg Gly Phe Arg Arg Ile Lys
1               5                   10                  15

Ser Gly Ser Leu Tyr Met Leu Cys Thr Gly Asn Ser Ser His Ser Ser
            20                  25                  30

Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser
        35                  40


<210> SEQ ID NO 17
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Asn Ser Gly Met His
1               5


<210> SEQ ID NO 18
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
```

-continued

<400> SEQUENCE: 18

Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> SEQ ID NO 19
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Asn Asp Asp Tyr
1

<210> SEQ ID NO 20
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

<210> SEQ ID NO 21
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

Asp Ala Ser Asn Arg Ala Thr
1 5

<210> SEQ ID NO 22
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

Gln Gln Ser Ser Asn Trp Pro Arg Thr
1 5

<210> SEQ ID NO 23
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

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Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

Ser


<210> SEQ ID NO 24
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105


<210> SEQ ID NO 25
<211> LENGTH: 439
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser
        115                 120                 125

Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
    130                 135                 140

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
145                 150                 155                 160

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
                165                 170                 175
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Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys
            180                 185                 190

Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp
        195                 200                 205

Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala
    210                 215                 220

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
225                 230                 235                 240

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
                245                 250                 255

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
            260                 265                 270

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
        275                 280                 285

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
    290                 295                 300

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
305                 310                 315                 320

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
                325                 330                 335

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
            340                 345                 350

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
        355                 360                 365

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
    370                 375                 380

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
385                 390                 395                 400

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
                405                 410                 415

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
            420                 425                 430

Ser Leu Ser Leu Ser Leu Gly
        435


<210> SEQ ID NO 26
<211> LENGTH: 440
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
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-continued

```
Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser
        115                 120                 125

Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
    130                 135                 140

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
145                 150                 155                 160

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
                165                 170                 175

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys
            180                 185                 190

Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp
        195                 200                 205

Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala
    210                 215                 220

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
225                 230                 235                 240

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
                245                 250                 255

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
            260                 265                 270

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
        275                 280                 285

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
    290                 295                 300

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
305                 310                 315                 320

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
                325                 330                 335

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
            340                 345                 350

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
        355                 360                 365

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
    370                 375                 380

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
385                 390                 395                 400

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
                405                 410                 415

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
            420                 425                 430

Ser Leu Ser Leu Ser Leu Gly Lys
        435                 440


<210> SEQ ID NO 27
<211> LENGTH: 214
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 27

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
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-continued

```
1               5                   10                  15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys
    210


<210> SEQ ID NO 28
<211> LENGTH: 673
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nivolumab HC fused to hCD25 variant a

<400> SEQUENCE: 28

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser
        115                 120                 125

Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
```

-continued

```
    130                 135                 140

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
145                 150                 155                 160

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
                165                 170                 175

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys
            180                 185                 190

Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp
        195                 200                 205

Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala
    210                 215                 220

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
225                 230                 235                 240

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
                245                 250                 255

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
            260                 265                 270

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
        275                 280                 285

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
    290                 295                 300

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
305                 310                 315                 320

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
                325                 330                 335

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
            340                 345                 350

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
        355                 360                 365

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
    370                 375                 380

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
385                 390                 395                 400

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
                405                 410                 415

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
            420                 425                 430

Ser Leu Ser Leu Ser Leu Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly
        435                 440                 445

Ser Gly Gly Gly Gly Ser Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile
    450                 455                 460

Pro His Ala Thr Phe Lys Ala Met Ala Tyr Lys Glu Gly Thr Met Leu
465                 470                 475                 480

Asn Cys Glu Cys Lys Arg Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu
                485                 490                 495

Tyr Met Leu Cys Thr Gly Asn Ser Ser His Ser Ser Trp Asp Asn Gln
            500                 505                 510

Cys Gln Cys Thr Ser Ser Ala Thr Arg Asn Thr Thr Lys Gln Val Thr
        515                 520                 525

Pro Gln Pro Glu Glu Gln Lys Glu Arg Lys Thr Thr Glu Met Gln Ser
    530                 535                 540
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```
Pro Met Gln Pro Val Asp Gln Ala Ser Leu Pro Gly His Cys Arg Glu
545                 550                 555                 560

Pro Pro Pro Trp Glu Asn Glu Ala Thr Glu Arg Ile Tyr His Phe Val
                565                 570                 575

Val Gly Gln Met Val Tyr Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu
            580                 585                 590

His Arg Gly Pro Ala Glu Ser Val Cys Lys Met Thr His Gly Lys Thr
        595                 600                 605

Arg Trp Thr Gln Pro Gln Leu Ile Cys Thr Gly Glu Met Glu Thr Ser
    610                 615                 620

Gln Phe Pro Gly Glu Glu Lys Pro Gln Ala Ser Pro Glu Gly Arg Pro
625                 630                 635                 640

Glu Ser Glu Thr Ser Cys Leu Val Thr Thr Thr Asp Phe Gln Ile Gln
                645                 650                 655

Thr Glu Met Ala Ala Thr Met Glu Thr Ser Ile Phe Thr Thr Glu Tyr
            660                 665                 670

Gln
```

