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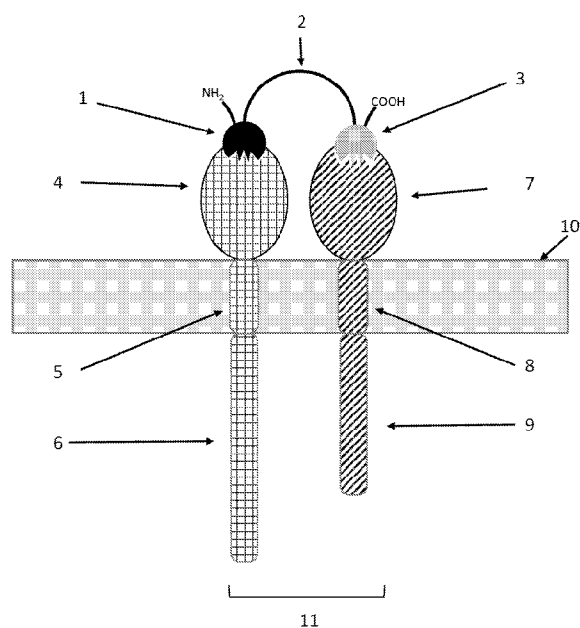


Figure 1

(57) Abstract: Provided herein are IL2R binding molecules that bind to IL2Rb and IL2Rg and comprise an IL2Rg sdAb and an anti-IL2Rg V_HH antibody.

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IL2Rb/IL2Rg Synthetic Cytokines

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 63/061,562, filed August 5, 2020, U.S. Provisional Application No. 63/078,745, filed September 15, 2020, U.S. Provisional Application No. 63/135,884, filed January 11, 2021, U.S. Provisional Application No. 63/136,095, filed January 11, 2021, and U.S. Provisional Application No. 63/136,098, filed January 11, 2021, the disclosures of which are hereby incorporated by reference in their entirety for all purposes.

BACKGROUND OF THE DISCLOSURE

The present disclosure relates to synthetic mimetics of naturally occurring IL2 which are agonists of the IL2 receptor (IL2R).

[0002] In one embodiment, the IL2Rb is the human IL2Rb. The human CD122 (hCD122) is expressed as a 551 amino acid pre-protein, the first 26 amino acids comprising a signal sequence which is post-translationally cleaved in the mature 525 amino acid protein. Amino acids 27-240 (amino acids 1-214 of the mature protein) correspond to the extracellular domain, amino acids 241-265 (amino acids 225-239 of the mature protein) correspond to the transmembrane domain and amino acids 266-551 (amino acids 240-525 of the mature protein) correspond to the intracellular domain. UniProt Reference Number 14784. The canonical full length hIL2Rb precursor is a polypeptide having the amino acid sequence:

MAAPALSWRLPLLILLPLATSWASAAVNGTSQFTCFYNSRANISCVWSQDGALQD
TSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCRE
GVRWRVMAIQDFKPFENLRMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEA
RTLSPGHTWEEAPLLTLKQKQEWICLETLPDTQYEFQVRVKPLQGEFTTWSPWSQ
PLAFRTKPAALGKDTIPWLGHLLVGLSGAFGHILVYLLINCRNTGPWLKKVLKCNT
PDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQ
DKVPEPASLSSNHSLTSCFTNQGYFFHLPDALEIEACQVYFTYDPYSEEDPDEGVA
GAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERM
PPSLQERVPRDWDQPPLGPPTPGVPDLVDFQPPPELVREAGEEVPDAGPREGVSFP
WSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV (SEQ ID NO: 1)

[0003] To generate sdAbs against hIL2Rb, the extracellular domain of the hIL2Rb protein was used as an immunogen. The extracellular domain of the mature (lacking the signal sequence) hIL2Rb possesses the amino acid sequence:

AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQ
 ASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRMAPISL
 QVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICL
 ETLPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDT (SEQ ID NO: 2)

[0004] For purposes of the present disclosure, the numbering of amino acid residues of the human IL2Rb polypeptides as described herein is made in accordance with the numbering of this canonical sequence (UniProt ID: P14784). Amino acids 1-26 of SEQ ID NO:1 are identified as the signal peptide of the IL2Rb, amino acids 27-240 of SEQ ID NO:1 are identified as the extracellular domain, amino acids 241-265 of SEQ ID NO:1 are identified as the transmembrane domain, and amino acids 266-551 of SEQ ID NO:1 are identified as the intracellular domain.

[0005] In one embodiment, the IL2Rb is the murine IL2Rb. The murine CD122 (mCD122) is expressed as a 539 amino acid precursor, the first 26 amino acids comprising a signal sequence which is post-translationally cleaved to provide the mature 525 amino acid protein. Amino acids 27-240 (amino acids 1-214 of the mature protein) correspond to the extracellular domain, amino acids 241-268 (amino acids 225-242 of the mature protein) correspond to the transmembrane domain and amino acids 269-539 (amino acids 243-513 of the mature protein) correspond to the intracellular domain. The canonical full length mIL2Rb precursor protein including the signal sequence is a polypeptide of the amino acid sequence:

MATIALPWSLSLYVFLLLLATPWASAAVKNCSHLECFYNSRANVSCMWSHEEALN
 VTTCHVHAKSNLRHWNTCELTLVRQASWACNLILGSFPESQSLTSVDLLDINVVC
 WEEKGWRRVKTCDFHPFDNLRLVAPHSLQVLHIDTQRCNISWKVSQVSHYIEPYLE
 FEARRLLGHSWEDASVLSLKQRQQWLFLEMLIPSTSYEVQVRVKAQRNNTGTWS
 PWSQPLTFRTRPADPMKEILPMSWLRVLLLVLGCFSGFFSCVYILVKCRYLGPWLKT
 VLKCHIPDPSEFFSQLSSQHGGDLQKWLSSPVPLSFFSPSGPAPEISPLEVLDGDSKAV
 QLLLLQKDSAPLPSPSGHSQASCFTNQGYFFHLPNALEIESCQVYFTYDPCVEEEVE
 EDGSRLPEGSPHPPLLPLAGEQDDYCAFPPRDDLFLFSPSLSTPNTAYGGSRAPEERS
 PLSLHEGLPSLASRDLMGLQRPLERMPEGDGEGLSANSSGEQASVPEGNLHGQDQD
 RGQGPILTLNTDAYLSLQELQAQDSVHLI (SEQ ID NO: 3)

[0006] To generate sdAbs against mIL2Rb, the extracellular domain of the mIL2Rb protein was used as an immunogen. The extracellular domain of the mature (lacking the signal sequence) hIL2Rb possesses the amino acid sequence (amino acids 27-240):

AVKNCSHLECFYNSRANVSCMWSHEEALNVTTCVHAKSNLRHWNKTCELTIVR
 QASWACNLILGSFPESQSLTSVDLLDINVVCWEEKGWRRVKTCDHFPHFDNLRLVAP
 HSLQVLHIDTQRCNISWKVSQVSHYIEPYLEFEARRRLLGHSWEDASVLSLKQRQQ
 WLFLEMLIPSTSYEVQVRVKAQRNNTGTWSPWSQPLTFRTRPADPMKE (SEQ ID
 NO: 4)

[0007] For purposes of the present disclosure, the numbering of amino acid residues of the murine IL2Rb polypeptides as described herein is made in accordance with the numbering of this canonical sequence (UniProt ID: P16297). Amino acids 1-26 of SEQ ID NO:3 are identified as the signal peptide of the IL2Rb, amino acids 27-240 of SEQ ID NO:3 are identified as the extracellular domain, amino acids 241-268 of SEQ ID NO:3 are identified as the transmembrane domain, and amino acids 269-539 of SEQ ID NO:3 are identified as the intracellular domain.

IL2Rg

[0008] The IL2Rg binding molecules of the present disclosure specifically bind to the extracellular domain of the IL2Rg.

Human IL2Rg

[0009] The IL2Rg binding molecules of the present disclosure specifically bind to the extracellular domain of the IL2Rg (CD132). In one embodiment, the IL2Rg is the human IL2Rg. The canonical full length IL2Rg (including the signal peptide) is a polypeptide possessing the amino acid sequence:

MLKPSLPFTSLLFLQLPLLGVGLNTTILTPNGNEDTTADFFLTMTPTDSLSVSTLPLPEVQ
 CFVFNVEYMNCTWNSSSEPQPTNLTLHYWYKNSDNDKVQKCSHYLFSEEITSGCQLQK
 KEIHLYQTFVVQLQDPREPRRQATQMLKLQNLVIPWAPENLTLLHKLSESQLELNWNNRF
 LNHCLEHLVQYRTDWDHSWTEQSVDRHKFSLPSVDGQKRYTFRVRSRFPNPLCGSAQH
 WSEWSHPIHWGSNTSKENPFLFALEAVVISVGSMLIISLLCVYFWLERTMPRIPTLKNL
 EDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEIPPKGGALGEGPGASPCNQHS
 PYWAPPCYTLKPET (SEQ ID NO: 5).

[0010] To generate sdAbs against the human IL2Rg, the extracellular domain of the hIL2Rg protein was used as an immunogen. The extracellular domain of the mature (lacking the signal sequence) hIL2Rg possesses the amino acid sequence:

LNTTILTPNGNEDTTADFFLTMTPTDSLSVSTLPLPEVQCFVFNVEYMNCTWNSSSEPQ
 PTNLTLHYWYKNSDNDKVQKCSHYLFSEEITSGCQLQKKEIHLYQTFVVQLQDPREPRRQ
 ATQMLKLQNLVIPWAPENLTLLHKLSESQLELNWNNRFLNHCLEHLVQYRTDWDHSWT
 EQSVDRHKFSLPSVDGQKRYTFRVRSRFPNPLCGSAQHWSEWSHPIHWGSNTSKENPFL
 FALEA (SEQ ID NO: 6)

[0011] For purposes of the present disclosure, the numbering of amino acid residues of the human IL2Rg (hIL2Rg) polypeptides as described herein is made in accordance with the numbering of this canonical sequence (UniProt ID: 31785; SEQ ID NO: 5). Amino acids 1-22 of SEQ ID NO:5 are identified as the signal peptide of hIL2Rg, amino acids 23-262 of SEQ ID NO: 5 are identified as the extracellular domain, amino acids 263-283 SEQ ID NO: 5 are identified as the transmembrane domain, and amino acids 284-269 of SEQ ID NO:5 are identified as the intracellular domain.

Murine IL2Rg

[0012] In one embodiment, the IL2Rg is the murine IL2Rg. The murine CD132 (mCD132) is expressed as a 369 amino acid precursor, the first 22 amino acids comprising a signal sequence which is post-translationally cleaved to provide the mature 353 amino acid protein. Amino acids 23-263 (amino acids 1-214 of the mature protein) correspond to the extracellular domain, amino acids 264-284 (amino acids 242-266 of the mature protein) correspond to the transmembrane domain and amino acids 285-369 (amino acids 263-347 of the mature protein) correspond to the intracellular domain. The canonical full length mIL2Rg precursor protein including the signal sequence is a polypeptide of the amino acid sequence:

MLKLLSPRSFLVLQLLLLRAGWSSKVLMSANEDIKADLILTSTAPEHLSAPTLPLPEV
QCFVFNIEYMNCTWNSSSEPQATNLTLHYRYKVSDNNTFQECSHYLSKEITSGCQIQK
EDIQLYQTFVVQLQDPQKPQRRVQKLNQLNLPVPRAPENLTLSNLSSESOLELRWKS
HIKERCLQYL VQYRSNRDRSWTELIVNHEPRFSLPSVDELKRYTFRVRSRYNPICGSSQ
QWSKWSQPVHWGSHTVEENPSLFALEAVLIPVGTMLIITLIFVYCWLERMPPPIPKN
LEDLVTEYQGNFSAWSGVSKGLTESLQPDYSERFCHVSEIPPKGKGGALGEGPGGSPCSLH
SPYWPPPCYSLKPEA (SEQ ID NO: 7)

[0013] In one embodiment, the IL2Rg is the murine IL2Rg. The murine CD132 (mCD132) is expressed as a 369 amino acid precursor, the first 22 amino acids comprising a signal sequence which is post-translationally cleaved to provide the mature 353 amino acid protein. Amino acids 23-263 (amino acids 1-214 of the mature protein) correspond to the extracellular domain, amino acids 264-284 (amino acids 242-266 of the mature protein) correspond to the transmembrane domain and amino acids 285-369 (amino acids 263-347 of the mature protein) correspond to the intracellular domain. The canonical full length mIL2Rg precursor protein including the signal sequence is a polypeptide of the amino acid sequence:

To generate sdAbs against mIL2Rg, the extracellular domain of the mIL2Rg protein was used as an immunogen. The extracellular domain of the mature (lacking the signal sequence) hIL2Rg possesses the amino acid sequence (amino acids 23-263):

WSSKVLMSANEDIKADLILTSTAPEHLSAPTLPLPEVQCFVFNIEYMNCTWNSSEPQA
 TNLTLYRYKVSDNNTFQECSHYLFSEKITSQCQIQKEDIQLYQTFVVQLQDPQKPQRRRA
 VQKLNLQNLVIPRAPENLTLSNLSESQLELRWKSRIKERCLQYLVQYRSNRDRSWTELI
 VNHEPRFSLPSVDELKRYTFRVRSRYNPICGSSQQWSKWSQPVHWGSHTVEENPSLFAL
 EA (SEQ ID NO: 8)

For purposes of the present disclosure, the numbering of amino acid residues of the murine IL2Rg polypeptides as described herein is made in accordance with the numbering of this canonical sequence (UniProt ID: P34902). Amino acids 1-22 of SEQ ID NO:7 are identified as the signal peptide of the IL2Rg, amino acids 23-263 of SEQ ID NO:7 are identified as the extracellular domain, amino acids 264-284 of SEQ ID NO:7 are identified as the transmembrane domain, and amino acids 285-369 of SEQ ID NO:7 are identified as the intracellular domain.

IL2

[0014] IL2 is a monomeric polypeptide which is an agonist of the IL2R. The amino acid sequence for human IL2 is set forth under UniProt ID: P60568 and is set forth below as SEQ ID NO: 9 below

MYRMQLLSIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLT
 RMLTFKIFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLE
 LKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT (SEQ ID NO: 9)

[0015] The amino acid sequence of mature murine IL2 is set forth under UniProt ID: P04351 and is set forth below as SEQ ID NO: 10.

MYSMQLASCVTLTLVLLVNSAPTSSSTSSSTAEEAQQQQQQQQQQQHLQQLMDLQELL
 SRMENYRNKLPRMLTFKIFYLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLEDAE
 NFISNIRVTVVKLKGSNTFECQFDDESATVVDFLRRWIAFCQSIISTSPQ (SEQ ID NO: 10)

[0016] IL2 is a pluripotent cytokine which is produced by antigen activated T cells. IL2 exerts a wide spectrum of effects on the immune system and plays important roles in regulating both immune activation, suppression and homeostasis. IL2 promotes the proliferation and expansion of activated T lymphocytes, induces proliferation and activation of naïve T cells, potentiates B cell growth, and promotes the proliferation and expansion of NK cells. Human interleukin 2 (IL2) is a 4 alpha-helix bundle cytokine of 133 amino acids. IL2 is a member of the IL2 family of cytokines which includes IL2, IL-4, IL-7, IL 9, IL-15 and IL21.

IL2/IL2 Receptor Interaction:

Monomeric IL2 forms a complex with both the trimeric “high affinity” form of the IL2 receptor and the dimeric intermediate affinity receptor (Wang, *et al.* (2005) *Science* 310:159-1163) through binding to the extracellular domains of the receptor components expressed on the cell surface. The binding of IL2 to CD25 induces a conformational change in IL2 facilitating increased binding to CD122. IL2 mutants, mimicking the CD25 binding-induced conformational change demonstrate increased binding to CD122 (Levin, *et al.* (2012) *Nature* 484(7395): 529-533). The association of CD132 provides formation of the dimeric intermediate-affinity or trimeric high-affinity receptor complexes which are associated with intracellular signaling. In addition to providing intracellular signaling via the JAK/STAT pathway (e.g. phosphorylation of STAT5) and other cellular systems, the interaction of hIL2 with the hIL2 high affinity trimeric receptor on a cell initiates a process by which CD122 is internalized, the membrane bound form of CD25 is released from the activated cell as a soluble protein (referred to as “soluble CD25” or “sCD25”) as well as triggering the release of IL2 endogenously produced by the activated cell which is capable of acting in an autocrine and/or paracrine fashion.

CD25 (also referred to interchangeably herein as IL2R α and IL2Ra) is a 55 kD polypeptide that is constitutively expressed in Treg cells and inducibly expressed on other T cells in response to activation. hIL2 binds to hCD25 with a K_d of approximately 10^{-8} M. CD25 is also referred to in the literature as the “low affinity” IL2 receptor. The human CD25 (“hCD25”) is expressed as a 272 amino acid pre-protein comprising a 21 amino acid signal sequence which is post-translationally removed to render a 251 amino acid mature protein. Amino acids 22-240 (amino acids 1-219 of the mature protein) correspond to the extracellular domain. Amino acids 241-259 (amino acids 220-238 of the mature protein) correspond to transmembrane domain. Amino acids 260-272 (amino acids 239-251 of the mature protein) correspond to intracellular domain. The intracellular domain of CD25 is comparatively small (13 amino acids) and has not been associated with any independent signaling activity. The IL2/CD25 complex has not been observed to produce a detectable intracellular signaling response. Human CD25 nucleic acid and protein sequences may be found as Genbank accession numbers NM__000417 and NP_0004Q8 respectively.

[0001] IL2 is a pluripotent cytokine which is produced by antigen activated T cells. IL2 exerts a wide spectrum of effects on the immune system and plays important roles in regulating both immune activation, suppression and homeostasis. IL2 promotes the

proliferation and expansion of activated T lymphocytes, induces proliferation and activation of naïve T cells, potentiates B cell growth, and promotes the proliferation and expansion of NK cells. Human interleukin 2 (IL2) is a 4 alpha-helix bundle cytokine of 133 amino acids. IL2 is a member of the IL2 family of cytokines which includes IL2, IL-4, IL-7, IL 9, IL-15 and IL21.

IL2 Receptor Expression On Various Cell Types

The IL2 receptors are expressed on the surface of most lymphatic cells, in particular on T cells, NK cells, and B cells, but the expression level is variable and is dependent on a variety of factors include the activation stage of the cell. Inactive T cells and NK cells express almost exclusively express the intermediate-affinity dimeric IL2 receptor, consisting of the two receptor subunits, CD122 and CD132 and demonstrates comparatively low responsiveness to IL2 since they predominantly express the intermediate affinity CD122/CD132 complex which has comparatively low affinity for IL2 relative to the CD25/CD122/CD132 high affinity receptor. In contrast, activated T cells and regulatory T cells express the trimeric high-affinity IL2 receptor consisting of CD25, CD122 and CD132. TCR activated T cells (*i.e.*, so called “antigen experienced” T cells) express the high-affinity trimeric IL2 receptor. T cells, including tumor infiltrating T cells (“TILs”) and tumor recognizing cells, upregulate CD25 and CD122 upon receiving a T cell receptor (TCR) signal (Kalia, et al. (2010) *Immunity* **32**(1): 91-103. The upregulation of CD25 and CD122 receptor in response to receiving a T cell receptor (TCR) signal renders the antigen activated T cell highly sensitive to the IL2 cytokine. Although, Tregs constitutively express CD25, and therefore express the high affinity trimeric IL2 receptor, TCR-activated T cells express higher levels of the trimeric receptor than regulatory T cells. As a consequence, the expansion of antigen activated T cells in antigen-challenged hosts significantly outpaces the expansion of Tregs. (Humblet-Baron, et al. (2016) *J Allergy Clin Immunol* **138**(1): 200-209 e208).

Recombinant hIL2 (sold under the trademark Proleukin) is indicated for the treatment of human adults with metastatic melanoma and metastatic renal cell carcinoma. Therapeutic application of High Dose hIL2 (HD-hIL2) induces tumor rejection in highly immune infiltrated melanomas and renal cell carcinomas (Atkins, *et al.* (1999) *J Clin Oncol* **17**(7):2105-2116). However, HD-hIL2 therapy is associated with severe dose limiting toxicity, including impaired neutrophil function, fever, hypotension, diarrhea and requires expert management. Dutcher, et al. (2014) *J Immunother Cancer* **2**(1): 26. HD-hIL2 treatment activates most lymphatic cells, including naïve T cells and NK cells, which predominantly express the intermediate affinity

CD122/CD132 dimeric receptor and CD25+ regulatory T cells (Tregs), which express the high affinity trimeric receptor (CD25/CD122/CD132). HD-hIL2 monotherapy may also induce generalized capillary leak syndrome which can lead to death. This limits the use of HD-IL2 therapy to mostly younger, very healthy patients with normal cardiac and pulmonary function. HD-IL2 therapy is typically applied in the hospital setting and frequently requires admission to an intensive care unit.

Clinical experience demonstrates that HD-IL2 treatment activates naïve T cells and NK cells, which predominantly express the intermediate affinity receptor as well as CD25+ regulatory T cells (Tregs) which mediate the activity of CD8+ T cells. Due to their constitutive expression of CD25, Tregs are particularly sensitive to IL2. To avoid preferential activation of Tregs, IL2 variants have been developed and introduced into clinical development, which are designed to avoid binding to CD25 and possess enhanced binding to the intermediate affinity CD122/CD132 receptor to activate NK cells and quiescent CD8+ T cells. Such IL2 muteins are often referred to in the literature as “non- α -IL2” or “ β/γ -biased IL2” muteins. However, such “non- α -IL2” or “ β/γ -biased IL2”, by virtue of their reduced binding to CD25, also avoid binding to the antigen activated T cells which have been identified as the primary mediators of anti-tumor T cell response (Peace, D. J. and Cheever, M. A. (1989) *J Exp Med* 169(1):161-173).

[0001] Additionally, preclinical experiments have implicated NK cells as the dominant mechanism for IL2 mediated acute toxicity. Assier E, et al. (2004) *J Immunol* 172:7661–7668. As NK cells express the intermediate affinity (CD122/CD132; β/γ -) IL2 receptor, the nature of such β/γ -IL2 muteins is to enhance the proliferation of such NK cells which may lead to enhanced toxicity. Additionally, although Tregs are associated with down-regulation of CD8+ T cells, Tregs have also been shown to limit the IL2 mediated off-tumor toxicity (Li, *et al.* (2017) *Nature Communications* 8(1):1762). Although nitric oxide synthase inhibitors have been suggested to ameliorate the symptoms of VLS, the common practice when VLS is observed is the withdrawal of IL2 therapy. To mitigate the VLS associated with HD IL2 treatment, low-dose IL2 regimens have been tested in patients. While low dose IL2 treatment regimens do partially mitigate the VLS toxicity, this lower toxicity was achieved at the expense of optimal therapeutic results in the treatment of neoplasms.

[0002] Considering the pluripotent effects of hIL2 and its demonstrated ability to modulate the activities of a wide variety of cell types associated with human disease, the search for IL2 muteins that retain certain desirable features of the native molecule while minimizing

undesirable features remain an active area of research with multiple IL2 muteins in clinical development nearly 40 years after its initial discovery.

SUMMARY OF THE DISCLOSURE

[0002] The present disclosure provides compositions useful in the pairing of cellular receptors to generate desirable effects useful in treatment of disease in mammalian subjects.

[0003] The present disclosure provides binding molecules that comprise a first domain that binds to IL2Rb of the IL2R receptor and a second domain that binds to IL2Rg of the IL2R receptor, such that upon contacting with a cell expressing IL2Rb and IL2Rg, the IL2R binding molecule causes the functional association of IL2Rb and IL2Rg, thereby resulting in functional dimerization of the receptors and downstream signaling.

[0004] Several advantages flow from the binding molecules described herein. As discussed above, the use of IL2 as a therapeutic in mammalian, particularly human, subjects, it may also trigger a number of adverse and undesirable effects by a variety of mechanisms including the presence of IL2Rb and IL2Rg on other cell types. The binding to IL2Rb and IL2Rg on the other cell types may result in undesirable effects and/or undesired signaling on cells expressing IL2Rb and IL2Rg.

[0005] The present disclosure is directed to methods and composition that facilitate the the modulation of the multiple effects characteristic of IL10 to provide that agents having a generate the activation and/or proliferative response of IL2 signaling in a desired cell population or tissue subtype, while exhibiting substantially reduced signaling activity and/or intracellular signaling.

[0006] In some embodiments, the IL2R binding molecules described herein are partial agonists of the IL2R. In some embodiments, the binding molecules described herein are designed such that the IL2R binding molecules are full agonists. In some embodiments, the IL2R binding molecules described herein are designed such that the IL2R binding molecules are super agonists.

[0007] In some embodiments, the IL2R binding molecules provide substantial IL2 intracellular signaling on the desired cell types, while providing significantly reduced IL2 signaling relative to wild-type IL2 on other undesired cell types. The architecture of the binding molecules of the present disclosure provide multiple means for the modulation of the signaling associated with the dimerization of IL2Rb and IL2g. In some embodiments, the selective may be achieve by selection of binding molecules having differing affinities or causing different E_{max} for IL2Rb and IL2Rg, as compared to the affinity of IL2 for IL2Rb and

IL2Rg. Because different cell types respond to the binding of ligands to its cognate receptor with different sensitivity, modulating the affinity of the dimeric ligand (or its individual binding moieties) for the IL2 receptor relative to wild-type IL2 binding facilitates the stimulation of desired activities while reducing undesired activities on non-target cells.

[0008] The present disclosure provides binding molecules that are agonists of the IL2R receptor, the binding molecule comprising:

- a first single domain antibody (sdAb) that specifically binds to the extracellular domain of IL2Rb of the IL2R (an “IL2Rb sdAb”), and
- a second single domain antibody that specifically binds to extracellular domain IL2Rg of the IL2R (an “IL2Rg sdAb”),

wherein the IL2Rb sdAb and IL2Rg sdAb are stably associated, and wherein contacting a cell expressing IL2Rb and IL2Rg with an effective amount of the binding molecule results in the dimerization of IL2Rb and IL2Rg, and results in intracellular signaling characteristic of the IL2R receptor when activated by its cognate ligand, IL2. In some embodiments, one or both of the sdAbs is a scFv. In some embodiments, one or both of the sdAbs is a VHH.

[0009] In some embodiments, one sdAb of the binding molecule is an scFv and the other sdAb is a VHH.

[0010] In some embodiments, one sdAb of the binding molecule is an scFv and the other sdAb is a VHH.

[0011] In some embodiments, the first and second sdAbs are covalently bound via a chemical linkage.

[0012] In some embodiments, the first and second sdAbs are provided as single continuous polypeptide.

[0013] In some embodiments, the first and second sdAbs are provided as single continuous polypeptide optionally comprising an intervening polypeptide linker between the amino acid sequences of the first and second sdAbs.

[0014] In some embodiments, the binding molecule optionally comprising a linker, is optionally expressed as a fusion protein with an additional amino acid sequence. In some embodiments, the additional amino acid sequence is a purification handle such as a chelating peptide or an additional protein such as a subunit of an Fc molecule.

[0015] The disclosure also provides an expression vector comprising a nucleic acid encoding the bispecific binding molecule operably linked to one or more expression control

sequences. The disclosure also provides an isolated host cell comprising the expression vector expression vector comprising a nucleic acid encoding the bispecific binding molecule operably linked to one or more expression control sequences functional in the host cell.

[0016] In another aspect, the disclosure provides a pharmaceutical composition comprising the IL2R binding molecule described herein and a pharmaceutically acceptable carrier.

[0017] In another aspect, the disclosure provides a method of treating an autoimmune or inflammatory disease, disorder, or condition or a viral infection in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an IL2R binding molecule described herein or a pharmaceutical composition described herein.

[0018] In another aspect, the disclosure provides a method of treating neoplastic disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an IL2R binding molecule described herein or a pharmaceutical composition described herein.

BRIEF DESCRIPTION OF THE FIGURES

[0001] Figure 1 of the attached drawings provides a schematic representation of one embodiment of the binding molecule of the present disclosure comprising a first single domain antibody (1) and a second single domain antibody (3) and a linker (2) depicted as interacting with a cell membrane (10) associated heterodimeric receptor comprising a first receptor subunit comprising an extracellular domain (4), and transmembrane domain (5) and an intracellular domain (6) interaction of a binding molecule and a second first receptor subunit comprising an extracellular domain (7), and transmembrane domain (8) and an intracellular domain (9) wherein the intracellular domain of the first receptor (6) and the intracellular domain of the second receptor (9) on of a binding molecule are within a proximal distance (11).

[0002] Figure 2 of the attached drawings provides a schematic representation of two illustrative configurations of binding molecules of the present disclosure. Panel A provides a schematic representation of an illustrative single polypeptide chain binding molecule comprising, from amino to carboxy, a first single domain antibody (1) and a second single domain antibody (3) and a linker (2). Panel B provides a schematic representation of a binding molecule comprising a first single domain antibody (1) and a second single domain antibody (3) and a linker (2) and a knob-into-hole Fc domain comprising a first subunit which is a Fc knob (13) and

a second subunit which is a Fc hole (14) wherein the single domain antibody is stably associated with the Fc domain via a IgG hinge sequence (12).

[0003] Figure 2 of the attached drawings provides a schematic representation of two illustrative configurations of binding molecules of the present disclosure. Panel A provides a schematic representation of an illustrative binding molecule comprising a first single domain antibody (1) and a second single domain antibody (3) and a linker (2). Panel B provides a schematic representation of a binding molecule comprising two polypeptide chains, the first polypeptide chain comprising (from amino to carboxy) a first single domain antibody (1), a linker sequence, a second single domain antibody (3), an IgG hinge sequence (12) and an Fc knob domain (13) and a second polypeptide comprising an Fc hole (14) wherein the first and second polypeptides are in stable association via the interaction of the knob-into-hole Fc domain.

[0004] Figure 3 of the attached drawings provides a schematic representations of two illustrative configurations of binding molecules of the present disclosure. Panel A provides a schematic representation of an illustrative binding molecule construct comprising two binding molecules each attached to a subunit of a knob-into-hole Fc domain, the construct comprising two polypeptide chains, the first polypeptide chain comprising, from amino to carboxy, a first single domain antibody (1), a linker (2) and a second single domain antibody (3), a IgG hinge sequence (12) and a Fc knob subunit (13) and a second polypeptide chain comprising, from amino to carboxy, a first single domain antibody (1), a linker (2) and a second single domain antibody (3), a IgG hinge sequence (12) and a Fc hole subunit (14) wherein the first and second polypeptides are in stable associate via the interaction of the knob-into-hole Fc domain. Panel B provides schematic representation of a an alternative arrangement of a binding molecule construct comprising two polypeptides a first polypeptide chain comprising, from amino to carboxy, a first single domain antibody (1), a linker (2) and a second single domain antibody (3), an IgG hinge sequence (12) and a Fc knob subunit (13) and a second polypeptide chain comprising, from amino to carboxy, a first second domain antibody (3), a linker (2) and a first single domain antibody (1), a IgG hinge sequence (12) and a Fc hole subunit (14), wherein the first and second polypeptides are in stable association via the interaction of the knob-into-hole Fc domain.

[0005] Figure 4, Panel A provides alternative schematic representations of configurations of the binding molecules of the present disclosure where one single domain antibody is attached to each subunit of a knob-into-hole Fc domain comprising two polypeptides, the first polypeptide

comprising from amino to carboxy, a first single domain antibody (1), an IgG hinge sequence (12) and a Fc knob subunit (13), the second polypeptide comprising from amino to carboxy, a second single domain antibody (3), an IgG hinge sequence (12) and a Fc hole subunit (13), wherein the first and second single domain antibodies are in stable association via the interaction of the knob-into-hole Fc domain.

[0006] Figure 4, Panel B provides a schematic representation of a binding molecule the binding domains are single domain antibodies associated via transition metal coordinate covalent complex. As illustrated, the binding molecule comprises two polypeptide subunits: the first subunit comprising a first single domain antibody (1) is attached via a first linker (15) to a first chelating peptide (17) and second subunit comprising a second single domain antibody (3) is attached via a second linker (16) to a second chelating peptide (18), wherein the first chelating peptide (17) and second chelating peptide (18) form a coordinate covalent complex with a single transition metal ion ("M"). The transition metal ion may be in a kinetically labile or kinetically inert oxidation state.

[0007] Provides data with respect to IL2R binding molecules of the present disclosure on the induction of IFN gamma in NK cells measured by luminescent. This data illustrates that the IL2 binding molecules by varying the sdAb components may provide substantial variations in activity significantly greater than wt IL2 in some instances. than wild type

[0008] Figure 6 of the attached drawings provides data from the evaluation of T cell outgrowth on PBMCs isolate from two separate donors. As can be seen the data, the IL2R binding molecules enables selective T cell proliferation activity with respect to NK cells, even though the NK cells express the b/g receptors. This demonstrates that variation in receptor binding affinity can be used to modulate the activity of the IL2R binding molecules in selective cell types.

DETAILED DESCRIPTION OF THE INVENTION

[0019] To facilitate the understanding of present disclosure, certain terms and phrases are defined below as well as throughout the specification. The definitions provided herein are non-limiting and should be read in view of the knowledge of one of skill in the art would know.

[0020] Before the present methods and compositions are described, it is to be understood that this invention is not limited to a particular method or composition described,

as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing embodiments only and is not intended to be limiting.

[0021] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0022] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, some potential and preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0023] It should be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the peptide" includes reference to one or more peptides and equivalents thereof, e.g. polypeptides, known to those skilled in the art, and so forth.

[0024] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

[0025] Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius (°C), and pressure is at or near atmospheric. Standard abbreviations are used, including the following: bp = base pair(s); kb = kilobase(s); pl = picoliter(s); s or sec = second(s); min = minute(s); h or hr = hour(s); AA

or aa = amino acid(s); kb = kilobase(s); nt = nucleotide(s); pg = picogram; ng = nanogram; μ g = microgram; mg = milligram; g = gram; kg = kilogram; dl or dL = deciliter; μ l or μ L = microliter; ml or mL = milliliter; l or L = liter; μ M = micromolar; mM = millimolar; M = molar; kDa = kilodalton; i.m. = intramuscular(ly); i.p. = intraperitoneal(ly); SC or SQ = subcutaneous(ly); QD = daily; BID = twice daily; QW = once weekly; QM = once monthly; HPLC = high performance liquid chromatography; BW = body weight; U = unit; ns = not statistically significant; PBS = phosphate-buffered saline; PCR = polymerase chain reaction; HSA = human serum albumin; MSA = mouse serum albumin; DMEM = Dulbecco's Modification of Eagle's Medium; EDTA = ethylenediaminetetraacetic acid.

[0026] It will be appreciated that throughout this disclosure reference is made to amino acids according to the single letter or three letter codes. For the reader's convenience, the single and three letter amino acid codes are provided in Table 1 below:

Table 1. Amino Acid Abbreviations		
G	Glycine	Gly
P	Proline	Pro
A	Alanine	Ala
V	Valine	Val
L	Leucine	Leu
I	Isoleucine	Ile
M	Methionine	Met
C	Cysteine	Cys
F	Phenylalanine	Phe
Y	Tyrosine	Tyr
W	Tryptophan	Trp
H	Histidine	His
K	Lysine	Lys
R	Arginine	Arg
Q	Glutamine	Gln
N	Asparagine	Asn
E	Glutamic Acid	Glu
D	Aspartic Acid	Asp
S	Serine	Ser
T	Threonine	Thr

[0027] Standard methods in molecular biology are described in the scientific literature (see, e.g., Sambrook and Russell (2001) *Molecular Cloning*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; and Ausubel, et al. (2001) *Current Protocols in Molecular Biology*, Vols. 1-4, John Wiley and Sons, Inc. New York, N.Y., which describes

cloning in bacterial cells and DNA mutagenesis (Vol. 1), cloning in mammalian cells and yeast (Vol. 2), glycoconjugates and protein expression (Vol. 3), and bioinformatics (Vol. 4)). The scientific literature describes methods for protein purification, including immunoprecipitation, chromatography, electrophoresis, centrifugation, and crystallization, as well as chemical analysis, chemical modification, post-translational modification, production of fusion proteins, and glycosylation of proteins (see, e.g., Coligan, et al. (2000) Current Protocols in Protein Science, Vols. 1-2, John Wiley and Sons, Inc., NY).

Definitions

[0028] Unless otherwise indicated, the following terms are intended to have the meaning set forth below. Other terms are defined elsewhere throughout the specification.

[0029] Activate: As used herein the term “activate” is used in reference to a receptor or receptor complex to reflect a biological effect, directly and/or by participation in a multicomponent signaling cascade, arising from the binding of an agonist ligand to a receptor responsive to the binding of the ligand.

[0030] ACT Cell Product: As used herein, the terms “cell product”, “adoptive cell transfer product” or “ACT cell product” are used interchangeably herein to refer to a population of cells comprising immune cells that have been manipulated ex vivo to be enriched for a desired subpopulation of immune cells for administration to a subject in need of treatment. One example of ACT cell product is a TIL cell product wherein the immune cells that have been manipulated ex vivo are lymphocytes isolated from a tissue sample of a subject suffering from a neoplastic disease. The tissue sample used as a source of the immune cells may be a neoplastic lesion or tumor mass for preparation of a TIL cell product. Alternatively, TILs may be isolated from circulating blood.

[0031] Activity: As used herein, the term “activity” is used with respect to a molecule to describe a property of the molecule with respect to a test system (e.g. an assay) or biological or chemical property (e.g. the degree of binding of the molecule to another molecule) or of a physical property of a material or cell (e.g. modification of cell membrane potential). Examples of such biological functions include but are not limited to catalytic activity of a biological agent, the ability to stimulate intracellular signaling, gene expression, cell proliferation, the ability to modulate immunological activity such as inflammatory response. “Activity” is typically expressed as a level of a biological activity per unit of agent tested such

as [catalytic activity]/[mg protein], [immunological activity]/[mg protein], international units (IU) of activity, [STAT5 phosphorylation]/[mg protein], [T-cell proliferation]/[mg protein], plaque forming units (pfu), etc. As used herein, the term “proliferative activity” refers to an activity that promotes cell proliferation and replication.

[0032] Administer/Administration: The terms “administration” and “administer” are used interchangeably herein to refer the act of contacting a subject, including contacting a cell, tissue, organ, or biological fluid of the subject *in vitro*, *in vivo* or *ex vivo* with an agent (e.g. an ortholog, an IL2 ortholog, an engineered cell expressing an orthogonal receptor, an engineered cell expressing an orthogonal IL2 receptor, a CAR-T cell expressing an orthogonal IL2 receptor, a chemotherapeutic agent, an antibody, or a pharmaceutical formulation comprising one or more of the foregoing). Administration of an agent may be achieved through any of a variety of art recognized methods including but not limited to the topical administration, intravascular injection (including intravenous or intraarterial infusion), intradermal injection, subcutaneous injection, intramuscular injection, intraperitoneal injection, inhalation and the like. The term “administration” includes contact of an agent to the cell, tissue or organ as well as the contact of an agent to a fluid, where the fluid is in contact with the cell, tissue or organ.

[0033] Affinity: As used herein the term “affinity” refers to the degree of specific binding of a first molecule (e.g., a ligand) to a second molecule (e.g., a receptor) and is measured by the binding kinetics expressed as K_d , a ratio of the dissociation constant between the molecule and its target (K_{off}) and the association constant between the molecule and its target (K_{on}).

[0034] Agonist: As used herein, the term “agonist” refers a first agent that specifically binds a second agent (“target”) and interacts with the target to cause or promote an increase in the activation of the target. In some instances, agonists are activators of receptor proteins that modulate cell activation, enhance activation, sensitize cells to activation by a second agent, or up-regulate the expression of one or more genes, proteins, ligands, receptors, biological pathways, that may result in cell proliferation or pathways that result in cell cycle arrest or cell death such as by apoptosis. In some embodiments, an agonist is an agent that binds to a receptor and alters the receptor state, resulting in a biological response. The response mimics the effect of the endogenous activator of the receptor. The term “agonist” includes partial agonists, full agonists and superagonists. An agonist may be described as a “full agonist” when such agonist which leads to a substantially full biological response (*i.e.*, the response

associated with the naturally occurring ligand/receptor binding interaction) induced by receptor under study, or a partial agonist. In contrast to agonists, antagonists may specifically bind to a receptor but do not result the signal cascade typically initiated by the receptor and may to modify the actions of an agonist at that receptor. Inverse agonists are agents that produce a pharmacological response that is opposite in direction to that of an agonist. A "superagonist" is a type of agonist that is capable of producing a maximal response greater than the endogenous agonist for the target receptor, and thus has an activity of more than 100% of the native ligand. A super agonist is typically a synthetic molecule that exhibits greater than 110%, alternatively greater than 120%, alternatively greater than 130%, alternatively greater than 140%, alternatively greater than 150%, alternatively greater than 160%, or alternatively greater than 170% of the response in an evaluable quantitative or qualitative parameter of the naturally occurring form of the molecule when evaluated at similar concentrations in a comparable assay.

[0035] Antagonist: As used herein, the term "antagonist" or "inhibitor" refers a molecule that opposes the action(s) of an agonist. An antagonist prevents, reduces, inhibits, or neutralizes the activity of an agonist, and an antagonist can also prevent, inhibit, or reduce constitutive activity of a target, e.g., a target receptor, even where there is no identified agonist. Inhibitors are molecules that decrease, block, prevent, delay activation, inactivate, desensitize, or down-regulate, e.g., a gene, protein, ligand, receptor, biological pathway, or cell.

[0036] Antibody: As used herein, the term "antibody" refers collectively to: (a) glycosylated and non-glycosylated immunoglobulins (including but not limited to mammalian immunoglobulin classes IgG1, IgG2, IgG3 and IgG4) that specifically binds to target molecule and (b) immunoglobulin derivatives including but not limited to IgG(1-4) Δ CH2, F(ab')₂, Fab, ScFv, V_H, V_L, tetrabodies, triabodies, diabodies, dsFv, F(ab')₃, scFv-Fc and (scFv)₂ that competes with the immunoglobulin from which it was derived for binding to the target molecule. The term antibody is not restricted to immunoglobulins derived from any particular mammalian species and includes murine, human, equine, and camelids antibodies (e.g., human antibodies). The term "antibody" encompasses antibodies isolatable from natural sources or from animals following immunization with an antigen and as well as engineered antibodies including monoclonal antibodies, bispecific antibodies, trispecific, chimeric antibodies, humanized antibodies, human antibodies, CDR-grafted, veneered, or deimmunized (e.g., to remove T-cell epitopes) antibodies. The term "human antibody" includes antibodies obtained from human beings as well as antibodies obtained from transgenic mammals comprising

human immunoglobulin genes such that, upon stimulation with an antigen the transgenic animal produces antibodies comprising amino acid sequences characteristic of antibodies produced by human beings. The term "antibody" should not be construed as limited to any particular means of synthesis and includes naturally occurring antibodies isolatable from natural sources and as well as engineered antibodies molecules that are prepared by "recombinant" means including antibodies isolated from transgenic animals that are transgenic for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed with a nucleic acid construct that results in expression of an antibody, antibodies isolated from a combinatorial antibody library including phage display libraries.

[0037] Binding molecule: As used herein, the term "binding molecule" refers to a molecule that can bind to the extracellular domain of two cell surface receptors. In some embodiments, a binding molecule specifically binds to two different receptors (or domains or subunits thereof) such that the receptors (or domains or subunits) are maintained in proximity to each other such that the receptors (or domains or subunits), including domains thereof (*e.g.*, intracellular domains) interact with each other and result in downstream signaling.

[0038] CDR: As used herein, the term "CDR" or "complementarity determining region" is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain immunoglobulin polypeptides. CDRs have been described by Kabat et al., *J. Biol. Chem.* 252:6609-6616 (1977); Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of proteins of immunological interest" (1991) (also referred to herein as Kabat 1991); by Chothia et al., *J. Mol. Biol.* 196:901-917 (1987) (also referred to herein as Chothia 1987); and MacCallum et al., *J. Mol. Biol.* 262:732-745 (1996), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. In the context of the present disclosure, the numbering of the CDR positions is provided according to Kabat numbering conventions.

[0039] Comparable: As used herein, the term "comparable" is used to describe the degree of difference in two measurements of an evaluable quantitative or qualitative parameter. For example, where a first measurement of an evaluable quantitative parameter and a second measurement of the evaluable parameter do not deviate beyond a range that the skilled artisan would recognize as not producing a statistically significant difference in effect between the

two results in the circumstances, the two measurements would be considered “comparable.” In some instances, measurements may be considered “comparable” if one measurement deviates from another by less than 30%, alternatively by less than 25%, alternatively by less than 20%, alternatively by less than 15%, alternatively by less than 10%, alternatively by less than 7%, alternatively by less than 5%, alternatively by less than 4%, alternatively by less than 3%, alternatively by less than 2%, or by less than 1%. In particular embodiments, one measurement is comparable to a reference standard if it deviates by less than 15%, alternatively by less than 10%, or alternatively by less than 5% from the reference standard.