<210> SEQ ID NO 29
<211> LENGTH: 656
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nivolumab HC fused to hCD25 variant b

<400> SEQUENCE: 29

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser
        115                 120                 125

Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
    130                 135                 140

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
145                 150                 155                 160

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
                165                 170                 175

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys
            180                 185                 190

Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp
        195                 200                 205
```

-continued

```
Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala
    210                 215                 220

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
225                 230                 235                 240

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
                245                 250                 255

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
            260                 265                 270

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
        275                 280                 285

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
    290                 295                 300

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
305                 310                 315                 320

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
                325                 330                 335

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
            340                 345                 350

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
        355                 360                 365

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
    370                 375                 380

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
385                 390                 395                 400

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
                405                 410                 415

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
            420                 425                 430

Ser Leu Ser Leu Ser Leu Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly
        435                 440                 445

Ser Gly Gly Gly Gly Ser Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile
    450                 455                 460

Pro His Ala Thr Phe Lys Ala Met Ala Tyr Lys Glu Gly Thr Met Leu
465                 470                 475                 480

Asn Cys Glu Cys Lys Arg Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu
                485                 490                 495

Tyr Met Leu Cys Thr Gly Asn Ser Ser His Ser Ser Trp Asp Asn Gln
            500                 505                 510

Cys Gln Cys Thr Ser Ser Ala Thr Arg Asn Thr Thr Lys Gln Val Thr
        515                 520                 525

Pro Gln Pro Glu Glu Gln Lys Glu Arg Lys Thr Thr Glu Met Gln Ser
    530                 535                 540

Pro Met Gln Pro Val Asp Gln Ala Ser Leu Pro Gly His Cys Arg Glu
545                 550                 555                 560

Pro Pro Pro Trp Glu Asn Glu Ala Thr Glu Arg Ile Tyr His Phe Val
                565                 570                 575

Val Gly Gln Met Val Tyr Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu
            580                 585                 590

His Arg Gly Pro Ala Glu Ser Val Cys Lys Met Thr His Gly Lys Thr
        595                 600                 605

Arg Trp Thr Gln Pro Gln Leu Ile Cys Thr Gly Glu Met Glu Thr Ser
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    610                 615                 620

Gln Phe Pro Gly Glu Glu Lys Pro Gln Ala Ser Pro Glu Gly Arg Pro
625                 630                 635                 640

Glu Ser Glu Thr Ser Cys Leu Val Thr Thr Thr Asp Phe Gln Ile Gln
                645                 650                 655


<210> SEQ ID NO 30
<211> LENGTH: 619
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nivolumab HC fused to hCD25 variant d

<400> SEQUENCE: 30

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser
        115                 120                 125

Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
    130                 135                 140

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
145                 150                 155                 160

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
                165                 170                 175

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys
            180                 185                 190

Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp
        195                 200                 205

Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala
    210                 215                 220

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
225                 230                 235                 240

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
                245                 250                 255

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
            260                 265                 270

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
        275                 280                 285

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
    290                 295                 300