[0040] Effective Concentration (EC): As used herein, the terms “effective concentration” or its abbreviation “EC” are used interchangeably to refer to the concentration of an agent (*e.g.*, an hIL2 mutein) in an amount sufficient to effect a change in a given parameter in a test system. The abbreviation “E” refers to the magnitude of a given biological effect observed in a test system when that test system is exposed to a test agent. When the magnitude of the response is expressed as a factor of the concentration (“C”) of the test agent, the abbreviation “EC” is used. In the context of biological systems, the term E_{max} refers to the maximal magnitude of a given biological effect observed in response to a saturating concentration of an activating test agent. When the abbreviation EC is provided with a subscript (*e.g.*, EC₄₀, EC₅₀, etc.) the subscript refers to the percentage of the E_{max} of the biological observed at that concentration. For example, the concentration of a test agent sufficient to result in the induction of a measurable biological parameter in a test system that is 30% of the maximal level of such measurable biological parameter in response to such test agent, this is referred to as the “EC₃₀” of the test agent with respect to such biological parameter. Similarly, the term “EC₁₀₀” is used to denote the effective concentration of an agent that results the maximal (100%) response of a measurable parameter in response to such agent. Similarly, the term EC₅₀ (which is commonly used in the field of pharmacodynamics) refers to the concentration of an agent sufficient to results in the half-maximal (50%) change in the measurable parameter. The term “saturating concentration” refers to the maximum possible quantity of a test agent that can dissolve in a standard volume of a specific solvent (*e.g.*, water) under standard conditions of temperature and pressure. In pharmacodynamics, a saturating concentration of a drug is typically used to denote the concentration sufficient of the drug such that all available receptors are occupied by the drug, and EC₅₀ is the drug concentration to give the half-maximal effect. The EC of a particular effective concentration of a test agent may be abbreviated with respect to the with respect to particular parameter and test system.

[0041] Extracellular Domain: As used herein the term "extracellular domain" or its abbreviation "ECD" refers to the portion of a cell surface protein (*e.g.* a cell surface receptor) which is outside of the plasma membrane of a cell. The term "ECD" may include the extra-cytoplasmic portion of a transmembrane protein or the extra-cytoplasmic portion of a cell surface (or membrane associated protein).

[0042] Identity: As used herein, the term "percent (%) sequence identity" or "substantially identical" used in the context of nucleic acids or polypeptides, refers to a sequence that has at least 50% sequence identity with a reference sequence. Alternatively, percent sequence identity can be any integer from 50% to 100%. In some embodiments, a sequence has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the reference sequence as determined with BLAST using standard parameters, as described below. For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters. A comparison window includes reference to a segment of any one of the number of contiguous positions, *e.g.*, a segment of at least 10 residues. In some embodiments, the comparison window has from 10 to 600 residues, *e.g.*, about 10 to about 30 residues, about 10 to about 20 residues, about 50 to about 200 residues, or about 100 to about 150 residues, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-410 and Altschul *et al.* (1977) *Nucleic Acids Res.* 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (NCBI) web site. The algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al. supra*). These initial neighborhood word hits act as seeds for initiating searches to find

longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word size (W) of 28, an expectation (E) of 10, M=1, N=-2, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word size (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)). The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.,* Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, an amino acid sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test amino acid sequence to the reference amino acid sequence is less than about 0.01, more preferably less than about 10^{-5} , and most preferably less than about 10^{-20} .

[0043] Intracellular Signaling: As used herein, the terms “intracellular signaling” and “downstream signaling” are used interchangeably to refer to the cellular signaling process that is caused by the interaction of the intracellular domains (ICDs) of two or more cell surface receptors that are in proximity of each other. In receptor complexes via the JAK/STAT pathway, the association of the ICDS of the receptor subunits brings the JAK domains of the ICDs into proximity which initiates a phosphorylation cascade in which STAT molecules are phosphorylated and translocate to the nucleus associating with particular nucleic acid sequences resulting in the activation and expression of particular genes in the cell. The binding molecules of the present disclosure provide intracellular signaling characteristic of the IL2R receptor when activated by its natural cognate IL2. To measure downstream signaling activity, a number of methods are available. For example, in some embodiments, one can

measure JAK/STAT signaling by the presence of phosphorylated receptors and/or phosphorylated STATs. In other embodiments, the expression of one or more downstream genes, whose expression levels can be affected by the level of downstream signaling caused by the binding molecule, can also be measured.

[0044] Ligand: As used herein, the term “ligand” refers to a molecule that exhibits specific binding to a receptor and results in a change in the biological activity of the receptor so as to effect a change in the activity of the receptor to which it binds. In one embodiment, the term “ligand” refers to a molecule, or complex thereof, that can act as an agonist or antagonist of a receptor. As used herein, the term “ligand” encompasses natural and synthetic ligands. “Ligand” also encompasses small molecules, *e.g.*, peptide mimetics of cytokines and peptide mimetics of antibodies. The complex of a ligand and receptor is termed a “ligand-receptor complex.”

[0045] As used herein, the term “linker” refers to a linkage between two elements, *e.g.*, protein domains. A linker can be a covalent bond or a peptide linker. The term “bond” refers to a chemical bond, *e.g.*, an amide bond or a disulfide bond, or any kind of bond created from a chemical reaction, *e.g.*, chemical conjugation. The term “peptide linker” refers to an amino acid or polypeptide that may be employed to link two protein domains to provide space and/or flexibility between the two protein domains.

[0046] Modulate: As used herein, the terms “modulate”, “modulation” and the like refer to the ability of a test agent to affect a response, either positive or negative or directly or indirectly, in a system, including a biological system or biochemical pathway.

[0047] Multimerization: As used herein, the term “multimerization” refers to two or more cell surface receptors, or domains or subunits thereof, being brought in close proximity to each other such that the receptors, or domains or subunits thereof, can interact with each other and cause intracellular signaling.

[0048] N-Terminus: As used herein in the context of the structure of a polypeptide, “N-terminus” (or “amino terminus”) and “C-terminus” (or “carboxyl terminus”) refer to the extreme amino and carboxyl ends of the polypeptide, respectively, while the terms “N-terminal” and “C-terminal” refer to relative positions in the amino acid sequence of the polypeptide toward the N-terminus and the C-terminus, respectively, and can include the residues at the N-terminus and C-terminus, respectively. The terms “immediately N-terminal” or “immediately C-terminal” are used to refer to a position of a first amino acid residue

relative to a second amino acid residue where the first and second amino acid residues are covalently bound to provide a contiguous amino acid sequence.

[0049] Nucleic Acid: The terms “nucleic acid”, “nucleic acid molecule”, “polynucleotide” and the like are used interchangeably herein to refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Non-limiting examples of polynucleotides include linear and circular nucleic acids, messenger RNA (mRNA), complementary DNA (cDNA), recombinant polynucleotides, vectors, probes, primers and the

[0050] Operably Linked: The term “operably linked” is used herein to refer to the relationship between nucleic acid sequences encoding differing functions when combined into a single nucleic acid sequence that, when introduced into a cell, provides a nucleic acid which is capable of effecting the transcription and/or translation of a particular nucleic acid sequence in a cell. For example, DNA for a signal sequence is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, “operably linked” means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, certain genetic elements such as enhancers need not be contiguous with respect to the sequence to which they provide their effect.

[0051] Partial Agonist: As used herein, the term “partial agonist” refers to a molecule that specifically binds that bind to and activate a given receptor but possess only partial activation the receptor relative to a full agonist. Partial agonists may display both agonistic and antagonistic effects. For example, when both a full agonist and partial agonist are present, the partial agonist acts as a competitive antagonist by competing with the full agonist for the receptor binding resulting in net decrease in receptor activation relative to the contact of the receptor with the full agonist in the absence of the partial agonist. Clinically, partial agonists can be used to activate receptors to give a desired submaximal response when inadequate amounts of the endogenous ligand are present, or they can reduce the overstimulation of receptors when excess amounts of the endogenous ligand are present. The maximum response (Emax) produced by a partial agonist is called its intrinsic activity and may be expressed on a percentage scale where a full agonist produced a 100% response. A In some embodiments,

the IL2R binding molecule has a reduced $E_{\max}$ compared to the $E_{\max}$ caused by IL2. $E_{\max}$ reflects the maximum response level in a cell type that can be obtained by a ligand (*e.g.*, a binding molecule described herein or the native cytokine (*e.g.*, IL2)). In some embodiments, the IL2R binding molecule described herein has at least 1% (*e.g.*, between 1% and 100%, between 10% and 100%, between 20% and 100%, between 30% and 100%, between 40% and 100%, between 50% and 100%, between 60% and 100%, between 70% and 100%, between 80% and 100%, between 90% and 100%, between 1% and 90%, between 1% and 80%, between 1% and 70%, between 1% and 60%, between 1% and 50%, between 1% and 40%, between 1% and 30%, between 1% and 20%, or between 1% and 10%) of the $E_{\max}$ caused by IL2. In other embodiments, the $E_{\max}$ of the IL2R binding molecule described herein is greater (*e.g.*, at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% greater) than the $E_{\max}$ of the natural ligand, IL2. In some embodiments, by varying the linker length of the IL2R binding molecule, the $E_{\max}$ of the IL2R binding molecule can be changed. The IL2R binding molecule can cause $E_{\max}$ in the most desired cell types, and a reduced $E_{\max}$ in other cell types.

[0052] Polypeptide: As used herein the terms “polypeptide,” “peptide,” and “protein”, used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include genetically coded and non-genetically coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified polypeptide backbones. The terms include fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence; fusion proteins with heterologous and homologous leader sequences; fusion proteins with or without N-terminus methionine residues; fusion proteins with immunologically tagged proteins; fusion proteins of immunologically active proteins (*e.g.* antigenic diphtheria or tetanus toxin fragments) and the like.

[0053] As used herein the terms “prevent”, “preventing”, “prevention” and the like refer to a course of action initiated with respect to a subject prior to the onset of a disease, disorder, condition or symptom thereof so as to prevent, suppress, inhibit or reduce, either temporarily or permanently, a subject’s risk of developing a disease, disorder, condition or the like (as determined by, for example, the absence of clinical symptoms) or delaying the onset thereof, generally in the context of a subject predisposed due to genetic, experiential or environmental factors to having a particular disease, disorder or condition. In certain instances, the terms “prevent”, “preventing”, “prevention” are also used to refer to the slowing of the progression of a disease, disorder or condition from a present its state to a more deleterious state.

[0054] Proximity: As used herein, the term “proximity” refers to the spatial proximity or physical distance between two cell surface receptors, or domains or subunits thereof, after a binding molecule described herein binds to the two cell surface receptors, or domains or subunits thereof. In some embodiments, after the binding molecule binds to the cell surface receptors, or domains or subunits thereof, the spatial proximity between the cell surface receptors, or domains or subunits thereof, can be, *e.g.*, less than about 500 angstroms, such as *e.g.*, a distance of about 5 angstroms to about 500 angstroms. In some embodiments, the spatial proximity amounts to less than about 5 angstroms, less than about 20 angstroms, less than about 50 angstroms, less than about 75 angstroms, less than about 100 angstroms, less than about 150 angstroms, less than about 250 angstroms, less than about 300 angstroms, less than about 350 angstroms, less than about 400 angstroms, less than about 450 angstroms, or less than about 500 angstroms. In some embodiments, the spatial proximity amounts to less than about 100 angstroms. In some embodiments, the spatial proximity amounts to less than about 50 angstroms. In some embodiments, the spatial proximity amounts to less than about 20 angstroms. In some embodiments, the spatial proximity amounts to less than about 10 angstroms. In some embodiments, the spatial proximity ranges from about 10 to 100 angstroms, from about 50 to 150 angstroms, from about 100 to 200 angstroms, from about 150 to 250 angstroms, from about 200 to 300 angstroms, from about 250 to 350 angstroms, from about 300 to 400 angstroms, from about 350 to 450 angstroms, or about 400 to 500 angstroms. In some embodiments, the spatial proximity amounts to less than about 250 angstroms, alternatively less than about 200 angstroms, alternatively less than about 150 angstroms, alternatively less than about 120 angstroms, alternatively less than about 100 angstroms, alternatively less than about 80 angstroms, alternatively less than about 70 angstroms, or alternatively less than about 50 angstroms.

[0055] Receptor: As used herein, the term “receptor” refers to a polypeptide having a domain that specifically binds a ligand that binding of the ligand results in a change to at least one biological property of the polypeptide. In some embodiments, the receptor is a “soluble” receptor that is not associated with a cell surface. In some embodiments, the receptor is a cell surface receptor that comprises an extracellular domain (ECD) and a membrane associated domain which serves to anchor the ECD to the cell surface. In some embodiments of cell surface receptors, the receptor is a membrane spanning polypeptide comprising an intracellular domain (ICD) and extracellular domain (ECD) linked by a membrane spanning domain typically referred to as a transmembrane domain (TM). The binding of the ligand to the

receptor results in a conformational change in the receptor resulting in a measurable biological effect. In some instances, where the receptor is a membrane spanning polypeptide comprising an ECD, TM and ICD, the binding of the ligand to the ECD results in a measurable intracellular biological effect mediated by one or more domains of the ICD in response to the binding of the ligand to the ECD. In some embodiments, a receptor is a component of a multi-component complex to facilitate intracellular signaling. For example, the ligand may bind a cell surface molecule having not associated with any intracellular signaling alone but upon ligand binding facilitates the formation of a multimeric complex that results in intracellular signaling.

[0056] Recombinant: As used herein, the term “recombinant” is used as an adjective to refer to the method by a polypeptide, nucleic acid, or cell that was modified using recombinant DNA technology. A recombinant protein is a protein produced using recombinant DNA technology and may be designated as such using the abbreviation of a lower case “r” (e.g., rhIL2) to denote the method by which the protein was produced. Similarly, a cell is referred to as a “recombinant cell” if the cell has been modified by the incorporation (e.g., transfection, transduction, infection) of exogenous nucleic acids (e.g., ssDNA, dsDNA, ssRNA, dsRNA, mRNA, viral or non-viral vectors, plasmids, cosmids and the like) using recombinant DNA technology. The techniques and protocols for recombinant DNA technology are well known in the art such as those can be found in Sambrook, *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y.) and other standard molecular biology laboratory manuals.

[0057] Response: The term “response,” for example, of a cell, tissue, organ, or organism, encompasses a quantitative or qualitative change in a evaluable biochemical or physiological parameter, (e.g., concentration, density, adhesion, proliferation, activation, phosphorylation, migration, enzymatic activity, level of gene expression, rate of gene expression, rate of energy consumption, level of or state of differentiation, where the change is correlated with activation, stimulation, or treatment, or with internal mechanisms such as genetic programming. In certain contexts, the terms “activation”, “stimulation”, and the like refer to cell activation as regulated by internal mechanisms, as well as by external or environmental factors. In contrast, the terms “inhibition”, “down-regulation” and the like refer to the opposite effects.

[0058] Single Domain Antibody (sdAb): The term “single-domain antibody” or “sdAbs,” refers to an antibody having a single (only one) monomeric variable antibody

domain. A sdAb is able to bind selectively to a specific antigen. A V_HH antibody, further defined below, is an example of a sdAb.

[0059] Specifically Binds: As used herein, the term “specifically bind” refers to the degree of selectivity or affinity for which one molecule binds to another. In the context of binding pairs (*e.g.*, a binding molecule described herein/receptor, a ligand/receptor, antibody/antigen, antibody/ligand, antibody/receptor binding pairs), a first molecule of a binding pair is said to specifically bind to a second molecule of a binding pair when the first molecule of the binding pair does not bind in a significant amount to other components present in the sample. A first molecule of a binding pair is said to specifically bind to a second molecule of a binding pair when the affinity of the first molecule for the second molecule is at least two-fold greater, alternatively at least five times greater, alternatively at least ten times greater, alternatively at least 20-times greater, or alternatively at least 100-times greater than the affinity of the first molecule for other components present in the sample.

[0060] Stably Associated: As used herein, the term “stably associated” or “in stable association with” are used to refer to the various means by which one molecule (*e.g.*, a polypeptide) may be associated with another molecule over an extended period of time. The stable association of one molecule to another may be effected by a variety of means, including covalent bonding and non-covalent interactions. In some embodiments, stable association of two molecules may be effected by covalent bonds such as peptide bonds. In other embodiments, stable association of two molecules may be effected by non-covalent interactions. Examples of non-covalent interactions which may provide a stable association between two molecules include electrostatic interactions (*e.g.*, hydrogen bonding, ionic bonding, halogen bonding, dipole-dipole interactions, Van der Waals forces and π -effects including cation- π interactions, anion- π interactions and π - π interactions) and hydrophobic/hydrophilic interactions. In some embodiments, the stable association of sdAbs of the binding molecules of the present disclosure may be effected by non-covalent interactions. In one embodiment, the non-covalent stable association of the sdAbs of the binding molecules may be achieved by conjugation of the sdAbs to “knob-into-hole” modified Fc monomers. An Fc “knob” monomer stably associates non-covalently with an Fc “hole” monomer. Conjugation of a first sdAb which specifically binds to the extracellular domain of a first subunit of a heterodimeric receptor to an “Fc knob” monomer and conjugation of a second sdAb which specifically binds to the extracellular domain of a second subunit of a heterodimeric receptor to an “Fc hole” monomer provides stable association of the first and second sdAbs.

[0061] Subject: The terms “recipient”, “individual”, “subject”, and “patient”, are used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. "Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, sheep, goats, pigs, etc. In some embodiments, the mammal is a human being.

[0062] Substantially: As used herein, the term “substantially” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher of a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In one embodiment, “substantially the same” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that produces an effect, e.g., a physiological effect, that is approximately the same as a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

[0063] Suffering From: As used herein, the term “suffering from” refers to a determination made by a physician with respect to a subject based on the available information accepted in the field for the identification of a disease, disorder or condition including but not limited to X-ray, CT-scans, conventional laboratory diagnostic tests (e.g., blood count), genomic data, protein expression data, immunohistochemistry, that the subject requires or will benefit from treatment. The term suffering from is typically used in conjunction with a particular disease state such as “suffering from a neoplastic disease” refers to a subject which has been diagnosed with the presence of a neoplasm.

[0064] Therapeutically Effective Amount: As used herein, the term The phrase “therapeutically effective amount” is used in reference to the administration of an agent to a subject, either alone or as part of a pharmaceutical composition or treatment regimen, in a single dose or as part of a series of doses in an amount capable of having any detectable, positive effect on any symptom, aspect, or characteristic of a disease, disorder or condition when administered to the subject. The therapeutically effective amount can be ascertained by measuring relevant physiological effects, and it may be adjusted in connection with a dosing regimen and in response to diagnostic analysis of the subject’s condition, and the like. The parameters for evaluation to determine a therapeutically effective amount of an agent are determined by the physician using art accepted diagnostic criteria including but not limited to

indicia such as age, weight, sex, general health, ECOG score, observable physiological parameters, blood levels, blood pressure, electrocardiogram, computerized tomography, X-ray, and the like. Alternatively, or in addition, other parameters commonly assessed in the clinical setting may be monitored to determine if a therapeutically effective amount of an agent has been administered to the subject such as body temperature, heart rate, normalization of blood chemistry, normalization of blood pressure, normalization of cholesterol levels, or any symptom, aspect, or characteristic of the disease, disorder or condition, modification of biomarker levels, increase in duration of survival, extended duration of progression free survival, extension of the time to progression, increased time to treatment failure, extended duration of event free survival, extension of time to next treatment, improvement objective response rate, improvement in the duration of response, and the like that are relied upon by clinicians in the field for the assessment of an improvement in the condition of the subject in response to administration of an agent.

[0065] Treat: The terms “treat”, “treating”, “treatment” and the like refer to a course of action (such as administering a binding molecule described herein, or a pharmaceutical composition comprising same) initiated with respect to a subject after a disease, disorder or condition, or a symptom thereof, has been diagnosed, observed, or the like in the subject so as to eliminate, reduce, suppress, mitigate, or ameliorate, either temporarily or permanently, at least one of the underlying causes of such disease, disorder, or condition afflicting a subject, or at least one of the symptoms associated with such disease, disorder, or condition. The treatment includes a course of action taken with respect to a subject suffering from a disease where the course of action results in the inhibition (*e.g.*, arrests the development of the disease, disorder or condition or ameliorates one or more symptoms associated therewith) of the disease in the subject.

[0066] V_HH: As used herein, the term “V_HH” is a type of sdAb that has a single monomeric heavy chain variable antibody domain. Such antibodies can be found in or produced from Camelid mammals (*e.g.*, camels, llamas) which are naturally devoid of light chains. V_HHs can be obtained from immunization of camelids (including camels, llamas, and alpacas (see, *e.g.*, Hamers-Casterman, *et al.* (1993) *Nature* 363:446-448) or by screening libraries (*e.g.*, phage libraries) constructed in V_HH frameworks. Antibodies having a given specificity may also be derived from non-mammalian sources such as V_HHs obtained from immunization of cartilaginous fishes including, but not limited to, sharks. In a particular embodiment, a V_HH in a bispecific V_HH² binding molecule described herein binds to a receptor

(e.g., the first receptor or the second receptor of the natural or non-natural receptor pairs) if the equilibrium dissociation constant between the V_HH and the receptor is greater than about 10⁶ M, alternatively greater than about 10⁸ M, alternatively greater than about 10¹⁰ M, alternatively greater than about 10¹¹ M, alternatively greater than about 10¹⁰ M, greater than about 10¹² M as determined by, e.g., Scatchard analysis (Munsen, et al. 1980 *Analyt. Biochem.* 107:220-239). Standardized protocols for the generation of single domain antibodies from camelids are well known in the scientific literature. See, e.g., Vincke, et al (2012) Chapter 8 in Methods in Molecular Biology, Walker, J. editor (Humana Press, Totowa NJ). Specific binding may be assessed using techniques known in the art including but not limited to competition ELISA, BIACORE® assays and/or KINEXA® assays. In some embodiments, a V_HH described herein can be humanized to contain human framework regions. Examples of human germlines that could be used to create humanized V_HHs include, but are not limited to, VH3-23 (e.g., UniProt ID: P01764), VH3-74 (e.g., UniProt ID: A0A0B4J1X5), VH3-66 (e.g., UniProt ID: A0A0C4DH42), VH3-30 (e.g., UniProt ID: P01768), VH3-11 (e.g., UniProt ID: P01762), and VH3-9 (e.g., UniProt ID: P01782).

[0067] V_HH²: As used herein, the term “V_HH²” and “bispecific V_HH²” and “VHH dimer” refers to are used interchangeably to refer to a subtype of the binding molecules of the present disclosure wherein the first and second sdAbs are both VHHs and first V_HH binding to a first receptor, or domain or subunit thereof, and a second V_HH binding to a second receptor, or domain or subunit thereof.

[0068] Wild Type: As used herein, the term “wild type” or “WT” or “native” is used to refer to an amino acid sequence or a nucleotide sequence that is found in nature and that has not been altered by the hand of man.

I. IL2R BINDING MOLECULES

II. HETERODIMERIZATION OF THE INTERMEDIATE AFFINITY INTERLEUKIN-2 RECEPTORS (IL-2R), IL-2R β AND IL-2R γ , INITIATES A SIGNALING CASCADE IN T AND NK CELLS THAT ULTIMATELY RESULTS IN PROLIFERATION AND PRODUCTION OF INTERFERON-GAMMA (IFN- γ). BINDING OF IL2 IN A DIMERIC COMPLEX WITH INTERMEDIATE AFFINITY OR IN THE HIGH AFFINITY TRIMERIC COMPLEX, INCLUDING IL-2R α , ON ACTIVATED T CELLS AND TREGS LEADS TO TRANS-PHOSPHORYLATION OF SIGNALING MOTIFS ON THE IL-2R β AND IL-2R γ INTRACELLULAR DOMAINS BY THE

ASSOCIATED JAK KINASES. THE FUNCTION OF THE IL2 IS TO BRING THE RECEPTOR CHAINS IN CLOSE PROXIMITY TO PRODUCE AN OPTIMAL LEVEL OF PHOSPHORYLATION AND ACTIVATION OF ASSOCIATED STAT TRANSCRIPTION FACTORS.

III. HERE WE ESTABLISHED A WAY TO BRING IL-2R β AND IL-2R γ CHAINS TOGETHER IN A CYTOKINE-INDEPENDENT MANNER THAT RESULTS IN FUNCTIONAL ACTIVATION. HUMAN IL-2R β AND IL-2R γ SPECIFIC HEAVY CHAIN SINGLE DOMAIN NANOBODIES (VHH) WERE GENERATED BY CAMEL IMMUNIZATION AND SCREENING OF VHH LIBRARIES PREPARED FROM PERIPHERAL BLOOD CELLS FOR BINDING. TEN IL-2R β VHH SEGMENTS AND SIX IL-2R γ VHH SEGMENTS, ALL WITH LOW NANOMOLAR AFFINITY, WERE IDENTIFIED AND COUPLED AS IL-2R β /IL-2R γ VHH DIMERS IN ALL POSSIBLE COMBINATIONS IN BOTH AMINO-CARBOXY AND CARBOXY-AMINO ORIENTATIONS YIELDING 120 DIFFERENT PROTEINS.

IV. FORTY-TWO OF THE IL-2R β /IL-2R γ SYNTHETIKINES FROM THE PANEL WERE FUNCTIONAL AND INDUCED PSTAT-5 PHOSPHORYLATION IN THE IL-2 DEPENDENT NK CELL LINE NKL.

V. THE BIOLOGICAL ACTIVITY OF THESE IL-2R β /IL-2R γ SYNTHETIKINES WAS FURTHER CONFIRMED ON PRIMARY CELLS. IL-2R β /IL-2R γ SYNTHETIKINES INDUCED PSTAT5 PHOSPHORYLATION ON NK CELLS ISOLATED FROM HUMAN PERIPHERAL BLOOD AND CULTURE WITH IL-2R β /IL-2R γ SYNTHETIKINES RESULTED IN PROLIFERATION AND PRODUCTION OF HIGH BUT VARIED LEVELS OF IFN- γ .

VI. THE ACTIVITY OF IL-2R β /IL-2R γ SYNTHETIKINES ON PRIMARY T CELLS WAS ALSO EXAMINED. SIMILAR TO NK CELLS, IL-2R β /IL-2R γ SYNTHETIKINES INDUCED PSTAT5 PHOSPHORYLATION, PROLIFERATION AND IFN- γ PRODUCTION BY CD4 POSITIVE AND CD8 POSITIVE T CELL BLASTS GENERATED AFTER CD3/CD28 ACTIVATION OF HUMAN PBMC. IN CONCLUSION, WE HAVE GENERATED A SERIES OF FUNCTIONAL IL-2R β /IL-2R γ SYNTHETIKINES EACH WITH UNIQUE SIGNALING STRENGTHS, WHICH WILL

NOW BE FURTHER ANALYZED FOR POTENTIAL THERAPEUTIC APPLICATION IN TUMOR IMMUNOLOGY AND AUTO-IMMUNITY.

VII.

[0069] The present disclosure provides binding molecules that are agonists of the IL2R receptor, the binding molecule comprising:

- a) a first single domain antibody (sdAb) that specifically binds to the extracellular domain of IL2Rb of the IL2R (an “IL2Rb sdAb”), and
- b) a second single domain antibody that specifically binds to extracellular domain IL2Rg of the IL2R (an “IL2Rg sdAb”),

wherein the IL2Rb sdAb and IL2Rg sdAb are stably associated and wherein contacting a cell expressing IL2Rb and IL2Rg with an effective amount of the binding molecule results in the dimerization of IL2Rb and IL2Rg, and results in intracellular signaling characteristic of the IL2R receptor when activated by its natural cognate IL2. In some embodiments, one or both of the sdAbs is a an scFv. In some embodiments, one or both of the sdAbs is a VHH.

[0070] As used herein, the term “IL2R receptor” or “IL2R” refers to the heterodimeric intermediate affinity receptor formed by subunits IL2Rb and IL2Rg, when associated with the cognate IL2.

[0071] The IL2 receptor (IL2R) includes CD25 subunit (CD25; also called IL2R α subunit or IL2Ra subunit), CD122 subunit (CD122; also called IL2R β subunit or IL2Rb subunit), and CD132 subunit (CD132; also called IL2R γ subunit or IL2Rg subunit). Provided herein is an IL2R binding protein that specifically binds to CD122 and CD132. In some embodiments, the IL2R binding protein binds to a mammalian cell expressing both CD122 and CD132. In some embodiments, the IL2R binding protein can be a bispecific VHH2 as described below. In other embodiments, the IL2R binding protein can include a first domain that is a VHH and a second domain which can be a fragment of IL2 or, for example, a scFv.

[0072] In some embodiments, the IL2R binding protein has a reduced E_{max} compared to the E_{max} caused by IL2. E_{max} reflects the maximum response level in a cell type that can be obtained by a ligand (*e.g.*, a binding protein described herein or the native cytokine (*e.g.*, IL2)). In some embodiments, the IL2R binding protein described herein has at least 1% (*e.g.*, between 1% and 100%, between 10% and 100%, between 20% and 100%, between 30% and 100%, between 40% and 100%, between 50% and 100%, between 60% and 100%, between 70% and

100%, between 80% and 100%, between 90% and 100%, between 1% and 90%, between 1% and 80%, between 1% and 70%, between 1% and 60%, between 1% and 50%, between 1% and 40%, between 1% and 30%, between 1% and 20%, or between 1% and 10%) of the $E_{\max}$ caused by IL2. In some embodiments, by varying the linker length of the IL2R binding protein, the $E_{\max}$ of the IL2R binding protein can be changed. The IL2R binding protein can cause $E_{\max}$ in the most desired cell types (e.g., CD8⁺ T cells), and a reduced $E_{\max}$ in other cell types (e.g., macrophages). In some embodiments, the $E_{\max}$ in macrophages caused by an IL2R binding protein described herein is between 1% and 100% (e.g., between 10% and 100%, between 20% and 100%, between 30% and 100%, between 40% and 100%, between 50% and 100%, between 60% and 100%, between 70% and 100%, between 80% and 100%, between 90% and 100%, between 1% and 90%, between 1% and 80%, between 1% and 70%, between 1% and 60%, between 1% and 50%, between 1% and 40%, between 1% and 30%, between 1% and 20%, or between 1% and 10%) of the $E_{\max}$ in T cells (e.g., CD8⁺ T cells) caused by the IL2R binding protein. In other embodiments, the $E_{\max}$ of the IL2R binding protein described herein is greater (e.g., at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% greater) than the $E_{\max}$ of the natural ligand, IL2.

[0073] The IL2R binding protein can be a bispecific VHH2 that has a first VHH binding to CD122 (an antiCD122 VHH antibody) and a second VHH binding to CD132 (an antiCD132 VHH antibody) and causes the dimerization of the two receptor subunits and downstream signaling when bound to a cell expressing CD122 and CD132, e.g., a T cell (e.g., a CD8⁺ T cell or a CD4⁺ T cell), a macrophage, and/or a Treg cell.

[0074] The IL2 receptor (IL2R) includes IL2Rb subunit (IL2Rb) and IL2Rg subunit (IL2Rg). Provided herein is an IL2R binding molecule that specifically binds to IL2Rb and IL2Rg. In some embodiments, the IL2R binding molecule binds to a mammalian cell expressing both IL2Rb and IL2Rg. In some embodiments, the IL2R binding molecule can be a bispecific VHH2 as described below.

Single Domain Antibody

The IL10R binding molecules of the present invention comprise two or more single domain antibodies. The term “single domain antibody” (sdAb) as used herein refers an antibody fragment consisting of a monomeric variable antibody domain that is able to bind specifically to an antigen and compete for binding with the parent antibody from which it is derived. The term “single domain antibody” includes scFv and VHH molecules. In some embodiments, one or both of the sdAbs of the cytokine receptor binding molecule is a an scFv. In some embodiments, one or both of the sdAbs is a VHH. In some embodiments, one or both of the sdAbs is a scFv.

[0001] The term single domain antibody includes engineered sdAbs including but not limited to chimeric sdAbs, CDR grafted sdAbs and humanized sdAbs. In some embodiments, the one or more of the sdAbs for incorporation into the IL10R binding molecules of the present disclosure are CDR grafted. CDRs obtained from antibodies, heavy chain antibodies, and sdAbs derived therefrom may be grafted onto alternative frameworks as described in Saerens, *et al.* (2005) J. Mol Biol 352:597-607 to generate CDR-grafted sdAbs. Any framework region can be used with the CDRs as described herein.

[0002] In some embodiments, one or more of the sdAbs for incorporation into the IL10R binding molecules is a chimeric sdAb, in which the CDRs are derived from one species (e.g., camel) and the framework and/or constant regions are derived from another species (e.g., human or mouse). In specific embodiments, the framework regions are human or humanized sequences. Thus, IL10R binding molecules comprising one or more humanized sdAbs are considered within the scope of the present disclosure.

[0003] In some embodiments, one or more of the sdAb of the cytokine receptor binding molecules of the present disclosure is a VHH. As used herein, the term “VHH” refers to a single domain antibody derived from camelid antibody typically obtained from immunization of camelids (including camels, llamas and alpacas (see, e.g., Hamers-Casterman, *et al.* (1993) Nature 363:446-448). VHHs are also referred to as heavy chain antibodies or Nanobodies® as Single domain antibodies may also be derived from non-mammalian sources such as VHHs obtained from IgNAR antibodies immunization of cartilaginous fishes including, but not limited to, sharks. A VHH is a type of single-domain antibody (sdAb) containing a single monomeric variable antibody domain. Like a full-length antibody, it is able to bind selectively to a specific antigen.

[0004] The complementary determining regions (CDRs) of VHHs are within a single-domain polypeptide. VHHs can be engineered from heavy-chain antibodies found in camelids.

An exemplary VHH has a molecular weight of approximately 12-15 kDa which is much smaller than traditional mammalian antibodies (150-160 kDa) composed of two heavy chains and two light chains. VHHs can be found in or produced from *Camelidae* mammals (e.g., camels, llamas, dromedary, alpaca, and guanaco) which are naturally devoid of light chains. Descriptions of sdAbs and VHHS can be found in, e.g., De Greve et al., *Curr Opin Biotechnol.* 61:96-101, 2019; Ciccarese, et al., *Front Genet.* 10:997, 2019; Chanier and Chames, *Antibodies (Basel)* 8(1), 2019; and De Vlieger et al., *Antibodies (Basel)* 8(1), 2018. The CDRs derived from camelid VHHs may be used to prepare CDR-grafted VHHs which may be incorporated in the IL10R binding molecules.

[0005] In some embodiments, the VHH for incorporation into the IL10R binding molecule of the present disclosure is a humanized VHH containing human framework regions. The techniques for humanization of camelid single domain antibodies are well known in the art. See, e.g., Vincke, et al. (2009) *General Strategy to Humanize a Camelid Single-domain Antibody and Identification of a Universal Humanized Nanobody Scaffold* J. Biol. Chem. 284(5)3273-3284. Human framework regions useful in the preparation of humanized VHHs include, but are not limited to, VH3-23 (e.g., UniProt ID: P01764), VH3-74 (e.g., UniProt ID: A0A0B4J1X5), VH3-66 (e.g., UniProt ID: A0A0C4DH42), VH3-30 (e.g., UniProt ID: P01768), VH3-11 (e.g., UniProt ID: P01762), and VH3-9 (e.g., UniProt ID: P01782).

Stably Associated:

[0006] The IL10R binding molecules of the present disclosure comprise a single domain antibody that selectively binds to the extracellular domain of IL10Ra (an "IL10Ra sdAb") in stable association with a single domain antibody that selectively binds to the extracellular domain of IL10Rb (an "IL10Rb sdAb"). As used herein, the term "stably associated" or "in stable association with" are used to refer to the various means by which one molecule (e.g., a polypeptide) may be thermodynamically and/or kinetically associated with another molecule. The stable association of one molecule to another may be achieved by a variety of means, including covalent bonding and non-covalent interactions.

[0007] In some embodiments, stable association of the IL10Ra sdAb and IL10Rb sdAb may be achieved by a covalent bond such as peptide bond. In some embodiments, the covalent linkage between the first and second binding domains is a covalent bond between the C-terminus of the first binding domain and the N-terminus of the second binding domain.

[0008] In some embodiments, the covalent linkage of the the IL10Ra sdAb and IL10Rb sdAb of the IL10R binding protein is effected by a coordinate covalent linkage. The present disclosure provides examples of single domain antibodies comprising a chelating peptide. The chelating peptide results in a coordinate covalent linkage to a transition metal ion. In some embodiments, a transition metal ion is capable of forming a coordinate covalent linkage with two or more chelating peptides. Consequently, the first and second binding domains may each comprise a chelating peptide and a stable association of the binding domains by each subunit forming a coordinate covalent complex with a transition metal ion. In some embodiments, the transition metal ion is selected from vanadium, manganese, iron, iridium, osmium, rhenium platinum, palladium, cobalt, chromium or ruthenium. A schematic illustration of this configuration is provided in Figure 4, Panel B of the attached drawings. It should be noted that in each of the configurations illustrated in Figure 4, Panels A and B, the N-terminal domain of the single domain antibody is presented to the environment enabling facilitating enhanced exposure of the CDRs of the sdAb to the target cytokine receptor ECD. The formation of the coordinate covalent linkage between the is favored when the transition metal ion is in a kinetically labile oxidation state, for example Co(II), Cr(II), or Ru(III). Following complexation, the oxidation state of the transition metal may be changed (oxidized or reduced) to a kinetically inert oxidation state, for example Co(III), Cr(III), or Ru(II), provide a kinetically inert coordinate covalent complex. The the formation of kinetically inert and kinetically labile coordinate covalent complexes between proteins comprising chelating peptides via a transition metal are described in more detail in Anderson, et al. United States Patent No. 5,439,928 issued August 8, 1995.

[0009] In some embodiments, the covalent linkage of the IL10Ra sdAb and IL10Rb sdAb of the IL10R binding molecule may further comprise a linker. Linkers are molecules selected from selected from the group including, but not limited to, peptide linkers and chemical linkers. In some embodiments, the linker a joins the C-terminus of the IL10Ra sdAb to the N-terminus of the IL10Rb sdAb. In some embodiments, the linker joins the C-terminus of the IL10Rb sdAb to the N-terminus of the IL10Ra sdAb.

Peptide Linkers

[0075] In some embodiments, the stable association of the first and second domains may be achieved by covalent linkage of the C-terminus of the first binding domain and the N-terminus of the second binding domain via a peptide linker. A peptide linker can include between 1 and 50 amino acids (e.g., between 2 and 50, between 5 and 50, between 10 and 50,

between 15 and 50, between 20 and 50, between 25 and 50, between 30 and 50, between 35 and 50, between 40 and 50, between 45 and 50, between 2 and 45, between 2 and 40, between 2 and 35, between 2 and 30, between 2 and 25, between 2 and 20, between 2 and 15, between 2 and 10, between 2 and 5 amino acids). Examples of flexible peptide linkers include glycine polymers (G)_n, glycine-alanine polymers, alanine-serine polymers, glycine-serine polymers (for example, (GmSo)_n, (GSGGS)_n, (GmSoGm)_n, (GmSoGmSoGm)_n, (GSGGS_m)_n, (GSGS_mG)_n and (GGGS_m)_n, and combinations thereof, where m, n, and o are each independently selected from an integer of at least 1 to 20, e.g., 1-18, 216, 3-14, 4-12, 5-10, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10), and other flexible linkers. Glycine and glycine-serine polymers are relatively unstructured, and therefore may serve as a neutral tether between components. Exemplary flexible linkers include the linkers of but are not limited to GGGG (SEQ ID NO: 11), GGGGS (SEQ ID NO: 12), GGSG (SEQ ID NO: 13), GSGGG (SEQ ID NO: 14), GSGSG (SEQ ID NO: 15), GSGGG (SEQ ID NO: 16), GGGSG (SEQ ID NO: 17) and GSSSG (SEQ ID NO: 18). In yet other embodiments, a peptide linker can contain 4 to 20 amino acids including mixtures of the above motifs of GGSG (SEQ ID NO: 13), e.g., GGSGGGSG (SEQ ID NO: 19), GGSGGGSGGGSG (SEQ ID NO: 20), GGSGGGSGGGSGGGSG (SEQ ID NO: 21), or GGSGGGSGGGSGGGSGGGSG (SEQ ID NO: 22). In other embodiments, a peptide linker can contain motifs of GGSG (SEQ ID NO: 13), e.g., GGSGGGSG (SEQ ID NO: 19), GGSGGGSGGGSG (SEQ ID NO: 20), GGSGGGSGGGSGGGSG (SEQ ID NO: 21), or GGSGGGSGGGSGGGSGGGSG (SEQ ID NO: 22).

[0076] In some embodiments, the covalent linkage of the first and second domains may be achieved by a chemical linker. Examples of chemical linkers include aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids, or combinations thereof.

[0077] In some embodiments, stable association the IL10Ra sdAb and IL10Rb sdAb of the IL10R binding protein is effected by non-covalent interaction. Examples of non-covalent interactions that provide a stable association between two molecules include electrostatic interactions (e.g., hydrogen bonding, ionic bonding, halogen bonding, dipole-dipole interactions, Van der Waals forces and p-effects including cation- π interactions, anion- π interactions and π - π interactions) and hydrophobic/hydrophilic interactions. In some embodiments, the stable association of sdAbs of the binding molecules of the present disclosure may be effected by non-covalent interactions.

[0078] In one embodiment, the non-covalent stable association of the IL10Ra sdAb and IL10Rb sdAb of the IL10R binding molecule may be achieved by conjugation a sdAb each monomer of a “knob-into-hole” engineered Fc dimer. The knob-into-hole modification refers to a modification at the interface between two immunoglobulin heavy chains in the CH3 domain, wherein: i) in a CH3 domain of a first heavy chain, an amino acid residue is replaced with an amino acid residue having a larger side chain (e.g., tyrosine or tryptophan) creating a projection from the surface (“knob”) and ii) in the CH3 domain of a second heavy chain, an amino acid residue is replaced with an amino acid residue having a smaller side chain (e.g., alanine or threonine), thereby generating a cavity (“hole”) within at interface in the second CH3 domain within which the protruding side chain of the first CH3 domain (“knob”) is received by the cavity in the second CH3 domain. The knob-into-hole modification is more fully described in Ridgway, et al. (1996) Protein Engineering 9(7):617-621 and U.S. Pat. No. 5,731,168, issued March 24, 1998, U.S. Pat. No. 7,642,228, issued Jan. 5, 2010, U.S. Pat. No. 7,695,936, issued Apr. 13, 2010, and US Patent No. 8,216,805, issued July 10, 2012. In one embodiment, the “knob-into-hole modification” comprises the amino acid substitution T366W and optionally the amino acid substitution S354C in one of the antibody heavy chains, and the amino acid substitutions T366S, L368A, Y407V and optionally Y349C in the other one of the antibody heavy chains. Furthermore, the Fc domains may be modified by the introduction of cysteine residues at positions S354 and Y349 which results in a stabilizing disulfide bridge between the two antibody heavy chains in the Fc region (Carter, et al. (2001) Immunol Methods 248, 7-15). The knob-into-hole format is used to facilitate the expression of a first polypeptide (e.g., an IL10Rb binding sdAb) on a first Fc monomer with a “knob” modification and a second polypeptide on the second Fc monomer possessing a “hole” modification to facilitate the expression of heterodimeric polypeptide conjugates. The knob-into-hole format is used to facilitate the expression of a first polypeptide on a first Fc monomer with a “knob” modification and a second polypeptide on the second Fc monomer possessing a “hole” modification to facilitate the expression of heterodimeric polypeptide conjugates. One embodiment of an IL10R binding molecule wherein the IL10Ra sdAb and IL10Rb sdAb are in stable, non-covalent association is wherein the each sdAb of the IL10R binding molecule covalently bonded, optionally including a linker, to each subunit of the knob-into-hole Fc dimer as illustrated in Figure 4, Panel A of the attached drawings.

Generation and Evaluation of IL2Rb Single Domain Antibodies

[00010] To generate sdAbs against the hIL2Ra, the extracellular domain of the hIL2Ra protein may be used as an immunogen. The extracellular domain of the mature (lacking the signal sequence) hIL2Ra possesses the amino acid sequence (amino acids 22-235 of SEQ ID NO:452) has the amino acid sequence

HGTELPSPPSVWFEEFFHHILHWTPIPNQSESTCYEVALLRYGIESWNSISNCSQTLSDLT
AVTLDLYHSNGYRARRVRAVDGSRHSNWTNTNTRFSVDEVTLTVGSVNLEIHNGFILGKIQLPRPKMA
PANDTYESIFSHFREYEIAIRKVPGNFTFTHKKVKHENFSLTSGEVGEFCVQVKPSVASRSNKGW
SKEECISLTRQYFTVTN (SEQ ID NO:453)

In some embodiments, when employed as an immunogen or a immunogenic composition, the hIL2Ra ECD may be provided as a domain of a fusion protein with an immunomodulatory protein.

[0001] To generate sdAbs against the mL10Ra, the extracellular domain of the mL10Ra protein may be used as an immunogen. The extracellular domain of the extracellular domain of mL10Ra possesses the amino acid sequence (amino acids 17-241 of SEQ ID NO:454) has the amino acid sequence

LEFIAYGTELPSPSYVWFEEFFQHILHWKPIPNQSESTYYEVALKQYGNSTWNDIHICRKAQ
ALSCDLTTFTLDLYHRSYGYRARRVRAVDNSQYSNWTNTTETRTVDEVILTVDSTLKAMDGIIYGTIHP
PRPTITPAGDEYEQVFKDLRVYKISIRKFSELKNATKRVKQETFTLTVPIGVRKFCVKVLPRLSRINKA
EWSEEQCLLITTEQYFTVTNLSI

(SEQ ID NO:455)

In some embodiments, when employed as an immunogen or a immunogenic composition, the mL2Ra ECD may be provided as a domain of a fusion protein with an immunomodulatory protein.