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
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-continued

```
305                 310                 315                 320

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
                325                 330                 335

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
            340                 345                 350

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
        355                 360                 365

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
    370                 375                 380

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
385                 390                 395                 400

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
                405                 410                 415

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
            420                 425                 430

Ser Leu Ser Leu Ser Leu Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly
        435                 440                 445

Ser Gly Gly Gly Gly Ser Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile
    450                 455                 460

Pro His Ala Thr Phe Lys Ala Met Ala Tyr Lys Glu Gly Thr Met Leu
465                 470                 475                 480

Asn Cys Glu Cys Lys Arg Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu
                485                 490                 495

Tyr Met Leu Cys Thr Gly Asn Ser Ser His Ser Ser Trp Asp Asn Gln
            500                 505                 510

Cys Gln Cys Thr Ser Ser Ala Thr Arg Asn Thr Thr Lys Gln Val Thr
        515                 520                 525

Pro Gln Pro Glu Glu Gln Lys Glu Arg Lys Thr Thr Glu Met Gln Ser
    530                 535                 540

Pro Met Gln Pro Val Asp Gln Ala Ser Leu Pro Gly His Cys Arg Glu
545                 550                 555                 560

Pro Pro Pro Trp Glu Asn Glu Ala Thr Glu Arg Ile Tyr His Phe Val
                565                 570                 575

Val Gly Gln Met Val Tyr Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu
            580                 585                 590

His Arg Gly Pro Ala Glu Ser Val Cys Lys Met Thr His Gly Lys Thr
        595                 600                 605

Arg Trp Thr Gln Pro Gln Leu Ile Cys Thr Gly
    610                 615


<210> SEQ ID NO 31
<211> LENGTH: 442
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Nivolumab HC hIgG1.3 lacking C-terminal K

<400> SEQUENCE: 31

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
```

-continued

```
        35                  40                  45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
        115                 120                 125

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
    130                 135                 140

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
145                 150                 155                 160

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
                165                 170                 175

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
            180                 185                 190

Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
        195                 200                 205

Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
    210                 215                 220

Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro
225                 230                 235                 240

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                245                 250                 255

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
            260                 265                 270

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        275                 280                 285

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    290                 295                 300

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
305                 310                 315                 320

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
                325                 330                 335

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
            340                 345                 350

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        355                 360                 365

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    370                 375                 380

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
385                 390                 395                 400

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
                405                 410                 415

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
            420                 425                 430

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440
```

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<210> SEQ ID NO 32
<211> LENGTH: 443
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nivolumab HC hIgG1.3

<400> SEQUENCE: 32

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
        115                 120                 125

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
    130                 135                 140

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
145                 150                 155                 160

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
                165                 170                 175

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
            180                 185                 190

Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
        195                 200                 205

Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
    210                 215                 220

Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro
225                 230                 235                 240

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                245                 250                 255

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
            260                 265                 270

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        275                 280                 285

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    290                 295                 300

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
305                 310                 315                 320

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
                325                 330                 335

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
            340                 345                 350
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```
Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        355                 360                 365

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    370                 375                 380

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
385                 390                 395                 400

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
                405                 410                 415

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
            420                 425                 430

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440


<210> SEQ ID NO 33
<211> LENGTH: 676
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nivolumab HC IgG1.3 fused to hCD25 variant a

<400> SEQUENCE: 33

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
        115                 120                 125

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
    130                 135                 140

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
145                 150                 155                 160

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
                165                 170                 175

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
            180                 185                 190

Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
        195                 200                 205

Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
    210                 215                 220

Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro
225                 230                 235                 240

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                245                 250                 255
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```
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
            260                 265                 270

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        275                 280                 285

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    290                 295                 300

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
305                 310                 315                 320

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
                325                 330                 335

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
            340                 345                 350

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        355                 360                 365

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    370                 375                 380

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
385                 390                 395                 400

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
                405                 410                 415

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
            420                 425                 430

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly Gly Ser Gly
        435                 440                 445

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Leu Cys Asp Asp Asp Pro
    450                 455                 460

Pro Glu Ile Pro His Ala Thr Phe Lys Ala Met Ala Tyr Lys Glu Gly
465                 470                 475                 480

Thr Met Leu Asn Cys Glu Cys Lys Arg Gly Phe Arg Arg Ile Lys Ser
                485                 490                 495

Gly Ser Leu Tyr Met Leu Cys Thr Gly Asn Ser Ser His Ser Ser Trp
            500                 505                 510

Asp Asn Gln Cys Gln Cys Thr Ser Ser Ala Thr Arg Asn Thr Thr Lys
        515                 520                 525

Gln Val Thr Pro Gln Pro Glu Glu Gln Lys Glu Arg Lys Thr Thr Glu
    530                 535                 540

Met Gln Ser Pro Met Gln Pro Val Asp Gln Ala Ser Leu Pro Gly His
545                 550                 555                 560

Cys Arg Glu Pro Pro Pro Trp Glu Asn Glu Ala Thr Glu Arg Ile Tyr
                565                 570                 575

His Phe Val Val Gly Gln Met Val Tyr Tyr Gln Cys Val Gln Gly Tyr
            580                 585                 590