[0001] A series of hIL10Ra sdAbs were generated in substantial accordance with the teaching of Examples 1-4 herein. Briefly, a camel was sequentially immunized with the ECD of the human IL10Ra over a period several weeks of by the subcutaneous an adjuvanted composition containing a recombinantly produced fusion proteins comprising the extracellular domain of hIL10Ra, the human IgG1 hinge domain and the human IgG1 heavy chain Fc. Following immunization, RNAs extracted from a blood sample of appropriate size VHH-hinge-CH2-CH3 species were transcribed to generate DNA sequences, digested to identify the approximately 400bp fragment comprising the nucleic acid sequence encoding the VHH domain was isolated. The isolated sequence was digested with restriction endonucleases to facilitate insertion into a phagemid vector for in frame with a sequence encoding a his-tag and transformed into E. coli to generate a phage library. Multiple rounds of bio-panning of the phage library were conducted to identify VHHs that bound to the ECD of IL10Ra (human or

mouse as appropriate). Individual phage clones were isolated for periplasmic extract ELISA (PE-ELISA) in a 96-well plate format and selective binding confirmed by colorimetric determination. The IL10Ra binding molecules that demonstrated specific binding to the IL10Ra antigen were isolated and sequenced and sequences analyzed to identify VHH sequences, CDRs and identify unique VHH clonotypes. As used herein, the term “clonotypes” refers a collection of binding molecules that originate from the same B-cell progenitor cell, in particular collection of antigen binding molecules that belong to the same germline family, have the same CDR3 lengths, and have 70% or greater homology in CDR3 sequence.

[0002] The amino acid sequences of VHH molecules demonstrating specific binding to the hIL10Ra ECD antigen (hIL10Ra VHHs) are provided in Table 5 and the CDRs isolated from such VHHs are provided in Table 2. Nucleic acid sequences encoding the VHHs of Table 5 are provided in Table 8.

[0003] To confirm and evaluate binding affinities of the binding of the IL10Ra sdAbs, a representative example from each clonotype generated was selected for evaluation of binding via SPR. Evaluation of binding affinity of the hIL10Ra VHHs for ECD corresponding to SEQ ID NOS 159, 161, 162, 163, 165, 167 and 170 was conducted using surface plasmon resonance (SPR) in substantial accordance with the teaching of Example 5. Buffer-subtracted sensograms were processed with Biacore T200 Evaluation Software and globally fit with a 1:1 Langmuir binding model (bulk shift set to zero) to extract kinetics and affinity constants (k_a , k_d , K_D). $R_{MAX} < 100$ RU indicates surface density compatible with kinetics analysis. Calculated R_{max} values were generated using the equation: $R_{max} = \text{Load (RU)} \times \text{valency of ligand} \times (\text{Molecular weight of analyte} / \text{Molecular weight of ligand})$. Surface activity was defined as the ratio of experimental/calculated R_{max} . The results of these binding affinity experiments are provided in Table 22 below. The data provided in Table 22 demonstrates that the IL10Ra single domain antibodies generated possessed specific binding to the ECD of hIL10Ra.

[0001] Tables 2 and 3 provides CDRs useful in the preparation of IL2Rb sdAbs for incorporation into the binding molecules of the present disclosure. In some embodiments, the IL2Rb sdAb is a single domain antibody comprising one or more anti-human IL2Rb CDRs in a row of Table 2, wherein each CDR independently comprises 0, 1, 2, or 3 amino acid changes relative to the sequence of Table 2. In some embodiments, the IL2Rb sdAb is a single domain antibody comprising one or more anti-murine IL2Rb CDRs in a row of Table 3, wherein each CDR independently comprises 0, 1, 2, or 3 amino acid changes relative to the sequence of Table 3.

[0079] In some embodiments, the IL2Rb sdAb comprises a sequence having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to a sequence of any one the of IL2Rb sdAbs provided in a row of Table 6. In certain embodiments, the binding molecule comprises a sequence that is substantially identical to a sequence of any one of listed in a row of Table 6.

[0080] In some embodiments, the IL2Rb sdAb comprises a sequence having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to a sequence of any one the of anti-murine IL2Rb sdAbs provided in a row of Table 7. In certain embodiments, the binding molecule comprises a sequence that is substantially identical to a sequence of any one of listed in a row of Table 7.

[0081] In another aspect, the disclosure provides an isolated nucleic acid encoding an IL2Rb sdAb described herein. Table 10 and Table 11 provide DNA sequences encoding the IL2Rb sdAbs of Table 6 and Table 7, respectively. In certain embodiments, the present disclosure provides an isolated nucleic acid comprising a sequence having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to a DNA sequence listed in a row Table 10 or Table 11.

Exemplary Anti IL2Rg Single Domain Antibodies

[0002] Table 4 and Table 5 provide CDRs useful in the preparation of IL2Rg sdAbs. In some embodiments, the anti- IL2Rg sdAb is a single domain antibody comprising one or more anti-human IL2Rg CDRs in a row of Table 4, wherein each CDR independently comprises 0, 1, 2, or 3 amino acid changes relative to the sequence of Table 4. In some embodiments, the IL2Rb sdAb is a single domain antibody comprising one or more anti-murine IL2Rg CDRs in a row of Table 5, wherein each CDR independently comprises 0, 1, 2, or 3 amino acid changes relative to the sequence of Table 5.

[0082] In some embodiments, the anti- IL2Rg sdAb comprises a sequence having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to a sequence of any one the of anti- IL2Rg sdAbs provided in a row of Table 8 or Table 9. In certain embodiments, the binding molecule comprises a sequence that is substantially identical to a sequence of any one of listed in a row of Table 8 or Table 9.

[0083] In another aspect, the disclosure provides an isolated nucleic acid encoding IL2Rg sdAb described herein. Table 12 and Table 13 provides DNA sequences encoding the anti- IL2Rg sdAbs of Table 8 or Table 9, respectively. In certain embodiments, the present

disclosure provides an isolated nucleic acid comprising a sequence having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to a DNA sequence listed in a row Table 12 or Table 13.

Anti IL2R VHH Dimer Bispecific Binding Molecules

A. “Forward Orientation”

[0084] In some embodiments, the IL2R binding molecule of the present disclosure comprises a polypeptide of the structure:



wherein L is a polypeptide linker of 1-50 amino acids and $x = 0$ or 1, and TAG is a chelating peptide or a subunit of an Fc domain and $y = 0$ or 1.

[0085] In some embodiments, a IL2R binding molecule of the foregoing structure comprises a polypeptide from amino to carboxy terminus:

- (a) an IL2Rb sdAb comprising:
 - a CDR1 having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity, or having 0, 1, 2, or 3 amino acid changes, optionally conservative amino acid changes relative, to the sequence of the sequence of any CDR1 in a row of Table 2 or Table 3.
 - a CDR2 having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity, or having 0, 1, 2, or 3 amino acid changes, optionally conservative amino acid changes relative, to the sequence of the sequence of any CDR2 in a row of Table 2 or Table 3; and
 - a CDR3 having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity, or having 0, 1, 2, or 3 amino acid changes, optionally conservative amino acid changes relative, to the sequence of the sequence of any CDR3 in a row of Table 2 or Table 3;
- (b) polypeptide linker from 1 – 50 amino acids, alternatively 1-40 amino acids, alternatively 1-30 amino acids, alternatively 1-20 amino acids, alternatively 1-15 amino acids, alternatively 1-10 amino acids, alternatively 1-8 amino acids, alternatively 1-6 amino acids, alternatively 1-4 amino acids; and
- (c) an IL2Rg sdAb comprising:

- a CDR1 having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity, or having 0, 1, 2, or 3 amino acid changes, optionally conservative amino acid changes relative, to the sequence of the sequence of any CDR1 in a row of Table 4 or Table 5;
- a CDR2 having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity, or having 0, 1, 2, or 3 amino acid changes, optionally conservative amino acid changes relative, to the sequence of the sequence of any CDR2 in a row of Table 4 or Table 5; and
- a CDR3 having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity, or having 0, 1, 2, or 3 amino acid changes, optionally conservative amino acid changes relative, to the sequence of the sequence of any CDR3 in a row of Table 4 or Table 5.

[0086] In some embodiments, the IL2R binding molecule comprises an IL2Rb sdAb comprising a CDR1, a CDR2, and a CDR3 listed in a row of Table 2 or Table 3, and an IL2Rg sdAb comprising a CDR1, a CDR2, and a CDR3 as listed in a row of Table 4 or Table 5.

[0087] In some embodiments, the IL2Rb sdAb of the IL2R binding molecule comprises a sequence having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to a sequence of any one the of IL2Rb sdAbs provided in Table 6 or Table 7. In some embodiments, the IL2Rg sdAb the IL2R binding molecule comprises a sequence having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to a sequence of any one the of IL2Rg sdAbs provided in Table 8 or Table 9.

[0088] *B* “Reverse Orientation”

[0089] In some embodiments, the IL2R binding molecule comprises a polypeptide of the structure:



wherein L is a polypeptide linker of 1-50 amino acids and $x = 0$ or 1, and TAG is a chelating peptide or a subunit of an Fe domain and $y = 0$ or 1.

[0090] In some embodiments, a IL2R binding molecule of the foregoing structure comprises a polypeptide from amino to carboxy terminus:

- (a) an IL2Rg sdAb comprising:
 - a CDR1 having at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity, or having 0, 1, 2, or 3 amino acid changes, optionally conservative amino acid changes relative, to the sequence of the sequence of any CDR1 in a row of Table 4 or Table 5;
 - a CDR2 having at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity, or having 0, 1, 2, or 3 amino acid changes, optionally conservative amino acid changes relative, to the sequence of the sequence of any CDR2 in a row of Table 4 or Table 5; and
 - a CDR3 having at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity, or having 0, 1, 2, or 3 amino acid changes, optionally conservative amino acid changes relative, to the sequence of the sequence of any CDR3 in a row of Table 4 or Table 5.
 - ;
- (b) polypeptide linker from 1 – 50 amino acids, alterantively 1-40 amino acids, alternatively 1-30 amino acids, alterantively 1-20 amino acids, alterantively 1-15 amino acids, alternatively 1-10 amino acids, alterantively 1-8 amino acids, alterantively 1-6 amino acids, alternatively 1-4 amino acids; and
- (c) an IL2Rb sdAb comprising:
 - a CDR1 having at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity, or having 0, 1, 2, or 3 amino acid changes, optionally conservative amino acid changes relative, to the sequence of the sequence of any CDR1 in a row of Table 2 or Table 3.
 - a CDR2 having at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity, or having 0, 1, 2, or 3 amino acid changes, optionally conservative amino acid changes relative, to the sequence of the sequence of any CDR2 in a row of Table 2 or Table 3; and
 - a CDR3 having at least 90% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity, or having 0, 1, 2, or 3 amino acid changes, optionally conservative amino acid changes relative, to the sequence of the sequence of any CDR3 in a row of Table 2 or Table 3.

[0091] In some embodiments, the binding molecule comprises an IL2Rg sdAb comprising a CDR1, a CDR2, and a CDR3 as listed in a row of Table 4 or Table 5, and the IL2Rb sdAb and a CDR1, a CDR2, and a CDR3 as listed in a row of Table 2 or Table 3.

[0092] In some embodiments, the IL2Rg sdAb comprises a sequence having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to a sequence listed in a row of Table 8 or Table 9. In some embodiments, the IL2Rb sdAb comprises a sequence having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to a sequence listed in a row of Table 6 or Table 7.

Linkers

[0093] A linker can be used to join the IL2Rg sdAb and the IL2Rb sdAb antibody. A linker is a linkage between two linker is a linkage between the two sdAbs in the binding molecule, e.g., protein domains. For example, a linker can simply be a covalent bond or a peptide linker. In some embodiments, the sdAbs in a binding molecule are joined directly (i.e., via a covalent bond). In a bispecific VHH2 binding molecule described herein, a linker is a linkage between the two VHs in the binding molecule. A In some embodiments, the linker is a peptide linker. A peptide linker can include between 1 and 50 amino acids (e.g., between 2 and 50, between 5 and 50, between 10 and 50, between 15 and 50, between 20 and 50, between 25 and 50, between 30 and 50, between 35 and 50, between 40 and 50, between 45 and 50, between 2 and 45, between 2 and 40, between 2 and 35, between 2 and 30, between 2 and 25, between 2 and 20, between 2 and 15, between 2 and 10, between 2 and 5 amino acids).

[0094] Examples of flexible linkers include glycine polymers (G)_n, glycine-alanine polymers, alanine-serine polymers, glycine-serine polymers (for example, (GmSo)_n, (GSGGS)_n, (GmSoGm)_n, (GmSoGmSoGm)_n, (GSGGS_m)_n, (GSGSmG)_n and (GGGS_m)_n, and combinations thereof, where m, n, and o are each independently selected from an integer of at least 1 to 20, e.g., 1-18, 216, 3-14, 4-12, 5-10, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10), and other flexible linkers. Glycine and glycine-serine polymers are relatively unstructured, and therefore may serve as a neutral tether between components Exemplary flexible linkers include, but are not limited to GGGG (SEQ ID NO: 11), GGGGS (SEQ ID NO: 12), GGSG (SEQ ID NO: 13), GGSGG (SEQ ID NO: 14), GSGSG (SEQ ID NO: 15), GSGGG (SEQ ID NO: 16), GGGSG (SEQ ID NO: 17) and GSSSG (SEQ ID NO: 18). In yet other embodiments, a peptide linker can contain 4 to 20 amino acids including mixtures of the above motifs of GGSG (SEQ ID

NO:19), e.g., GGSGGGSG (SEQ ID NO:20), GGSGGGSGGGSG (SEQ ID NO:21), GGSGGGSGGGSGGGSG (SEQ ID NO:22), or GGSGGGSGGGSGGGSGGGSG (SEQ ID NO:23). In other embodiments, a peptide linker can contain motifs of GGSG (SEQ ID NO:19), e.g., GGSGGGSG (SEQ ID NO:20), GGSGGGSGGGSG (SEQ ID NO:21), GGSGGGSGGGSGGGSG (SEQ ID NO:22), or GGSGGGSGGGSGGGSGGGSG (SEQ ID NO:23).

[0095] A linker can also be a chemical linker, such as a synthetic polymer, e.g., a polyethylene glycol (PEG) polymer.

[0096] The length of the linker between two sdAb in a binding molecule can be used to modulate the proximity of the two sdAb of the binding molecule. By varying the length of the linker, the overall size and length of the binding molecule can be tailored to bind to specific cell receptors or domains or subunits thereof. For example, if the binding molecule is designed to bind to two receptors or domains or subunits thereof that are located close to each other on the same cell, then a short linker can be used. In another example, if the binding molecule is designed to bind to two receptors or domains or subunits thereof that are located on two different cells, then a long linker can be used.

[0097] In some embodiments, a linker joins the C-terminus of the IL2Rb sdAb in the binding molecule to the N-terminus of the IL2Rg sdAb in the binding molecule. In other embodiments, a linker joins the C-terminus of the IL2Rg sdAb in the binding molecule to the N-terminus of the IL2Rb sdAb in the binding molecule.

Modulation of sdAb Binding Affinity:

[0098] In some embodiments, the activity and/or specificity of the IL2R binding molecule of the present disclosure may be modulated by the respective binding affinities of the sdAbs for their respective receptor subunits.

[0099] It will be appreciated by one of skill in the art that the binding of the first sdAb of the IL2R binding molecule to the first receptor subunit ECD on the cell surface will enhance the probability of a binding interaction between the second sdAb of the IL2R binding molecule with the ECD of the second receptor subunit. This cooperative binding effect may result in a IL2R binding molecule which has a very high affinity for the receptor

and a very slow “off rate” from the receptor. Typical VHH single domain antibodies have an affinity for their targets of from about 10^{-5} M to about 10^{-10} M. In those instances such slow off-rate kinetics are desirable in the IL2R binding molecule, the selection of sdAbs having high affinities (about 10^{-7} M to about 10^{-10} M) for incorporation into the IL2R binding molecule are favored.

[0100] Naturally occurring cytokine ligands for typically do not exhibit a similar affinity for each subunit of a heterodimeric receptor. Consequently, in designing a IL2R binding molecule which is a mimetic of the cognate cytokine IL2 as contemplated by some embodiments of the present disclosure, selection of sdAbs for the first and second IL2R receptor subunit have an affinity similar to (e.g., having an affinity about 10 fold, alternatively about 20 fold, or alternatively about 50 fold higher or lower than) the cognate IL2 for the respective receptor subunit may be used.

[0101] In some embodiments, the IL2R binding molecules of the present disclosure are partial agonists of the IL2R receptor. As such, the activity of the binding molecule may be modulated by selecting sdAb which have greater or lesser affinity for either one or both of the IL2R receptor subunits. As some heterodimeric cytokine receptors are comprised of a “proprietary subunit” (i.e., a subunit which is not naturally a subunit of another multimeric receptor) and a second “common” subunit (such as CD132) which is a shared component of multiple cytokine receptors), selectivity for the formation of such receptor may be enhanced by employing first sdAb which has a higher affinity for the proprietary receptor subunit and second sdAB which exhibits a lower affinity for the common receptor subunit. Additionally, the common receptor subunit may be expressed on a wider variety of cell types than the proprietary receptor subunit. In some embodiments wherein the receptor is a heterodimeric receptor comprising a proprietary subunit and a common subunit, the first sdAb of the IL2R binding molecule exhibits a significantly greater (more than 10 times greater, alternatively more than 100 times greater, alternatively more than 1000 times greater) affinity for the proprietary receptor than the second sdAb of the IL2R binding molecule for the common receptor subunit. In one embodiment, the present disclosure provides a IL2R binding molecule wherein the affinity of the IL2Rb sdAb of has an affinity of more than 10 times greater, alternatively more than 100 times greater, alternatively more than 1000 times greater) affinity IL2Rg sdAb common receptor subunit.

Illustrative IL2R Binding Molecules

[0102] A series of illustrative IL2R binding molecules of the present disclosure were prepared in accordance with the teaching of the Examples. Briefly, camel with a fragments of the extracellular domains of IL2Rb and IL2Rg of the IL2R receptor and single domain antibody sequences isolated in accordance with the teaching of the Examples. Nucleic acid sequences were isolated from the antibody producing cells of the camels and these were used for the construction of nucleic acid sequences optimized for the expression control system were generated. In particular, modification of nucleic acid sequences to facilitate insertion into the expression vector were performed, for example avoid undesired restriction sites and codon optimized for the host cell line in accordance with procedures well known in the art.

Binding Experiments

[0103] All experiments were conducted in 10 mM Hepes, 150 mM NaCl, 0.05% (v/v) Polysorbate 20 (PS20) and 3 mM EDTA (HBS-EP+ buffer) on a Biacore T200 instrument equipped with a Protein A chip (Cytiva). Mono-Fc VHH ligands were flowed at 5 μ L/min for variable time ranging from 18 to 300 seconds, reaching the capture loads listed in the tables below.

[0104] Following ligand capture, injections of a 2-fold dilution series of his-tagged cytokine receptors typically comprising at least five concentrations between 1 μ M and 1 nM were performed in either high performance or single cycle kinetics mode. Surface regeneration was achieved by flowing 10 mM glycine-HCl, pH 1.5 (60 seconds, 50 μ L/min). Buffer-subtracted sensograms were processed with Biacore T200 Evaluation Software and globally fit with a 1:1 Langmuir binding model (bulk shift set to zero) to extract kinetics and affinity constants (k_a , k_d , K_D). $R_{MAX} < 100$ RU indicates surface density compatible with kinetics analysis.

[0105] Calculated R_{max} were generated using the equation $R_{max} = \text{Load (RU)} \times \text{valency of ligand} \times (\text{Molecular weight of analyte} / \text{Molecular weight of ligand})$. Surface activity was defined as the ratio experimental/calculated R_{max} . See tables 16 and 17 below for sample information and experimental results.

III. MODIFICATIONS TO EXTEND DURATION OF ACTION *IN VIVO*

[0106] The IL2R binding molecule described herein can be modified to provide for an extended lifetime in vivo and/or extended duration of action in a subject. In some embodiments, the binding molecule can be conjugated to carrier molecules to provide desired pharmacological properties such as an extended half-life. In some embodiments, the binding molecule can be covalently linked to the Fc domain of IgG, albumin, or other molecules to extend its half-life, e.g., by pegylation, glycosylation, and the like as known in the art. In some embodiments, the IL2R binding molecule modified to provide an extended duration of action in a mammalian subject has a half-life in a mammalian of greater than 4 hours, alternatively greater than 5 hours, alternatively greater than 6 hours, alternatively greater than 7 hours, alternatively greater than 8 hours, alternatively greater than 9 hours, alternatively greater than 10 hours, alternatively greater than 12 hours, alternatively greater than 18 hours, alternatively greater than 24 hours, alternatively greater than 2 days, alternatively greater than 3 days, alternatively greater than 4 days, alternatively greater than 5 days, alternatively greater than 6 days, alternatively greater than 7 days, alternatively greater than 10 days, alternatively greater than 14 days, alternatively greater than 21 days, or alternatively greater than 30 days.

[0107] Modifications of the IL2R binding molecule to provide an extended duration of action in a mammalian subject include (but are not limited to);

- conjugation of the IL2R binding molecule to one or more carrier molecules,
- conjugation IL2R binding molecule to protein carriers molecules, optionally in the form of a fusion protein with additional polypeptide sequences (e.g, IL2R binding molecule-Fc fusions) and
- conjugation to polymers, (e.g. water soluble polymers to provide a PEGylated IL2R binding molecule).

[0108] It should be noted that the more than one type of modification that provides for an extended duration of action in a mammalian subject may be employed with respect to a given IL2R binding molecule. For example, IL2R binding molecule of the present disclosure may comprise both amino acid substitutions that provide for an extended duration of action as well as conjugation to a carrier molecule such as a polyethylene glycol (PEG) molecule.

Protein Carrier Molecules:

[0109] Examples of protein carrier molecules which may be covalently attached to the IL2R binding molecule to provide an extended duration of action in vivo include, but are not limited to albumins, antibodies and antibody fragments such and Fc domains of IgG molecules

Fc Fusions:

[0110] In some embodiments, the IL2R binding molecule is conjugated to a functional domain of an Fc-fusion chimeric polypeptide molecule. Fc fusion conjugates have been shown to increase the systemic half-life of biopharmaceuticals, and thus the biopharmaceutical product can require less frequent administration. Fc binds to the neonatal Fc receptor (FcRn) in endothelial cells that line the blood vessels, and, upon binding, the Fc fusion molecule is protected from degradation and re-released into the circulation, keeping the molecule in circulation longer. This Fc binding is believed to be the mechanism by which endogenous IgG retains its long plasma half-life. More recent Fc-fusion technology links a single copy of a biopharmaceutical to the Fc region of an antibody to optimize the pharmacokinetic and pharmacodynamic properties of the biopharmaceutical as compared to traditional Fc-fusion conjugates. The "Fc region" useful in the preparation of Fc fusions can be a naturally occurring or synthetic polypeptide that is homologous to an IgG C-terminal domain produced by digestion of IgG with papain. IgG Fc has a molecular weight of approximately 50 kDa. The binding molecule described herein can be conjugated to the entire Fc region, or a smaller portion that retains the ability to extend the circulating half- life of a chimeric polypeptide of which it is a part. In addition, full-length or fragmented Fc regions can be variants of the wild-type molecule. In a typical presentation, each monomer of the dimeric Fc can carry a heterologous polypeptide, the heterologous polypeptides being the same or different.

[0111] Illustrative examples of Fc formats useful for IL2R binding molecules of the present disclosure are provided schematically in Figures 1-Figure 4B of the attached drawings.

Linkage of Binding Molecule to Fc

[0112] As indicated, the linkage of the IL2R binding molecule to the Fc subunit may incorporate a linker molecule as described below between the sdAb and Fc subunit. In some

embodiments, the IL2R binding molecule is expressed as a fusion protein with the Fc domain incorporating an amino acid sequence of a hinge region of an IgG antibody. The Fc domains engineered in accordance with the foregoing may be derived from IgG1, IgG2, IgG3 and IgG4 mammalian IgG species. In some embodiments, the Fc domains may be derived from human IgG1, IgG2, IgG3 and IgG4 IgG species. In some embodiments, the hinge region is the hinge region of an IgG1. In one particular embodiment, the IL2R binding is linked to an Fc domain using an human IgG1 hinge domain.

Knob-Into-Hole Fc Format

[0113] In some embodiments, when the IL2R binding molecule described herein is to be administered in the format of an Fc fusion, particularly in those situations when the polypeptide chains conjugated to each subunit of the Fc dimer are different, the Fc fusion may be engineered to possess a “knob-into-hole modification.” The knob-into-hole modification is more fully described in Ridgway, et al. (1996) Protein Engineering 9(7):617-621 and United States Patent No. 5,731,168, issued March 24, 1998. The knob-into-hole modification refers to a modification at the interface between two immunoglobulin heavy chains in the CH3 domain, wherein: i) in a CH3 domain of a first heavy chain, an amino acid residue is replaced with an amino acid residue having a larger side chain (e.g., tyrosine or tryptophan) creating a projection from the surface (“knob”), and ii) in the CH3 domain of a second heavy chain, an amino acid residue is replaced with an amino acid residue having a smaller side chain (e.g., alanine or threonine), thereby generating a cavity (“hole”) at interface in the second CH3 domain within which the protruding side chain of the first CH3 domain (“knob”) is received by the cavity in the second CH3 domain. In one embodiment, the “knob-into-hole modification” comprises the amino acid substitution T366W and optionally the amino acid substitution S354C in one of the antibody heavy chains, and the amino acid substitutions T366S, L368A, Y407V and optionally Y349C in the other one of the antibody heavy chains. Furthermore, the Fc domains may be modified by the introduction of cysteine residues at positions S354 and Y349 which results in a stabilizing disulfide bridge between the two antibody heavy chains in the Fc region (Carter, et al. (2001) Immunol Methods 248, 7-15).

[0114] The knob-into-hole format is used to facilitate the expression of a first polypeptide on a first Fc monomer with a “knob” modification and a second polypeptide on the second Fc monomer possessing a “hole” modification to facilitate the expression of

heterodimeric polypeptide conjugates. In some embodiments, the IL2R binding molecule covalently linked to a single subunit of the Fc as illustrated in Figure 4A, a IL2R binding molecule is provided on each of the subunits of the Fc as illustrated in Figure 4B.

Albumin Carrier Molecules

[0115] In some embodiments, the IL2R binding molecule conjugated to an is albumin molecule (e.g., human serum albumin) which is known in the art to facilitate extended exposure in vivo. In one embodiment of the invention, the IL2R binding molecule is conjugated to albumin via chemical linkage or expressed as a fusion protein with an albumin molecule referred to herein as an IL2R binding molecule albumin fusion.” The term “albumin” as used in the context of hIL2 mutein albumin fusions include albumins such as human serum albumin (HSA), cyno serum albumin, and bovine serum albumin (BSA). In some embodiments, the HSA the HSA comprises a C34S or K573P amino acid substitution relative to the wild-type HSA sequence According to the present disclosure, albumin can be conjugated to a IL2R binding molecule at the carboxyl terminus, the amino terminus, both the carboxyl and amino termini, and internally (see, e.g., US 5,876,969 and US 7,056,701). In the HAS IL2R binding molecule contemplated by the present disclosure, various forms of albumin can be used, such as albumin secretion pre-sequences and variants thereof, fragments and variants thereof, and HSA variants. Such forms generally possess one or more desired albumin activities. In additional embodiments, the present disclosure involves fusion proteins comprising a IL2R binding molecule fused directly or indirectly to albumin, an albumin fragment, and albumin variant, etc., wherein the fusion protein has a higher plasma stability than the unfused drug molecule and/or the fusion protein retains the therapeutic activity of the unfused drug molecule. As an alternative to chemical linkage between the IL2R binding molecule and the albumin molecule the IL2R binding molecule – albumin complex may be provided as a fusion protein comprising an albumin polypeptide sequence and an IL2R binding molecule recombinantly expressed in a host cell as a single polypeptide chain, optionally comprising a linker molecule between the albumin and IL2R binding molecule. Such fusion proteins may be readily prepared through recombinant technology to those of ordinary skill in the art. Nucleic acid sequences encoding such fusion proteins may be ordered from any of a variety of commercial sources. The nucleic acid sequence encoding the fusion protein is incorporated into an expression vector operably linked to one or more expression control elements, the vector introduced into a suitable host cell and the fusion protein isolated from the host cell culture by techniques well known in the art.

Polymeric Carriers

[0116] In some embodiments, extended in vivo duration of action of the IL2R binding molecule may be achieved by conjugation to one or more polymeric carrier molecules such as XTEN polymers or water soluble polymers.

XTEN Conjugates

[0117] The IL2R binding molecule may further comprise an XTEN polymer. The XTEN polymer may be conjugated (either chemically or as a fusion protein) the hIL2 mutein provides extended duration of action akin to PEGylation and may be produced as a recombinant fusion protein in *E. coli*. XTEN polymers suitable for use in conjunction with the IL2R binding molecule of the present disclosure are provided in Podust, et al. (2016) “Extension of in vivo half-life of biologically active molecules by XTEN protein polymers”, *J Controlled Release* 240:52-66 and Haeckel et al. (2016) “XTEN as Biological Alternative to PEGylation Allows Complete Expression of a Protease-Activatable Killin-Based Cytostatic” *PLOS ONE* | DOI:10.1371/journal.pone.0157193 June 13, 2016. The XTEN polymer may fusion protein may incorporate a protease sensitive cleavage site between the XTEN polypeptide and the hIL2 mutein such as an MMP-2 cleavage site.

Water Soluble Polymers

[0118] In some embodiments, the IL2R binding molecule can be conjugated to one or more water-soluble polymers. Examples of water soluble polymers useful in the practice of the present disclosure include polyethylene glycol (PEG), poly-propylene glycol (PPG), polysaccharides (polyvinylpyrrolidone, copolymers of ethylene glycol and propylene glycol, poly(oxyethylated polyol), polyolefinic alcohol), polysaccharides), poly-alpha-hydroxy acid), polyvinyl alcohol (PVA), polyphosphazene, polyoxazolines (POZ), poly(N-acryloylmorpholine), or a combination thereof.

[0119] In some embodiments, IL2R binding molecule can be conjugated to one or more polyethylene glycol molecules or “PEGylated.” Although the method or site of PEG attachment to the binding molecule may vary, in certain embodiments the PEGylation does not alter, or only minimally alters, the activity of the binding molecule.

[0120] PEGs suitable for conjugation to a polypeptide sequence are generally soluble in water at room temperature, and have the general formula

[0121] $R(O-CH_2-CH_2)_nO-R$,

[0122] where R is hydrogen or a protective group such as an alkyl or an alkanol group, and where n is an integer from 1 to 1000. When R is a protective group, it generally has from 1 to 8 carbons. The PEG can be linear or branched. Branched PEG derivatives, “star-PEGs” and multi-armed PEGs are contemplated by the present disclosure.

[0123] In some embodiments, selective PEGylation of the IL2R binding molecule, for example, by the incorporation of non-natural amino acids having side chains to facilitate selective PEG conjugation, may be employed. Specific PEGylation sites can be chosen such that PEGylation of the binding molecule does not affect its binding to the target receptors.

[0124] In some instances, the sequences of IL2R binding molecules provided herein possess an N-terminal glutamine (“1Q”) residue. N-terminal glutamine residues have been observed to spontaneously cyclize to form pyroglutamate (pE) at or near physiological conditions. (See e.g., Liu, et al (2011) J. Biol. Chem. 286(13): 11211–11217). In some embodiments, the formation of pyroglutamate complicates N-terminal PEG conjugation particularly when aldehyde chemistry is used for N-terminal PEGylation. Consequently, when PEGylating the IL2R binding molecules of the present disclosure, particularly when aldehyde chemistry is to be employed, the IL2R binding molecules possessing an amino acid at position 1 (e.g., 1Q) are substituted at position 1 with an alternative amino acid or are deleted at position 1 (e.g., des-1Q). In some embodiments, the IL2R binding molecules of the present disclosure comprise an amino acid substitution selected from the group Q1E and Q1D.

[0125] In certain embodiments, the increase in half-life is greater than any decrease in biological activity. PEGs suitable for conjugation to a polypeptide sequence are generally soluble in water at room temperature, and have the general formula $R(O-CH_2-CH_2)_nO-R$, where R is hydrogen or a protective group such as an alkyl or an alkanol group, and where n is an integer from 1 to 1000. When R is a protective group, it generally has from 1 to 8 carbons. The PEG conjugated to the polypeptide sequence can be linear or branched. Branched PEG derivatives, “star-PEGs” and multi-armed PEGs are contemplated by the present disclosure.

[0126] A molecular weight of the PEG used in the present disclosure is not restricted to any particular range. The PEG component of the binding molecule can have a molecular mass greater than about 5kDa, greater than about 10kDa, greater than about 15kDa, greater than about 20kDa, greater than about 30kDa, greater than about 40kDa, or greater than about 50kDa. In some embodiments, the molecular mass is from about 5kDa to about 10kDa, from

about 5kDa to about 15kDa, from about 5kDa to about 20kDa, from about 10kDa to about 15kDa, from about 10kDa to about 20kDa, from about 10kDa to about 25kDa, or from about 10kDa to about 30kDa. Linear or branched PEG molecules having molecular weights from about 2,000 to about 80,000 daltons, alternatively about 2,000 to about 70,000 daltons, alternatively about 5,000 to about 50,000 daltons, alternatively about 10,000 to about 50,000 daltons, alternatively about 20,000 to about 50,000 daltons, alternatively about 30,000 to about 50,000 daltons, alternatively about 20,000 to about 40,000 daltons, or alternatively about 30,000 to about 40,000 daltons. In one embodiment of the disclosure, the PEG is a 40kD branched PEG comprising two 20 kD arms.

[0127] The present disclosure also contemplates compositions of conjugates wherein the PEGs have different n values, and thus the various different PEGs are present in specific ratios. For example, some compositions comprise a mixture of conjugates where $n=1, 2, 3$ and 4 . In some compositions, the percentage of conjugates where $n=1$ is 18-25%, the percentage of conjugates where $n=2$ is 50-66%, the percentage of conjugates where $n=3$ is 12-16%, and the percentage of conjugates where $n=4$ is up to 5%. Such compositions can be produced by reaction conditions and purification methods known in the art. Chromatography may be used to resolve conjugate fractions, and a fraction is then identified which contains the conjugate having, for example, the desired number of PEGs attached, purified free from unmodified protein sequences and from conjugates having other numbers of PEGs attached.

[0128] PEGs suitable for conjugation to a polypeptide sequence are generally soluble in water at room temperature, and have the general formula $R(O-CH_2-CH_2)_nO-R$, where R is hydrogen or a protective group such as an alkyl or an alkanol group, and where n is an integer from 1 to 1000. When R is a protective group, it generally has from 1 to 8 carbonst

[0129] Two widely used first generation activated monomethoxy PEGs (mPEGs) are succinimidyl carbonate PEG (SC-PEG; see, e.g., Zalipsky, et al. (1992) *Biotechnol. Appl. Biochem* 15:100-114) and benzotriazole carbonate PEG (BTC-PEG; see, e.g., Dolence, et al. US Patent No. 5,650,234), which react preferentially with lysine residues to form a carbamate linkage but are also known to react with histidine and tyrosine residues. Use of a PEG-aldehyde linker targets a single site on the N-terminus of a polypeptide through reductive amination.

[0130] Pegylation most frequently occurs at the alpha-amino group at the N-terminus of the polypeptide, the epsilon amino group on the side chain of lysine residues, and the

imidazole group on the side chain of histidine residues. Since most recombinant polypeptides possess a single alpha and a number of epsilon amino and imidazole groups, numerous positional isomers can be generated depending on the linker chemistry. General PEGylation strategies known in the art can be applied herein.

[0131] The PEG can be bound to a binding molecule of the present disclosure via a terminal reactive group (a “spacer”) which mediates a bond between the free amino or carboxyl groups of one or more of the polypeptide sequences and polyethylene glycol. The PEG having the spacer which can be bound to the free amino group includes N-hydroxysuccinylimide polyethylene glycol, which can be prepared by activating succinic acid ester of polyethylene glycol with N-hydroxysuccinylimide.

[0132] In some embodiments, the PEGylation of the binding molecules is facilitated by the incorporation of non-natural amino acids bearing unique side chains to facilitate site specific PEGylation. The incorporation of non-natural amino acids into polypeptides to provide functional moieties to achieve site specific PEGylation of such polypeptides is known in the art. See e.g., Ptacin et al., PCT International Application No. PCT/US2018/045257 filed August 3, 2018 and published February 7, 2019 as International Publication Number WO 2019/028419A1.

[0133] The PEG conjugated to the polypeptide sequence can be linear or branched. Branched PEG derivatives, “star-PEGs” and multi-armed PEGs are contemplated by the present disclosure. Specific embodiments PEGs useful in the practice of the present disclosure include a 10kDa linear PEG-aldehyde (e.g., Sunbright® ME-100AL, NOF America Corporation, One North Broadway, White Plains, NY 10601 USA), 10kDa linear PEG-NHS ester (e.g., Sunbright® ME-100CS, Sunbright® ME-100AS, Sunbright® ME-100GS, Sunbright® ME-100HS, NOF), a 20kDa linear PEG-aldehyde (e.g., Sunbright® ME-200AL, NOF), a 20kDa linear PEG- NHS ester (e.g., Sunbright® ME-200CS, Sunbright® ME-200AS, Sunbright® ME-200GS, Sunbright® ME-200HS, NOF), a 20kDa 2-arm branched PEG-aldehyde the 20 kDa PEG-aldehyde comprising two 10kDa linear PEG molecules (e.g., Sunbright® GL2-200AL3, NOF), a 20kDa 2-arm branched PEG-NHS ester the 20 kDa PEG-NHS ester comprising two 10kDa linear PEG molecules (e.g., Sunbright® GL2-200TS, Sunbright® GL200GS2, NOF), a 40kDa 2-arm branched PEG-aldehyde the 40 kDa PEG-aldehyde comprising two 20kDa linear PEG molecules (e.g., Sunbright® GL2-400AL3), a 40kDa 2-arm branched PEG-NHS ester the 40 kDa PEG-NHS ester comprising two 20kDa

linear PEG molecules (e.g., Sunbright® GL2-400AL3, Sunbright® GL2-400GS2, NOF), a linear 30kDa PEG-aldehyde (e.g., Sunbright® ME-300AL) and a linear 30kDa PEG-NHS ester.

[0134] In some embodiments, a linker can be used to join the IL2R binding molecule and the PEG molecule. Suitable linkers include “flexible linkers” which are generally of sufficient length to permit some movement between the modified polypeptide sequences and the linked components and molecules. The linker molecules are generally about 6-50 atoms long. The linker molecules may also be, for example, aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids, or combinations thereof. Suitable linkers can be readily selected and can be of any suitable length, such as 1 amino acid (e.g., Gly), 2, 3, 4, 5, 6, 7, 8, 9, 10, 10-20, 20-30, 30-50 or more than 50 amino acids. Examples of flexible linkers are described in Section IV. Further, a multimer (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 10-20, 20-30, or 30-50) of these linker sequences may be linked together to provide flexible linkers that may be used to conjugate two molecules. Alternative to a polypeptide linker, the linker can be a chemical linker, e.g., a PEG-aldehyde linker. In some embodiments, the binding molecule is acetylated at the N-terminus by enzymatic reaction with N-terminal acetyltransferase and, for example, acetyl CoA. Alternatively, or in addition to N-terminal acetylation, the binding molecule can be acetylated at one or more lysine residues, e.g., by enzymatic reaction with a lysine acetyltransferase. See, for example Choudhary et al. (2009) *Science* 325 (5942):834-840.

Fatty Acid Carriers

[0135] In some embodiments an IL2R binding molecule having an extended duration of action in a mammalian subject and useful in the practice of the present disclosure is achieved by covalent attachment of the IL2R binding molecule to a fatty acid molecule as described in Resh (2016) *Progress in Lipid Research* 63: 120–131. Examples of fatty acids that may be conjugated include myristate, palmitate and palmitoleic acid. Myristoylate is typically linked to an N-terminal glycine but lysines may also be myristoylated. Palmitoylation is typically achieved by enzymatic modification of free cysteine -SH groups such as DHHC proteins catalyze S-palmitoylation. Palmitoleylation of serine and threonine residues is typically achieved enzymatically using PORCN enzymes. In some embodiments, the IL2R binding molecule is acetylated at the N-terminus by enzymatic reaction with N-terminal acetyltransferase and, for example, acetyl CoA. Alternatively, or in addition to N-terminal

acetylation, the IL2R binding molecule is acetylated at one or more lysine residues, e.g., by enzymatic reaction with a lysine acetyltransferase. See, for example Choudhary et al. (2009) Science 325 (5942):834L2 ortho840.

Modifications to Provide Additional Functions

[0136] In some embodiments, embodiment, the IL2R binding molecule may comprise a functional domain of a chimeric polypeptide. IL2R binding molecule fusion proteins of the present disclosure may be readily produced by recombinant DNA methodology by techniques known in the art by constructing a recombinant vector comprising a nucleic acid sequence comprising a nucleic acid sequence encoding the IL2R binding molecule in frame with a nucleic acid sequence encoding the fusion partner either at the N-terminus or C-terminus of the IL2R binding molecule, the sequence optionally further comprising a nucleic acid sequence in frame encoding a linker or spacer polypeptide.

FLAG Tags

[0137] In other embodiments, the IL2R binding molecule can be modified to include an additional polypeptide sequence that functions as an antigenic tag, such as a FLAG sequence. FLAG sequences are recognized by biotinylated, highly specific, anti-FLAG antibodies, as described herein (see e.g., Blancar et al. (1992) Science 256:1014 and LeClair, et al. (1992) PNAS-USA 89:8145). In some embodiments, the binding molecule further comprises a C-terminal c-myc epitope tag.

Chelating Peptides

[0002] In one embodiment, the present disclosure provides a IL10Rb1 binding molecule comprising one or more transition metal chelating polypeptide sequences known as chelating peptides. A chelating peptide is a polypeptide of the formula:



wherein "His" is the amino acid histidine; "AA" is an amino acid other than proline; a = an integer from 0 to 10; b = an integer from 0 to 4; c = an integer from 0 to 10; and random, block and alternating copolymers thereof. In some embodiments, the chelating peptide has an amino acid sequence selected from the group consisting of: SEQ ID NOS: 507-521. The incorporation of such a transition metal chelating domain facilitates purification immobilized metal affinity chromatography (IMAC) as described in Smith, et al. United States Patent No. 4,569,794 issued February 11, 1986. Examples of transition metal

chelating polypeptides useful in the practice of the present IL12RB1 binding molecule are described in Smith, et al. *supra* and Dobeli, et al. United States Patent No. 5,320,663 issued May 10, 1995, the entire teachings of which are hereby incorporated by reference. Particular transition metal chelating polypeptides useful in the practice of the present IL12RB1 binding molecule are polypeptides comprising 3-6 contiguous histidine residues such as a six-histidine (His)₆ peptide and are frequently referred to in the art as “His-tags.” In addition to providing a purification “handle” for the recombinant proteins or to facilitate immobilization on SPR sensor chips, such the conjugation of the hIL12RB1 binding molecule to a chelating peptide facilitates the targeted delivery to IL12RB1 expressing cells of transition metal ions as kinetically inert or kinetically labile complexes in substantial accordance with the teaching of Anderson, et al., (United States Patent No. 5,439,829 issued August 8, 1995 and Hale, J.E (1996) *Analytical Biochemistry* 231(1):46-49. Particular transition metal chelating polypeptides useful in the practice of the present disclosure are peptides comprising 3-6 contiguous histidine residues such as a six-histidine peptide (His)₆ and are frequently referred to in the art as “His-tags.” In some embodiments, a purification handle is a polypeptide having the sequence Ala-Ser-His-His-His-His-His-His (“ASH6”) (SEQ ID NO: 23) or Gly-Ser-His-His-His-His-His-His-His (“GSH8”) (SEQ ID NO: 24).

Targeting Moieties:

[0138] In some embodiments, IL2R binding molecule is conjugated to molecule which provides (“targeting domain”) to facilitate selective binding to particular cell type or tissue expressing a cell surface molecule that specifically binds to such targeting domain, optionally incorporating a linker molecule of from 1-40 (alternatively 2-20, alternatively 5-20, alternatively 10-20) amino acids between IL2R binding molecule sequence and the sequence of the targeting domain of the fusion protein.

[0139] In other embodiments, a chimeric polypeptide including a IL2R binding molecule and an antibody or antigen-binding portion thereof can be generated. The antibody or antigen-binding component of the chimeric protein can serve as a targeting moiety. For example, it can be used to localize the chimeric protein to a particular subset of cells or target molecule. Methods of generating cytokine-antibody chimeric polypeptides are described, for example, in U.S. Pat. No. 6,617,135.

[0140] In some embodiments, the targeting moiety is an antibody that specifically binds to at least one cell surface molecule associated with a tumor cell (i.e. at least one tumor

antigen) wherein the cell surface molecule associated with a tumor cell is selected from the group consisting of GD2, BCMA, CD19, CD33, CD38, CD70, GD2, IL3RB2, CD19, mesothelin, Her2, EpCam, Muc1, ROR1, CD133, CEA, EGRFRVIII, PSCA, GPC3, Pan-ErbB and FAP.

Elimination of N-Linked Glycosylation Sites

[0141] In some embodiments, it is possible that an amino acid sequence (particularly a CDR sequence) of the IL10Ra or IL10Rb sdAb may contain a glycosylation motif, particularly an N-linked glycosylation motif of the sequence Asn-X-Ser (N-X-S) or Asn-X-Thr (N-X-T), wherein X is any amino acid except for proline. In such instances, it is desirable to eliminate such N-linked glycosylation motifs by modifying the sequence of the N-linked glycosylation motif to prevent glycosylation. In some embodiments, the elimination of the Asn-X-Ser (N-X-S) N-linked glycosylation motif may be achieved by the incorporation of conservative amino acid substitution of the Asn (N) residue and/or Ser (S) residue of the Asn-X-Ser (N-X-S) N-linked glycosylation motif. In some embodiments, the elimination of the Asn-X-Thr (N-X-T) N-linked glycosylation motif may be achieved by the incorporation of conservative amino acid substitution of the Asn (N) residue and/or Thr (T) residue of the Asn-X-Thr (N-X-T) N-linked glycosylation motif. In some embodiments, elimination of the glycosylation site is not required when the IL10R binding molecule comprising the IL10Ra or IL10Rb sdAb is expressed in procaryotic host cells. Since procaryotic cells do not provide a mechanism for glycosylation of recombinant proteins, when employing a procaryotic expression system to produce a recombinant IL10R binding molecule comprising the IL10Ra or IL10Rb sdAb the modification of the sequence to eliminate the N-linked glycosylation sites may be obviated.