Arg Ala Leu His Arg Gly Pro Ala Glu Ser Val Cys Lys Met Thr His
        595                 600                 605

Gly Lys Thr Arg Trp Thr Gln Pro Gln Leu Ile Cys Thr Gly Glu Met
    610                 615                 620

Glu Thr Ser Gln Phe Pro Gly Glu Glu Lys Pro Gln Ala Ser Pro Glu
625                 630                 635                 640

Gly Arg Pro Glu Ser Glu Thr Ser Cys Leu Val Thr Thr Thr Asp Phe
                645                 650                 655
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```
Gln Ile Gln Thr Glu Met Ala Ala Thr Met Glu Thr Ser Ile Phe Thr
            660                 665                 670

Thr Glu Tyr Gln
        675


<210> SEQ ID NO 34
<211> LENGTH: 659
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nivolumab HC IgG1.3 fused to hCD25 variant b

<400> SEQUENCE: 34

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
        115                 120                 125

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
    130                 135                 140

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
145                 150                 155                 160

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
                165                 170                 175

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
            180                 185                 190

Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
        195                 200                 205

Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
    210                 215                 220

Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro
225                 230                 235                 240

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                245                 250                 255

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
            260                 265                 270

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        275                 280                 285

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    290                 295                 300

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
305                 310                 315                 320
```

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```
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
                325                 330                 335

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
            340                 345                 350

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        355                 360                 365

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    370                 375                 380

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
385                 390                 395                 400

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
                405                 410                 415

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
            420                 425                 430

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly Gly Ser Gly
        435                 440                 445

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Leu Cys Asp Asp Asp Pro
    450                 455                 460

Pro Glu Ile Pro His Ala Thr Phe Lys Ala Met Ala Tyr Lys Glu Gly
465                 470                 475                 480

Thr Met Leu Asn Cys Glu Cys Lys Arg Gly Phe Arg Arg Ile Lys Ser
                485                 490                 495

Gly Ser Leu Tyr Met Leu Cys Thr Gly Asn Ser Ser His Ser Ser Trp
            500                 505                 510

Asp Asn Gln Cys Gln Cys Thr Ser Ser Ala Thr Arg Asn Thr Thr Lys
        515                 520                 525

Gln Val Thr Pro Gln Pro Glu Glu Gln Lys Glu Arg Lys Thr Thr Glu
    530                 535                 540

Met Gln Ser Pro Met Gln Pro Val Asp Gln Ala Ser Leu Pro Gly His
545                 550                 555                 560

Cys Arg Glu Pro Pro Pro Trp Glu Asn Glu Ala Thr Glu Arg Ile Tyr
                565                 570                 575

His Phe Val Val Gly Gln Met Val Tyr Tyr Gln Cys Val Gln Gly Tyr
            580                 585                 590

Arg Ala Leu His Arg Gly Pro Ala Glu Ser Val Cys Lys Met Thr His
        595                 600                 605

Gly Lys Thr Arg Trp Thr Gln Pro Gln Leu Ile Cys Thr Gly Glu Met
    610                 615                 620

Glu Thr Ser Gln Phe Pro Gly Glu Glu Lys Pro Gln Ala Ser Pro Glu
625                 630                 635                 640

Gly Arg Pro Glu Ser Glu Thr Ser Cys Leu Val Thr Thr Thr Asp Phe
                645                 650                 655

Gln Ile Gln


<210> SEQ ID NO 35
<211> LENGTH: 619
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nivolumab HC IgG1.3 fused to hCD25 variant d

<400> SEQUENCE: 35

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
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```
1               5                   10                  15

Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser
        115                 120                 125

Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
    130                 135                 140

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
145                 150                 155                 160

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
                165                 170                 175

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys
            180                 185                 190

Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp
        195                 200                 205

Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala
    210                 215                 220

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
225                 230                 235                 240

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
                245                 250                 255

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
            260                 265                 270

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
        275                 280                 285

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
    290                 295                 300

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
305                 310                 315                 320

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
                325                 330                 335

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
            340                 345                 350

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
        355                 360                 365

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
    370                 375                 380

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
385                 390                 395                 400

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
                405                 410                 415
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```
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
            420                 425                 430

Ser Leu Ser Leu Ser Leu Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly
        435                 440                 445

Ser Gly Gly Gly Gly Ser Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile
    450                 455                 460

Pro His Ala Thr Phe Lys Ala Met Ala Tyr Lys Glu Gly Thr Met Leu
465                 470                 475                 480

Asn Cys Glu Cys Lys Arg Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu
                485                 490                 495

Tyr Met Leu Cys Thr Gly Asn Ser Ser His Ser Ser Trp Asp Asn Gln
            500                 505                 510

Cys Gln Cys Thr Ser Ser Ala Thr Arg Asn Thr Thr Lys Gln Val Thr
        515                 520                 525

Pro Gln Pro Glu Glu Gln Lys Glu Arg Lys Thr Thr Glu Met Gln Ser
    530                 535                 540

Pro Met Gln Pro Val Asp Gln Ala Ser Leu Pro Gly His Cys Arg Glu
545                 550                 555                 560

Pro Pro Pro Trp Glu Asn Glu Ala Thr Glu Arg Ile Tyr His Phe Val
                565                 570                 575

Val Gly Gln Met Val Tyr Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu
            580                 585                 590

His Arg Gly Pro Ala Glu Ser Val Cys Lys Met Thr His Gly Lys Thr
        595                 600                 605

Arg Trp Thr Gln Pro Gln Leu Ile Cys Thr Gly
    610                 615


<210> SEQ ID NO 36
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 36

Asn Tyr Tyr Met Tyr
1               5


<210> SEQ ID NO 37
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 37

Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe Lys
1               5                   10                  15

Asn


<210> SEQ ID NO 38
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 38

Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr
1               5                   10
```