RECOMBINANT PRODUCTION

[0142] Alternatively, the IL2R binding molecules of the present disclosure are produced by recombinant DNA technology. In the typical practice of recombinant production of polypeptides, a nucleic acid sequence encoding the desired polypeptide is incorporated into an expression vector suitable for the host cell in which expression will be accomplished, the nucleic acid sequence being operably linked to one or more expression control sequences encoding by the vector and functional in the target host cell. The recombinant protein may be recovered through disruption of the host cell or from the cell medium if a secretion leader sequence (signal peptide) is incorporated into the polypeptide.

Construction of Nucleic Acid Sequences Encoding the IL2R binding molecule

[0143] In some embodiments, the IL2R binding molecule is produced by recombinant methods using a nucleic acid sequence encoding the IL2R binding molecule (or fusion protein comprising the IL2R binding molecule). The nucleic acid sequence encoding the desired hIL2R binding molecule can be synthesized by chemical means using an oligonucleotide synthesizer.

[0144] The nucleic acid molecules are not limited to sequences that encode polypeptides; some or all of the non-coding sequences that lie upstream or downstream from a coding sequence (e.g., the coding sequence of IL-2) can also be included. Those of ordinary skill in the art of molecular biology are familiar with routine procedures for isolating nucleic acid molecules. They can, for example, be generated by treatment of genomic DNA with restriction endonucleases, or by performance of the polymerase chain reaction (PCR). In the event the nucleic acid molecule is a ribonucleic acid (RNA), molecules can be produced, for example, by in vitro transcription.

[0145] The nucleic acid molecules encoding the IL2R binding molecule (and fusions thereof) may contain naturally occurring sequences or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide. These nucleic acid molecules can consist of RNA or DNA (for example, genomic DNA, cDNA, or synthetic DNA, such as that produced by phosphoramidite-based synthesis), or combinations or modifications of the nucleotides within these types of nucleic acids. In

addition, the nucleic acid molecules can be double-stranded or single-stranded (i.e., either a sense or an antisense strand).

[0146] Nucleic acid sequences encoding the IL2R binding molecule may be obtained from various commercial sources that provide custom made nucleic acid sequences. Amino acid sequence variants of the IL2R binding molecules of the present disclosure are prepared by introducing appropriate nucleotide changes into the coding sequence based on the genetic code which is well known in the art. Such variants represent insertions, substitutions, and/or specified deletions of residues as noted. Any combination of insertion, substitution, and/or specified deletion is made to arrive at the final construct, provided that the final construct possesses the desired biological activity as defined herein.

[0147] Methods for constructing a DNA sequence encoding a IL2R binding molecule and expressing those sequences in a suitably transformed host include, but are not limited to, using a PCR-assisted mutagenesis technique. Mutations that consist of deletions or additions of amino acid residues to a IL2R binding molecule can also be made with standard recombinant techniques. In the event of a deletion or addition, the nucleic acid molecule encoding a IL2R binding molecule is optionally digested with an appropriate restriction endonuclease. The resulting fragment can either be expressed directly or manipulated further by, for example, ligating it to a second fragment. The ligation may be facilitated if the two ends of the nucleic acid molecules contain complementary nucleotides that overlap one another, but blunt-ended fragments can also be ligated. PCR-generated nucleic acids can also be used to generate various mutant sequences.

[0148] A IL2R binding molecule of the present disclosure may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, e.g. a signal sequence or other polypeptide having a specific cleavage site at the N-terminus or C-terminus of the mature IL2R binding molecule. In general, the signal sequence may be a component of the vector, or it may be a part of the coding sequence that is inserted into the vector. The heterologous signal sequence selected preferably is one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. The inclusion of a signal sequence depends on whether it is desired to secrete the IL2R binding molecule from the recombinant cells in which it is made. If the chosen cells are prokaryotic, it generally is preferred that the DNA sequence not encode a signal sequence. When the recombinant host cell is a yeast cell such as *Saccharomyces cerevisiae*, the alpha mating factor secretion signal

sequence may be employed to achieve extracellular secretion of the IL2R binding molecule into the culture medium as described in Singh, United States Patent No. 7,198,919 B1 issued April 3, 2007.

[0149] In the event the IL2R binding molecule to be expressed is to be expressed as a chimera (e.g., a fusion protein comprising a IL2R binding molecule and a heterologous polypeptide sequence), the chimeric protein can be encoded by a hybrid nucleic acid molecule comprising a first sequence that encodes all or part of the IL2R binding molecule and a second sequence that encodes all or part of the heterologous polypeptide. For example, subject IL2R binding molecules described herein may be fused to a hexa-/octa-histidine tag to facilitate purification of bacterially expressed protein, or to a hemagglutinin tag to facilitate purification of protein expressed in eukaryotic cells. By first and second, it should not be understood as limiting to the orientation of the elements of the fusion protein and a heterologous polypeptide can be linked at either the N-terminus and/or C-terminus of the IL2R binding molecule. For example, the N-terminus may be linked to a targeting domain and the C-terminus linked to a hexa-histidine tag purification handle.

[0150] The complete amino acid sequence of the polypeptide (or fusion/chimera) to be expressed can be used to construct a back-translated gene. A DNA oligomer containing a nucleotide sequence coding a IL2R binding molecule can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

Codon Optimization:

[0151] In some embodiments, the nucleic acid sequence encoding the IL2R binding molecule may be “codon optimized” to facilitate expression in a particular host cell type. Techniques for codon optimization in a wide variety of expression systems, including mammalian, yeast and bacterial host cells, are well known in the art and there are online tools to provide for a codon optimized sequences for expression in a variety of host cell types. See e.g. Hawash, et al., (2017) 9:46-53 and Mauro and Chappell in Recombinant Protein Expression in Mammalian Cells: Methods and Protocols, edited by David Hacker (Human Press New York). Additionally, there are a variety of web based on-line software packages that are freely available to assist in the preparation of codon optimized nucleic acid sequences.

Expression Vectors:

[0152] Once assembled (by synthesis, site-directed mutagenesis or another method), the nucleic acid sequence encoding an IL2R binding molecule will be inserted into an expression vector. A variety of expression vectors for uses in various host cells are available and are typically selected based on the host cell for expression. An expression vector typically includes, but is not limited to, one or more of the following: an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Vectors include viral vectors, plasmid vectors, integrating vectors, and the like. Plasmids are examples of non-viral vectors.

[0153] To facilitate efficient expression of the recombinant polypeptide, the nucleic acid sequence encoding the polypeptide sequence to be expressed is operably linked to transcriptional and translational regulatory control sequences that are functional in the chosen expression host.

Selectable Marker:

[0154] Expression vectors usually contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media.

Regulatory Control Sequences:

[0155] Expression vectors for a IL2R binding molecules of the present disclosure contain a regulatory sequence that is recognized by the host organism and is operably linked to nucleic acid sequence encoding the IL2R binding molecule. The terms “regulatory control sequence,” “regulatory sequence” or “expression control sequence” are used interchangeably herein to refer to promoters, enhancers, and other expression control elements (e.g., polyadenylation signals). See, for example, Goeddel (1990) in *Gene Expression Technology: Methods in Enzymology* 185 (Academic Press, San Diego CA USA). Regulatory sequences include those that direct constitute expression of a nucleotide sequence in many types of host cells and those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. In selecting an expression

control sequence, a variety of factors understood by one of skill in the art are to be considered. These include, for example, the relative strength of the sequence, its controllability, and its compatibility with the actual DNA sequence encoding the subject a IL2R binding molecule, particularly as regards potential secondary structures.

Promoters:

[0156] In some embodiments, the regulatory sequence is a promoter, which is selected based on, for example, the cell type in which expression is sought. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of particular nucleic acid sequence to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g., the presence or absence of a nutrient or a change in temperature. A large number of promoters recognized by a variety of potential host cells are well known.

[0157] A T7 promoter can be used in bacteria, a polyhedrin promoter can be used in insect cells, and a cytomegalovirus or metallothionein promoter can be used in mammalian cells. Also, in the case of higher eukaryotes, tissue-specific and cell type-specific promoters are widely available. These promoters are so named for their ability to direct expression of a nucleic acid molecule in a given tissue or cell type within the body. Skilled artisans are well aware of numerous promoters and other regulatory elements which can be used to direct expression of nucleic acids.

[0158] Transcription from vectors in mammalian host cells may be controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as human adenovirus serotype 5), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus (such as murine stem cell virus), hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter, PGK (phosphoglycerate kinase), or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication.

Enhancers:

[0159] Transcription by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to

300 bp, which act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent, having been found 5' and 3' to the transcription unit, within an intron, as well as within the coding sequence itself. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, alpha-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the expression vector at a position 5' or 3' to the coding sequence but is preferably located at a site 5' from the promoter. Expression vectors used in eukaryotic host cells will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. Construction of suitable vectors containing one or more of the above-listed components employs standard techniques.

[0160] In addition to sequences that facilitate transcription of the inserted nucleic acid molecule, vectors can contain origins of replication, and other genes that encode a selectable marker. For example, the neomycin-resistance (neoR) gene imparts G418 resistance to cells in which it is expressed, and thus permits phenotypic selection of the transfected cells. Additional examples of marker or reporter genes include beta-lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding beta-galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT). Those of skill in the art can readily determine whether a given regulatory element or selectable marker is suitable for use in a particular experimental context.

[0161] Proper assembly of the expression vector can be confirmed by nucleotide sequencing, restriction mapping, and expression of a biologically active polypeptide in a suitable host.

Host Cells:

[0162] The present disclosure further provides prokaryotic or eukaryotic cells that contain and express a nucleic acid molecule that encodes a IL2R binding molecule. A cell of the present disclosure is a transfected cell, i.e., a cell into which a nucleic acid molecule, for example a nucleic acid molecule encoding a mutant IL-2 polypeptide, has been introduced by

means of recombinant DNA techniques. The progeny of such a cell are also considered within the scope of the present disclosure.

[0163] Host cells are typically selected in accordance with their compatibility with the chosen expression vector, the toxicity of the product coded for by the DNA sequences of this invention, their secretion characteristics, their ability to fold the polypeptides correctly, their fermentation or culture requirements, and the ease of purification of the products coded for by the DNA sequences. Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells.

[0164] In some embodiments the recombinant IL2R binding molecule can also be made in eukaryotes, such as yeast or human cells. Suitable eukaryotic host cells include insect cells (examples of Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf9 cells) include the pAc series (Smith et al. (1983) Mol. Cell Biol. 3:2156-2165) and the pVL series (Lucklow and Summers (1989) Virology 170:31-39)); yeast cells (examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari et al. (1987) EMBO J. 6:229-234), pMFa (Kurjan and Herskowitz (1982) Cell 30:933-943), pJRY88 (Schultz et al. (1987) Gene 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and pPicZ (Invitrogen Corporation, San Diego, Calif.)); or mammalian cells (mammalian expression vectors include pCDM8 (Seed (1987) Nature 329:840) and pMT2PC (Kaufman et al. (1987) EMBO J. 6:187:195)).

[0165] Examples of useful mammalian host cell lines are mouse L cells (L-M[TK-], ATCC#CRL-2648), monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (HEK293 or HEK293 cells subcloned for growth in suspension culture; baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO); mouse sertoli cells (TM4); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1 587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells; MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2). In mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus, and Simian Virus 40.

[0166] The IL2R binding molecule may be produced in a prokaryotic host, such as the bacterium *E. coli*, or in a eukaryotic host, such as an insect cell (e.g., an Sf21 cell), or mammalian cells (e.g., COS cells, NIH 3T3 cells, or HeLa cells). These cells are available from many sources, including the American Type Culture Collection (Manassas, Va.). In selecting an expression system, it matters only that the components are compatible with one another. Artisans or ordinary skill are able to make such a determination. Furthermore, if guidance is required in selecting an expression system, skilled artisans may consult Ausubel et al. (Current Protocols in Molecular Biology, John Wiley and Sons, New York, N.Y., 1993) and Pouwels et al. (Cloning Vectors: A Laboratory Manual, 1985 Suppl. 1987).

[0167] In some embodiments, a IL2R binding molecule obtained will be glycosylated or unglycosylated depending on the host organism used to produce the mutein. If bacteria are chosen as the host then the a IL2R binding molecule produced will be unglycosylated. Eukaryotic cells, on the other hand, will typically result in glycosylation of the IL2R binding molecule.

[0168] For other additional expression systems for both prokaryotic and eukaryotic cells, see Chapters 16 and 17 of Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual (2nd ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y.). See, Goeddel (1990) in Gene Expression Technology: Methods in Enzymology 185 (Academic Press, San Diego, Calif.).

Transfection:

[0169] The expression constructs of the can be introduced into host cells to thereby produce a IL2R binding molecule disclosed herein. The expression vector comprising a nucleic acid sequence encoding IL2R binding molecule is introduced into the prokaryotic or eukaryotic host cells via conventional transformation or transfection techniques. Suitable methods for transforming or transfecting host cells can be found in Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y.) and other standard molecular biology laboratory manuals. To facilitate transfection of the target cells, the target cell may be exposed directly with the non-viral vector may under conditions that facilitate uptake of the non-viral vector. Examples of conditions which facilitate uptake of foreign nucleic acid by mammalian cells are well known in the art and include but are not limited to chemical means (such as Lipofectamine®, Thermo-Fisher Scientific), high salt, and magnetic fields (electroporation).

Cell Culture: Cells may be cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. Mammalian host cells may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), Sigma), RPMI 1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics, trace elements, and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression and will be apparent to the ordinarily skilled artisan.

Recovery of Recombinant Proteins:

[0170] Recombinantly produced IL2R binding molecule polypeptides can be recovered from the culture medium as a secreted polypeptide if a secretion leader sequence is employed. Alternatively, the IL2R binding molecule polypeptides can also be recovered from host cell lysates. A protease inhibitor, such as phenyl methyl sulfonyl fluoride (PMSF) may be employed during the recovery phase from cell lysates to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants.

[0171] Various purification steps are known in the art and find use, e.g. affinity chromatography. Affinity chromatography makes use of the highly specific binding sites usually present in biological macromolecules, separating molecules on their ability to bind a particular ligand. Covalent bonds attach the ligand to an insoluble, porous support medium in a manner that overtly presents the ligand to the protein sample, thereby using natural specific binding of one molecular species to separate and purify a second species from a mixture. Antibodies are commonly used in affinity chromatography. Size selection steps may also be used, e.g. gel filtration chromatography (also known as size-exclusion chromatography or molecular sieve chromatography) is used to separate proteins according to their size. In gel filtration, a protein solution is passed through a column that is packed with semipermeable porous resin. The semipermeable resin has a range of pore sizes that determines the size of proteins that can be separated with the column.

[0172] A recombinantly IL2R binding molecule by the transformed host can be purified according to any suitable method. Recombinant IL2R binding molecules can be isolated from inclusion bodies generated in *E. coli*, or from conditioned medium from either mammalian or yeast cultures producing a given mutein using cation exchange, gel filtration, and or reverse phase liquid chromatography. The substantially purified forms of the recombinant IL2R binding molecule can be purified from the expression system using routine biochemical procedures, and can be used, e.g., as therapeutic agents, as described herein.

[0173] In some embodiments, where the IL2R binding molecule is expressed with a purification tag as discussed above, this purification handle may be used for isolation of the IL2R binding molecule from the cell lysate or cell medium. Where the purification tag is a chelating peptide, methods for the isolation of such molecules using immobilized metal affinity chromatography are well known in the art. See, e.g., Smith, et al. United States Patent 4,569,794.

[0174] The biological activity of the IL2R binding molecules recovered can be assayed for activating by any suitable method known in the art and may be evaluated as substantially purified forms or as part of the cell lysate or cell medium when secretion leader sequences are employed for expression.

METHODS OF USE

[0175] The present disclosure provides methods of use of IL10R binding molecules of the present disclosure in the treatment of a subject suffering from a neoplastic disease by the administration to the subject of therapeutically effective amount of an IL10R binding molecule, a nucleic acid encoding an IL10R binding molecule, a recombinant viral or non-viral vector encoding an IL10R binding molecules, or a recombinantly modified cell that expresses an IL10R binding molecules

[0176] The determination of whether a subject is “suffering from a neoplastic disease” refers to a determination made by a physician with respect to a subject based on the available information accepted in the field for the identification of a disease, disorder or condition including but not limited to X-ray, CT-scans, conventional laboratory diagnostic tests (e.g. blood count, etc.), genomic data, protein expression data, immunohistochemistry, that the subject requires or will benefit from treatment.

[0177] The adaptive immune system recognizes the display of certain cell surface proteins in response to tumor mutations facilitating the recognition and elimination of neoplastic cells. Tumors that possess a higher tumor mutation burden (TMB) are more likely to exhibit such “tumor antigens.” Indeed, clinical experience shows that tumors comprised of neoplastic cells exhibiting a high tumor mutation burden are more likely to respond to immune therapies, including immune checkpoint blockade (Rizvi, *et al.* (2015) *Science* 348(6230): 124-128; Marabelle, *et al.* (2020) *Lancet Oncol* 21(10):1353-1365). Tumor mutation burden is useful as a biomarker to identify tumors with an increased sensitivity to immune therapies such as those provided in the present disclosure.

[0178] In some embodiments, the neoplastic disease is characterized by the presence in the subject of a benign neoplasm. . Examples of benign neoplasms amenable to treatment using the compositions and methods of the present disclosure include but are not limited to adenomas, fibromas, hemangiomas, and lipomas. Examples of pre-malignant neoplasms amenable to treatment using the compositions and methods of the present disclosure include but are not limited to hyperplasia, atypia, metaplasia, and dysplasia. Examples of malignant neoplasms amenable to treatment using the compositions and methods of the present disclosure

include but are not limited to carcinomas (cancers arising from epithelial tissues such as the skin or tissues that line internal organs), leukemias, lymphomas, and sarcomas typically derived from bone fat, muscle, blood vessels or connective tissues). Also included in the term neoplasms are viral induced neoplasms such as warts and EBV induced disease (i.e., infectious mononucleosis), scar formation, hyperproliferative vascular disease including intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion and the like.

[0179] The term “neoplastic disease” includes cancers characterized by solid tumors and non-solid tumors including but not limited to breast cancers; sarcomas (including but not limited to osteosarcomas and angiosarcomas and fibrosarcomas), leukemias, lymphomas, genitourinary cancers (including but not limited to ovarian, urethral, bladder, and prostate cancers); gastrointestinal cancers (including but not limited to colon esophageal and stomach cancers); lung cancers; myelomas; pancreatic cancers; liver cancers; kidney cancers; endocrine cancers; skin cancers; and brain or central and peripheral nervous (CNS) system tumors, malignant or benign, including gliomas and neuroblastomas, astrocytomas, myelodysplastic disorders; cervical carcinoma-in-situ; intestinal polyposes; oral leukoplakias; histiocytoses, hyperproliferative scars including keloid scars, hemangiomas; hyperproliferative arterial stenosis, psoriasis, inflammatory arthritis; hyperkeratoses and papulosquamous eruptions including arthritis.

[0180] The term neoplastic disease includes carcinomas. The term “carcinoma” refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. The term neoplastic disease includes adenocarcinomas. An “adenocarcinoma” refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures.

[0181] As used herein, the term “hematopoietic neoplastic disorders” refers to neoplastic diseases involving hyperplastic/neoplastic cells of hematopoietic origin, e.g., arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof.

[0182] Myeloid neoplasms include, but are not limited to, myeloproliferative neoplasms, myeloid and lymphoid disorders with eosinophilia, myeloproliferative/myelodysplastic neoplasms, myelodysplastic syndromes, acute myeloid leukemia and related precursor neoplasms, and acute leukemia of ambiguous lineage. Exemplary myeloid disorders amenable to treatment in accordance with the present disclosure

include, but are not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML).

[0183] Lymphoid neoplasms include, but are not limited to, precursor lymphoid neoplasms, mature B-cell neoplasms, mature T-cell neoplasms, Hodgkin's Lymphoma, and immunodeficiency-associated lymphoproliferative disorders. Exemplary lymphic disorders amenable to treatment in accordance with the present disclosure include, but are not limited to, acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's macroglobulinemia (WM).

[0184] In some instances, the hematopoietic neoplastic disorder arises from poorly differentiated acute leukemias (e.g., erythroblastic leukemia and acute megakaryoblastic leukemia). As used herein, the term "hematopoietic neoplastic disorders" refers malignant lymphomas including, but are not limited to, non-Hodgkins lymphoma and variants thereof, peripheral T cell lymphomas, adult T-cell leukemia/lymphoma (ATL), cutaneous T cell lymphoma (CTCL), large granular lymphocytic leukemia (LGL), Hodgkin's disease and Reed-Stenberg disease.

[0185] In some embodiments, the compositions and methods of the present disclosure are useful in the treatment of neoplastic disease associated with the formation of solid tumors exhibiting an intermediate or high tumor mutational burden (TMB). In some embodiments, the compositions and compositions and methods of the present disclosure are useful in the treatment of immune sensitive solid tumors exhibiting an intermediate or high tumor mutational burden (TMB). Examples of neoplastic diseases associated with the formation of solid tumors having an intermediate or high tumor mutational burden amenable to treatment with the compositions and methods of the present disclosure include, but are not limited to, non-small cell lung cancer and renal cell cancer. In one embodiment, the compositions and methods are useful in the treatment of non-small cell lung cancer (NSCLC) exhibiting an intermediate or high TMB. NSCLC cells typically harbor a significant number of mutations and are therefore more sensitive to immune therapies. The current standard of care for NSCLC is stratified by the cancer initiating mechanisms and generally follows the recommendations of NCCN or ASCO. A large proportion of NSCLC has increased TMB and is therefore initially more sensitive to immune therapies. However, most tumors eventually relapse on immune checkpoint inhibition. Patients with relapsed tumors typically show reduced T cell infiltration in the tumor, systemic T cell exhaustion and a suppressed immune response compared to the

lesions prior to immune checkpoint inhibition. Therefore, improved immune therapies are required, re-activating and expanding the exhausted, rare tumor infiltrating T cells.

[0186] Combination Of IL10R binding molecules with Supplemental Therapeutic Agents:

[0187] The present disclosure provides for the use of the IL10R binding molecules of the present disclosure in combination with one or more additional active agents ("supplemental agents"). Such further combinations are referred to interchangeably as "supplemental combinations" or "supplemental combination therapy" and those therapeutic agents that are used in combination with IL10R binding molecules of the present disclosure are referred to as "supplemental agents." As used herein, the term "supplemental agents" includes agents that can be administered or introduced separately, for example, formulated separately for separate administration (e.g., as may be provided in a kit) and/or therapies that can be administered or introduced in combination with the hIL10R binding molecules.