-continued

<210> SEQ ID NO 39
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 39

Arg Ala Ser Lys Gly Val Ser Thr Ser Gly Tyr Ser Tyr Leu His
1 5 10 15

<210> SEQ ID NO 40
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 40

Leu Ala Ser Tyr Leu Glu Ser
1 5

<210> SEQ ID NO 41
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 41

Gln His Ser Arg Asp Leu Pro Leu Thr
1 5

<210> SEQ ID NO 42
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: humanized mouse

<400> SEQUENCE: 42

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe
50 55 60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
65 70 75 80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 43
<211> LENGTH: 111
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: humanized mouse

<400> SEQUENCE: 43

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```
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Lys Gly Val Ser Thr Ser
            20                  25                  30

Gly Tyr Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
        35                  40                  45

Arg Leu Leu Ile Tyr Leu Ala Ser Tyr Leu Glu Ser Gly Val Pro Ala
    50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65                  70                  75                  80

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Ser Arg
                85                  90                  95

Asp Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110


<210> SEQ ID NO 44
<211> LENGTH: 446
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: humanized mouse variable domain with human IgG4
      constant domain

<400> SEQUENCE: 44

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe
    50                  55                  60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
    210                 215                 220

Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
225                 230                 235                 240
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```
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly
        435                 440                 445


<210> SEQ ID NO 45
<211> LENGTH: 447
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: humanized mouse variable domain with human IgG4
      constant domain

<400> SEQUENCE: 45

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe
    50                  55                  60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
```

-continued

```
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
    210                 215                 220

Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
        435                 440                 445


<210> SEQ ID NO 46
<211> LENGTH: 218
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: humanized mouse variable domain with human
      kappa constant domain

<400> SEQUENCE: 46

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Lys Gly Val Ser Thr Ser
            20                  25                  30
```

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Gly Tyr Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
        35                  40                  45

Arg Leu Leu Ile Tyr Leu Ala Ser Tyr Leu Glu Ser Gly Val Pro Ala
    50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65                  70                  75                  80

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Ser Arg
                85                  90                  95

Asp Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
            100                 105                 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
        115                 120                 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
    130                 135                 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145                 150                 155                 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
                165                 170                 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
            180                 185                 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
        195                 200                 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215


<210> SEQ ID NO 47
<211> LENGTH: 680
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pembrolizumab HC fused to hCD25 variant a

<400> SEQUENCE: 47

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe
    50                  55                  60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160
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Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
    210                 215                 220

Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Gly Gly
        435                 440                 445

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Leu Cys
    450                 455                 460

Asp Asp Asp Pro Pro Glu Ile Pro His Ala Thr Phe Lys Ala Met Ala
465                 470                 475                 480

Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg Gly Phe Arg
                485                 490                 495

Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu Cys Thr Gly Asn Ser Ser
            500                 505                 510

His Ser Ser Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser Ala Thr Arg
        515                 520                 525

Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu Gln Lys Glu Arg
    530                 535                 540

Lys Thr Thr Glu Met Gln Ser Pro Met Gln Pro Val Asp Gln Ala Ser
545                 550                 555                 560

Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn Glu Ala Thr
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                565                 570                 575

Glu Arg Ile Tyr His Phe Val Val Gly Gln Met Val Tyr Tyr Gln Cys
            580                 585                 590

Val Gln Gly Tyr Arg Ala Leu His Arg Gly Pro Ala Glu Ser Val Cys
        595                 600                 605

Lys Met Thr His Gly Lys Thr Arg Trp Thr Gln Pro Gln Leu Ile Cys
    610                 615                 620

Thr Gly Glu Met Glu Thr Ser Gln Phe Pro Gly Glu Glu Lys Pro Gln
625                 630                 635                 640

Ala Ser Pro Glu Gly Arg Pro Glu Ser Glu Thr Ser Cys Leu Val Thr
                645                 650                 655

Thr Thr Asp Phe Gln Ile Gln Thr Glu Met Ala Ala Thr Met Glu Thr
            660                 665                 670

Ser Ile Phe Thr Thr Glu Tyr Gln
        675                 680


<210> SEQ ID NO 48
<211> LENGTH: 663
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pembrolizumab HC fused to hCD25 variant b

<400> SEQUENCE: 48

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe
    50                  55                  60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
    210                 215                 220

Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
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225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Gly Gly
        435                 440                 445

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Leu Cys
    450                 455                 460

Asp Asp Asp Pro Pro Glu Ile Pro His Ala Thr Phe Lys Ala Met Ala
465                 470                 475                 480

Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg Gly Phe Arg
                485                 490                 495

Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu Cys Thr Gly Asn Ser Ser
            500                 505                 510

His Ser Ser Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser Ala Thr Arg
        515                 520                 525

Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu Gln Lys Glu Arg
    530                 535                 540

Lys Thr Thr Glu Met Gln Ser Pro Met Gln Pro Val Asp Gln Ala Ser
545                 550                 555                 560

Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn Glu Ala Thr
                565                 570                 575

Glu Arg Ile Tyr His Phe Val Val Gly Gln Met Val Tyr Tyr Gln Cys
            580                 585                 590

Val Gln Gly Tyr Arg Ala Leu His Arg Gly Pro Ala Glu Ser Val Cys
        595                 600                 605

Lys Met Thr His Gly Lys Thr Arg Trp Thr Gln Pro Gln Leu Ile Cys
    610                 615                 620

Thr Gly Glu Met Glu Thr Ser Gln Phe Pro Gly Glu Glu Lys Pro Gln
625                 630                 635                 640
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Ala Ser Pro Glu Gly Arg Pro Glu Ser Glu Thr Ser Cys Leu Val Thr
645 650 655

Thr Thr Asp Phe Gln Ile Gln
660

<210> SEQ ID NO 49
<211> LENGTH: 626
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pembrolizumab HC fused to hCD25 variant d

<400> SEQUENCE: 49

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe
50 55 60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
65 70 75 80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
210 215 220

Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
260 265 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305 310 315 320

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Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Gly Gly
        435                 440                 445

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Leu Cys
    450                 455                 460

Asp Asp Asp Pro Pro Glu Ile Pro His Ala Thr Phe Lys Ala Met Ala
465                 470                 475                 480

Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg Gly Phe Arg
                485                 490                 495

Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu Cys Thr Gly Asn Ser Ser
            500                 505                 510

His Ser Ser Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser Ala Thr Arg
        515                 520                 525

Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu Gln Lys Glu Arg
    530                 535                 540

Lys Thr Thr Glu Met Gln Ser Pro Met Gln Pro Val Asp Gln Ala Ser
545                 550                 555                 560

Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn Glu Ala Thr
                565                 570                 575

Glu Arg Ile Tyr His Phe Val Val Gly Gln Met Val Tyr Tyr Gln Cys
            580                 585                 590

Val Gln Gly Tyr Arg Ala Leu His Arg Gly Pro Ala Glu Ser Val Cys
        595                 600                 605

Lys Met Thr His Gly Lys Thr Arg Trp Thr Gln Pro Gln Leu Ile Cys
    610                 615                 620

Thr Gly
625


<210> SEQ ID NO 50
<211> LENGTH: 449
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pembrolizumab HC hIgG1.3 lacking C terminal K

<400> SEQUENCE: 50

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30
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Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe
    50                  55                  60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430
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Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly


<210> SEQ ID NO 51
<211> LENGTH: 450
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pembrolizumab HC hIgG1.3

<400> SEQUENCE: 51

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe
    50                  55                  60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
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                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly Lys
    450


<210> SEQ ID NO 52
<211> LENGTH: 683
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pembrolizumab HC hIgG1.3 hCD25 variant a fusion

<400> SEQUENCE: 52

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe
    50                  55                  60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
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```
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
    450                 455                 460

Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile Pro His Ala Thr Phe Lys
465                 470                 475                 480

Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg
                485                 490                 495

Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu Cys Thr Gly
            500                 505                 510

Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser
        515                 520                 525

Ala Thr Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu Gln
    530                 535                 540

Lys Glu Arg Lys Thr Thr Glu Met Gln Ser Pro Met Gln Pro Val Asp
545                 550                 555                 560

Gln Ala Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn
                565                 570                 575

Glu Ala Thr Glu Arg Ile Tyr His Phe Val Val Gly Gln Met Val Tyr
            580                 585                 590

Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu His Arg Gly Pro Ala Glu
        595                 600                 605

Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Trp Thr Gln Pro Gln
    610                 615                 620
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-continued

```
Leu Ile Cys Thr Gly Glu Met Glu Thr Ser Gln Phe Pro Gly Glu Glu
625                 630                 635                 640

Lys Pro Gln Ala Ser Pro Glu Gly Arg Pro Glu Ser Glu Thr Ser Cys
                645                 650                 655

Leu Val Thr Thr Thr Asp Phe Gln Ile Gln Thr Glu Met Ala Ala Thr
            660                 665                 670

Met Glu Thr Ser Ile Phe Thr Thr Glu Tyr Gln
        675                 680


<210> SEQ ID NO 53
<211> LENGTH: 666
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pembrolizumab HC hIgG1.3 hCD25 variant b fusion

<400> SEQUENCE: 53

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe
    50                  55                  60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
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Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
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Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
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Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
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Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
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Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
    450                 455                 460

Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile Pro His Ala Thr Phe Lys
465                 470                 475                 480

Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg
                485                 490                 495

Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu Cys Thr Gly
            500                 505                 510

Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser
        515                 520                 525

Ala Thr Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu Gln
    530                 535                 540

Lys Glu Arg Lys Thr Thr Glu Met Gln Ser Pro Met Gln Pro Val Asp
545                 550                 555                 560

Gln Ala Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn
                565                 570                 575

Glu Ala Thr Glu Arg Ile Tyr His Phe Val Val Gly Gln Met Val Tyr
            580                 585                 590

Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu His Arg Gly Pro Ala Glu
        595                 600                 605

Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Trp Thr Gln Pro Gln
    610                 615                 620

Leu Ile Cys Thr Gly Glu Met Glu Thr Ser Gln Phe Pro Gly Glu Glu
625                 630                 635                 640

Lys Pro Gln Ala Ser Pro Glu Gly Arg Pro Glu Ser Glu Thr Ser Cys
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<211> LENGTH: 629

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pembrolizumab HC hIgG1.3 hCD25 variant d fusion

<400> SEQUENCE: 54

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Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe
    50                  55                  60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365
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Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
    450                 455                 460

Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile Pro His Ala Thr Phe Lys
465                 470                 475                 480

Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg
                485                 490                 495

Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu Cys Thr Gly
            500                 505                 510

Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser
        515                 520                 525

Ala Thr Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu Gln
    530                 535                 540

Lys Glu Arg Lys Thr Thr Glu Met Gln Ser Pro Met Gln Pro Val Asp
545                 550                 555                 560

Gln Ala Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn
                565                 570                 575

Glu Ala Thr Glu Arg Ile Tyr His Phe Val Val Gly Gln Met Val Tyr
            580                 585                 590

Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu His Arg Gly Pro Ala Glu
        595                 600                 605

Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Trp Thr Gln Pro Gln
    610                 615                 620

Leu Ile Cys Thr Gly
625
```

1. A polypeptide construct comprising a targeting moiety and a CD25 moiety, each of which comprises one or more amino acid sequences.

2. The polypeptide construct of claim **1** wherein the targeting moiety comprises an antibody, or an antigen binding fragment thereof.

3. The polypeptide construct of claim **2** wherein the targeting moiety comprises an antibody that binds specifically to a target selected from the group consisting of PD-1, NKG2a, CD8a, FcRL6, CRTAM and LAG3, or an antigen binding fragment thereof.

4. The polypeptide construct of claim **3** wherein the targeting moiety is an anti-PD-1 antibody or an antigen binding fragment thereof, wherein the anti-PD-1 antibody or antigen binding fragment comprises one or more heavy chains.

5. The polypeptide construct of claim **2**, wherein the amino acid sequence of the CD25 moiety is appended to the C-terminus of at least one heavy chain of the antibody or antigen binding fragment thereof.

6. The polypeptide construct of claim **5** wherein the amino acid sequence of the CD25 moiety is appended to the C-terminus of both heavy chains of the anti-PD-1 antibody.

7. The polypeptide construct of claim **4**, wherein the anti-PD-1 antibody or antigen binding fragment comprises nivolumab, pembrolizumab, a PD-1 binding fragment of nivolumab, or a PD-1 binding fragment of pembrolizumab.

8. The polypeptide construct of claim **7** wherein the PD-1 antibody comprises:

a. a heavy chain comprising a heavy chain variable domain comprising:
   i. a CDRH1 of SEQ ID NO: 17;
   ii. a CDRH2 of SEQ ID NO: 18;
   iii. a CDRH3 of SEQ ID NO: 19; and
b. a light chain comprising a light chain variable domain comprising:
   i. a CDRL1 of SEQ ID NO: 20;
   ii. a CDRL2 of SEQ ID NO: 21;
   iii. a CDRL3 of SEQ ID NO: 22.

9. The polypeptide construct of claim **8** comprising;
a. a heavy chain variable domain comprising the sequence of SEQ ID NO: 23; and
b. a light chain variable domain comprising the sequence of SEQ ID NO: 24.

10. The polypeptide construct of claim **9** comprising;
a. a heavy chain comprising the sequence of SEQ ID NO: 25; and
b. a light chain comprising the sequence of SEQ ID NO: 27.

11. The polypeptide construct of claim **1**, wherein the CD25 moiety comprises the sequence of SEQ ID NO: 14.

12. The polypeptide construct of claim **11** wherein the human CD25 comprises the sequence of SEQ ID NO: 12.

13. The polypeptide construct of claim **12** wherein the human CD25 comprises the sequence of SEQ ID NO: 11.

14. The polypeptide construct of claim **1**, further comprising a linker between the targeting moiety and CD25 moiety comprising the sequence of SEQ ID NO: 7.

15. The polypeptide construct of claim **14** comprising a first construct comprising:
a. a heavy chain comprising the sequence of SEQ ID NO: 28, 29 or 30; and
b. two light chains comprising the sequence of SEQ ID NO: 27;

or a second construct comprising:
a. two heavy chains comprising the same sequence, said sequence being selected from the group consisting of SEQ ID NO: 28, 29 or 30; and
b. two light chains each comprising the sequence of SEQ ID NO: 27;

or a third construct comprising:
a. a heavy chain comprising the sequence of SEQ ID NO: 25; and
b. a heavy chain comprising the sequence of SEQ ID NO: 28, 29 or 30; and
c. two light chains comprising the sequence of SEQ ID NO: 27.

16. (canceled)

17. (canceled)

18. The polypeptide construct of claim **15** wherein the sequence of both antibody heavy chains are modified by the knob-into-hole approach to promote heterodimeric heavy chain pairing.

19. A pharmaceutical composition comprising a polypeptide construct of claim **1**.

20. A nucleic acid encoding one or more polypeptide chains of the polypeptide construct of claim **1**.

21. An expression vector comprising the nucleic acid of claim **20**.

22. A host cell comprising the expression vector of claim **21**.

23. A method of making the polypeptide construct of claim **1** comprising:
a. culturing the host cell of claim **22** under conditions that allow production of the polypeptide construct; and
b. isolating the polypeptide construct.

24. A method of treating a disease in a human subject comprising administering to the subject the polypeptide construct of claim **1**.

25. The method of claim **24** wherein the disease is cancer.

26. (canceled)

27. (canceled)

28. (canceled)

29. The method of claim **24** wherein human IL-2 is also administered to the subject.

30. A method of treating a disease in a human subject comprising:
a. obtaining tumor infiltrating lymphocytes (TIL) from the subject;
b. measuring IL-2 expression level in the TIL; and
c. administering the polypeptide construct of claim **1** only to subjects whose TIL exhibit IL-2 expression above a threshold level.

31. The method of claim **30** wherein the disease is cancer.

32. (canceled)

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