[0188] As used herein, the term "in combination with" when used in reference to the administration of multiple agents to a subject refers to the administration of a first agent at least one additional (i.e. second, third, fourth, fifth, etc.) agent to a subject. For purposes of the present invention, one agent (e.g. hIL10R binding molecule) is considered to be administered in combination with a second agent (e.g. a modulator of an immune checkpoint pathway) if the biological effect resulting from the administration of the first agent persists in the subject at the time of administration of the second agent such that the therapeutic effects of the first agent and second agent overlap. For example, the PD1 immune checkpoint inhibitors (e.g. nivolumab or pembrolizumab) are typically administered by IV infusion every two weeks or every three weeks while the hIL10R binding molecules of the present disclosure are typically administered more frequently, e.g. daily, BID, or weekly. However, the administration of the first agent (e.g. pembrolizumab) provides a therapeutic effect over an extended time and the administration of the second agent (e.g. an hIL10R binding molecule) provides its therapeutic effect while the therapeutic effect of the first agent remains ongoing such that the second agent is considered to be administered in combination with the first agent, even though the first agent may have been administered at a point in time significantly distant (e.g. days or weeks) from the time of administration of the second agent. In one embodiment, one agent is considered to be administered in combination with a second agent if the first and second agents are administered simultaneously (within 30 minutes of each other), contemporaneously or sequentially. In some embodiments, a first agent is deemed to be administered

“contemporaneously” with a second agent if first and second agents are administered within about 24 hours of each other, preferably within about 12 hours of each other, preferably within about 6 hours of each other, preferably within about 2 hours of each other, or preferably within about 30 minutes of each other. The term “in combination with” shall also be understood to apply to the situation where a first agent and a second agent are co-formulated in single pharmaceutically acceptable formulation and the co-formulation is administered to a subject. In certain embodiments, the hIL10R binding molecule and the supplemental agent(s) are administered or applied sequentially, e.g., where one agent is administered prior to one or more other agents. In other embodiments, the hIL10R binding molecule and the supplemental agent(s) are administered simultaneously, e.g., where two or more agents are administered at or about the same time; the two or more agents may be present in two or more separate formulations or combined into a single formulation (i.e., a co-formulation). Regardless of whether the agents are administered sequentially or simultaneously, they are considered to be administered in combination for purposes of the present disclosure.

[0189] Supplemental Agents Useful In The Treatment of Neoplastic Disease:

[0190] In some embodiments, the supplemental agent is a chemotherapeutic agent. In some embodiments the supplemental agent is a “cocktail” of multiple chemotherapeutic agents. In some embodiments the chemotherapeutic agent or cocktail is administered in combination with one or more physical methods (e.g. radiation therapy). The term “chemotherapeutic agents” includes but is not limited to alkylating agents such as thiotepa and cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, anthramycin, azaserine, bleomycins such as bleomycin A₂, cactinomycin, calicheamicin, carubicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin and derivatives such as demethoxy-daunorubicin, 11-deoxydaunorubicin, 13-deoxydaunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as

mitomycin C, N-methyl mitomycin C; mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate, dideazatetrahydrofolic acid, and folinic acid; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanol, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (Ara-C); cyclophosphamide; thiotepa; taxoids, e.g., paclitaxel, nab-paclitaxel and doxetaxel; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum and platinum coordination complexes such as cisplatin, oxaplatin and carboplatin; vinblastine; etoposide (VP- 16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT11; topoisomerase inhibitors; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; taxanes such as paclitaxel, docetaxel, cabazitaxel; carminomycin, adriamycins such as 4'-epiadriamycin, 4- adriamycin-14-benzoate, adriamycin-14-octanoate, adriamycin-14-naphthaleneacetate; cholechicine and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0191] The term "chemotherapeutic agents" also includes anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens, including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, onapristone, and toremifene; and antiandrogens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0192] In some embodiments, a supplemental agent is one or more chemical or biological agents identified in the art as useful in the treatment of neoplastic disease, including, but not limited to, a cytokines or cytokine antagonists such as IL-12, INF α , or anti-epidermal growth factor receptor, irinotecan; tetrahydrofolate antimetabolites such as pemetrexed; antibodies against tumor antigens, a complex of a monoclonal antibody and toxin, a T-cell adjuvant, bone marrow transplant, or antigen presenting cells (e.g., dendritic cell therapy), anti-tumor vaccines, replication competent viruses, signal transduction inhibitors (e.g., Gleevec® or Herceptin®) or an immunomodulator to achieve additive or synergistic suppression of tumor growth, non-steroidal anti-inflammatory drugs (NSAIDs), cyclooxygenase-2 (COX-2) inhibitors, steroids, TNF antagonists (e.g., Remicade® and Enbrel®), interferon- β 1a (Avonex®), and interferon- β 1b (Betaseron®) as well as combinations of one or more of the foregoing as practiced in known chemotherapeutic treatment regimens including but not limited to TAC, FOLFOX, TPC, FEC, ADE, FOLFOX-6, EPOCH, CHOP, CMF, CVP, BEP, OFF, FLOX, CVD, TC, FOLFIRI, PCV, FOLFOXIRI, ICE-V, XELOX, and others that are readily appreciated by the skilled clinician in the art.

[0193] In some embodiments, the hIL10R binding molecule is administered in combination with BRAF/MEK inhibitors, kinase inhibitors such as sunitinib, PARP inhibitors such as olaparib, EGFR inhibitors such as osimertinib (Ahn, *et al.* (2016) J Thorac Oncol 11:S115), IDO inhibitors such as epacadostat, and oncolytic viruses such as talimogene laherparepvec (T-VEC).

[0194] In some embodiments, a “supplemental agent” is a therapeutic antibody (including bi-specific and tri-specific antibodies which bind to one or more tumor associated antigens including but not limited to bispecific T cell engagers (BITes), dual affinity retargeting (DART) constructs, and trispecific killer engager (TriKE) constructs).

[0195] In some embodiments, the therapeutic antibody is an antibody that binds to at least one tumor antigen selected from the group consisting of HER2 (e.g. trastuzumab, pertuzumab, ado-trastuzumab emtansine), nectin-4 (e.g. enfortumab), CD79 (e.g. polatuzumab vedotin), CTLA4 (e.g. ipilimumab), CD22 (e.g. moxetumomab pasudotox), CCR4 (e.g. magamulizumab), IL23p19 (e.g. tildrakizumab), PDL1 (e.g. durvalumab, avelumab, atezolizumab), IL17a (e.g. ixekizumab), CD38 (e.g. daratumumab), SLAMF7 (e.g. elotuzumab), CD20 (e.g. rituximab, tositumomab, ibritumomab and ofatumumab), CD30 (e.g. brentuximab vedotin), CD33 (e.g. gemtuzumab ozogamicin), CD52 (e.g. alemtuzumab), EpCam, CEA, fpA33, TAG-72, CAIX, PSMA, PSA, folate binding protein, GD2 (e.g.

dinutuximab), GD3, IL6 (e.g. siltuximab) GM2, Le^y, VEGF (e.g. bevacizumab), VEGFR, VEGFR2 (e.g. ramucirumab), PDGFR α (e.g. olartumumab), EGFR (e.g. cetuximab, panitumumab and necitumumab), ERBB2 (e.g. trastuzumab), ERBB3, MET, IGF1R, EPHA3, TRAIL R1, TRAIL R2, RANKL RAP, tenascin, integrin α V β 3, and integrin α 4 β 1.

[0196] Examples of antibody therapeutics which are FDA approved and may be used as supplemental agents for use in the treatment of neoplastic disease include those provided in the table below.

Name	Tradename(s)	Target; format	Indication
[fam]-trastuzumab deruxtecan	Enhertu	HER2; Humanized IgG1 ADC	HER2+ breast cancer
Enfortumab vedotin	Padcev	Nectin-4; Human IgG1 ADC	Urothelial cancer
Polatuzumab vedotin	Polivy	CD79b; Humanized IgG1 ADC	Diffuse large B-cell lymphoma
Cemiplimab	Libtayo	PD-1; Human mAb	Cutaneous squamous cell carcinoma
Moxetumomab pasudotox	Lumoxiti	CD22; Murine IgG1 dsFv immunotoxin	Hairy cell leukemia
Mogamulizumab	Poteligeo	CCR4; Humanized IgG1	Cutaneous T cell lymphoma
Tildrakizumab	Ilumya	IL23p19; Humanized IgG1	Plaque psoriasis
Ibalizumab	Trogarzo	CD4; Humanized IgG4	HIV infection
Durvalumab	IMFINZI	PD-L1; Human IgG1	Bladder cancer
Inotuzumab ozogamicin	BESPONSA	CD22; Humanized IgG4, ADC	Hematological malignancy
Avelumab	Bavencio	PD-L1; Human IgG1	Merkel cell carcinoma
Atezolizumab	Tecentriq	PD-L1; Humanized IgG1	Bladder cancer
Olaratumab	Lartruvo	PDGFR α ; Human IgG1	Soft tissue sarcoma
Ixekizumab	Taltz	IL-17a; Humanized IgG4	Psoriasis
Daratumumab	Darzalex	CD38; Human IgG1	Multiple myeloma

Name	Tradename(s)	Target, format	Indication
Elotuzumab	Empliciti	SLAMF7; Humanized IgG1	Multiple myeloma
Necitumumab	Portrazza	EGFR; Human IgG1	Non-small cell lung cancer
Dinutuximab	Unituxin	GD2; Chimeric IgG1	Neuroblastoma
Nivolumab	Opdivo	PD1; Human IgG4	Melanoma, non-small cell lung cancer
Blinatumomab	Blinicyto	CD19, CD3; Murine bispecific tandem scFv	Acute lymphoblastic leukemia
Pembrolizumab	Keytruda	PD1; Humanized IgG4	Melanoma
Ramucirumab	Cyramza	VEGFR2; Human IgG1	Gastric cancer
Siltuximab	Sylvant	IL-6; Chimeric IgG1	Castleman disease
Obinutuzumab	Gazyva	CD20; Humanized IgG1; Glycoengineered	Chronic lymphocytic leukemia
Ado-trastuzumab emtansine	Kadcyla	HER2; Humanized IgG1, ADC	Breast cancer
Pertuzumab	Perjeta	HER2; Humanized IgG1	Breast Cancer
Brentuximab vedotin	Adcetris	CD30; Chimeric IgG1, ADC	Hodgkin lymphoma, systemic anaplastic large cell lymphoma
Ipilimumab	Yervoy	CTLA-4; Human IgG1	Metastatic melanoma
Ofatumumab	Arzerra	CD20; Human IgG1	Chronic lymphocytic leukemia
Certolizumab pegol	Cimzia	TNF; Humanized Fab, pegylated	Crohn disease
Catumaxomab	Removab	EPCAM/CD3; Rat/mouse bispecific mAb	Malignant ascites
Panitumumab	Vectibix	EGFR; Human IgG2	Colorectal cancer
Bevacizumab	Avastin	VEGF; Humanized IgG1	Colorectal cancer

Name	Tradename(s)	Target, format	Indication
Cetuximab	Erbitux	EGFR; Chimeric IgG1	Colorectal cancer
Tositumomab-1131	Bexxar	CD20; Murine IgG2a	Non-Hodgkin lymphoma
Ibritumomab tiuxetan	Zevalin	CD20; Murine IgG1	Non-Hodgkin lymphoma
Gemtuzumab ozogamicin	Mylotarg	CD33; Humanized IgG4, ADC	Acute myeloid leukemia
Trastuzumab	Herceptin	HER2; Humanized IgG1	Breast cancer
Infliximab	Remicade	TNF; Chimeric IgG1	Crohn disease
Rituximab	MabThera, Rituxan	CD20; Chimeric IgG1	Non-Hodgkin lymphoma
Edrecolomab	Panorex	EpCAM; Murine IgG2a	Colorectal cancer

In some embodiments, where the antibody is a bispecific antibody targeting a first and second tumor antigen such as HER2 and HER3 (abbreviated HER2 x HER3), FAP x DR-5 bispecific antibodies, CEA x CD3 bispecific antibodies, CD20 x CD3 bispecific antibodies, EGFR-EDV-miR16 trispecific antibodies, gp100 x CD3 bispecific antibodies, Ny-eso x CD3 bispecific antibodies, EGFR x cMet bispecific antibodies, BCMA x CD3 bispecific antibodies, EGFR-EDV bispecific antibodies, CLEC12A x CD3 bispecific antibodies, HER2 x HER3 bispecific antibodies, Lgr5 x EGFR bispecific antibodies, PD1 x CTLA-4 bispecific antibodies, CD123 x CD3 bispecific antibodies, gpA33 x CD3 bispecific antibodies, B7-H3 x CD3 bispecific antibodies, LAG-3 x PD1 bispecific antibodies, DLL4 x VEGF bispecific antibodies, Cadherin-P x CD3 bispecific antibodies, BCMA x CD3 bispecific antibodies, DLL4 x VEGF bispecific antibodies, CD20 x CD3 bispecific antibodies, Ang-2 x VEGF-A bispecific antibodies,

CD20 x CD3 bispecific antibodies, CD123 x CD3 bispecific antibodies, SSTR2 X CD3 bispecific antibodies, PD1 x CTLA-4 bispecific antibodies, HER2 x HER2 bispecific antibodies, GPC3 x CD3 bispecific antibodies, PSMA x CD3 bispecific antibodies, LAG-3 x PD-L1 bispecific antibodies, CD38 x CD3 bispecific antibodies, HER2 x CD3 bispecific antibodies, GD2 x CD3 bispecific antibodies, and CD33 x CD3 bispecific antibodies. Such therapeutic antibodies may be further conjugated to one or more chemotherapeutic agents

(e.g. antibody drug conjugates or ADCs) directly or through a linker, especially acid, base or enzymatically labile linkers.

In some embodiments, a supplemental agent is one or more non-pharmacological modalities (e.g., localized radiation therapy or total body radiation therapy or surgery). By way of example, the present disclosure contemplates treatment regimens wherein a radiation phase is preceded or followed by treatment with a treatment regimen comprising an IL10R binding molecule and one or more supplemental agents. In some embodiments, the present disclosure further contemplates the use of an IL10R binding molecule in combination with surgery (e.g. tumor resection). In some embodiments, the present disclosure further contemplates the use of an IL10R binding molecule in combination with bone marrow transplantation, peripheral blood stem cell transplantation or other types of transplantation therapy.

In some embodiments, a “supplemental agent” is an immune checkpoint modulator for the treatment and/or prevention neoplastic disease in a subject as well as diseases, disorders or conditions associated with neoplastic disease. The term “immune checkpoint pathway” refers to biological response that is triggered by the binding of a first molecule (e.g. a protein such as PD1) that is expressed on an antigen presenting cell (APC) to a second molecule (e.g. a protein such as PDL1) that is expressed on an immune cell (e.g. a T-cell) which modulates the immune response, either through stimulation (e.g. upregulation of T-cell activity) or inhibition (e.g. downregulation of T-cell activity) of the immune response. The molecules that are involved in the formation of the binding pair that modulate the immune response are commonly referred to as “immune checkpoints.” The biological responses modulated by such immune checkpoint pathways are mediated by intracellular signaling pathways that lead to downstream immune effector pathways, such as cell activation, cytokine production, cell migration, cytotoxic factor secretion, and antibody production. Immune checkpoint pathways are commonly triggered by the binding of a first cell surface expressed molecule to a second cell surface molecule associated with the immune checkpoint pathway (e.g. binding of PD1 to PDL1, CTLA4 to CD28, etc.). The activation of immune checkpoint pathways can lead to stimulation or inhibition of the immune response.

In one embodiment, the immune checkpoint pathway modulator is an antagonist of a negative immune checkpoint pathway that inhibits the binding of PD1 to PDL1 and/or PDL2 (“PD1 pathway inhibitor”). PD1 pathway inhibitors result in the stimulation of a range of favorable immune response such as reversal of T-cell exhaustion, restoration cytokine

production, and expansion of antigen-dependent T-cells. PD1 pathway inhibitors have been recognized as effective variety of cancers receiving approval from the USFDA for the treatment of variety of cancers including melanoma, lung cancer, kidney cancer, Hodgkins lymphoma, head and neck cancer, bladder cancer and urothelial cancer.

The term PD1 pathway inhibitors includes monoclonal antibodies that interfere with the binding of PD1 to PDL1 and/or PDL2. Antibody PD1 pathway inhibitors are well known in the art. Examples of commercially available PD1 pathway inhibitors that monoclonal antibodies that interfere with the binding of PD1 to PDL1 and/or PDL2 include nivolumab (Opdivo®, BMS-936558, MDX1106, commercially available from BristolMyers Squibb, Princeton NJ), pembrolizumab (Keytruda®/MK-3475, lambrolizumab, commercially available from Merck and Company, Kenilworth NJ), and atezolizumab (Tecentriq®, Genentech/Roche, South San Francisco CA). Additional PD1 pathway inhibitors antibodies are in clinical development including but not limited to durvalumab (MEDI4736, Medimmune/AstraZeneca), pidilizumab (CT-011, CureTech), PDR001 (Novartis), BMS-936559 (MDX1105, BristolMyers Squibb), and avelumab (MSB0010718C, Merck Serono/Pfizer) and SHR-1210 (Incyte). Additional antibody PD1 pathway inhibitors are described in United States Patent No. 8,217,149 (Genentech, Inc) issued July 10, 2012; United States Patent No. 8,168,757 (Merck Sharp and Dohme Corp.) issued May 1, 2012, United States Patent No. 8,008,449 (Medarex) issued August 30, 2011, United States Patent No. 7,943,743 (Medarex, Inc) issued May 17, 2011.

In some embodiments, the methods of the disclosure may include the combination of the administration of an IL10R binding molecules with supplemental agents in the form of cell therapies for the treatment of neoplastic, autoimmune or inflammatory diseases. Examples of cell therapies that are amenable to use in combination with the methods of the present disclosure include but are not limited to engineered T cell products comprising one or more activated CAR-T cells, engineered TCR cells, tumor infiltrating lymphocytes (TILs), engineered Treg cells. Cell Therapy Agents and Methods as Supplementary Agents:

In some embodiments, the methods of the disclosure may include the combination of the administration of an IL2R binding molecules with supplementary agents in the form of cell therapies for the treatment of neoplastic, autoimmune or inflammatory diseases. Examples of cell therapies that are amenable to use in combination with the methods of the present disclosure include but are not limited to engineered T cell products comprising one or more activated CAR-T cells, engineered TCR cells, tumor infiltrating lymphocytes (TILs),

engineered Treg cells. As engineered T-cell products are commonly activated *ex vivo* prior to their administration to the subject and therefore provide upregulated levels of CD25, cell products comprising such activated engineered T cells types are amenable to further support via the administration of an CD25 biased IL2R binding molecule as described herein.

In some embodiments of the methods of the present disclosure, the supplementary agent is a “chimeric antigen receptor T-cell” and “CAR-T cell” are used interchangeably to refer to a T-cell that has been recombinantly modified to express a chimeric antigen receptor. As used herein, the terms “chimeric antigen receptor” and “CAR” are used interchangeably to refer to a chimeric polypeptide comprising multiple functional domains arranged from amino to carboxy terminus in the sequence: (a) an antigen binding domain (ABD), (b) a transmembrane domain (TD); and (c) one or more cytoplasmic signaling domains (CSDs) wherein the foregoing domains may optionally be linked by one or more spacer domains. The CAR may also further comprise a signal peptide sequence which is conventionally removed during post-translational processing and presentation of the CAR on the cell surface of a cell transformed with an expression vector comprising a nucleic acid sequence encoding the CAR. CARs useful in the practice of the present invention are prepared in accordance with principles well known in the art. See e.g., Eshhaar et al. United States Patent No. 7,741,465 B1 issued June 22, 2010; Sadelain, et al (2013) *Cancer Discovery* 3(4):388-398; Jensen and Riddell (2015) *Current Opinions in Immunology* 33:9-15; Gross, et al. (1989) *PNAS(USA)* 86(24):10024-10028; Curran, et al. (2012) *J Gene Med* 14(6):405-15. Examples of commercially available CAR-T cell products that may be modified to incorporate an orthogonal receptor of the present invention include axicabtagene ciloleucel (marketed as Yescarta® commercially available from Gilead Pharmaceuticals) and tisagenlecleucel (marketed as Kymriah® commercially available from Novartis).

In some embodiments, the CAR-T cells comprise an antigen binding domain (ABD) refers to a polypeptide that specifically binds to an antigen expressed on the surface of a target cell. In some embodiments, the CAR-T cells useful as supplementary agents comprise and ABD is a polypeptide that specifically binds to a cell surface molecule associated with a tumor cell is selected from the group consisting of GD2, BCMA, CD19, CD33, CD38, CD70, GD2, IL3R α , CD19, mesothelin, Her2, EpCam, Muc1, ROR1, CD133, CEA, EGRFRVIII, PSCA, GPC3, Pan-ErbB and FAP. In some embodiments, the ABD is an antibody (as defined hereinabove to include molecules such as one or more VHHs, scFvs, etc.) that specifically binds to at least one cell surface molecule associated with a tumor cell

(i.e. at least one tumor antigen) wherein the cell surface molecule associated with a tumor cell is selected from the group consisting of GD2, BCMA, CD19, CD33, CD38, CD70, GD2, IL3R α , CD19, mesothelin, Her2, EpCam, Muc1, ROR1, CD133, CEA, EGFRvIII, PSCA, GPC3, Pan-ErbB and FAP.

In some embodiments, the engineered T cell is allogeneic with respect to the individual that is treated. Graham et al. (2018) Cell 7(10) E155. In some embodiments an allogeneic engineered T cell is fully HLA matched. However not all patients have a fully matched donor and a cellular product suitable for all patients independent of HLA type provides an alternative. If the T cells used in the practice of the present invention are allogeneic T cells, such cells may be modified to reduce graft versus host disease. For example, the engineered cells of the present invention may be TCR $\alpha\beta$ receptor knock-outs achieved by gene editing techniques. TCR $\alpha\beta$ is a heterodimer and both alpha and beta chains need to be present for it to be expressed. A single gene codes for the alpha chain (TRAC), whereas there are 2 genes coding for the beta chain, therefore TRAC loci KO has been deleted for this purpose. A number of different approaches have been used to accomplish this deletion, e.g. CRISPR/Cas9; meganuclease; engineered I-CreI homing endonuclease, etc. See, for example, Eyquem et al. (2017) Nature 543:113–117, in which the TRAC coding sequence is replaced by a CAR coding sequence; and Georgiadis et al. (2018) Mol. Ther. 26:1215–1227, which linked CAR expression with TRAC disruption by clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 without directly incorporating the CAR into the TRAC loci. An alternative strategy to prevent GVHD modifies T cells to express an inhibitor of TCR $\alpha\beta$ signaling, for example using a truncated form of CD3 ζ as a TCR inhibitory molecule.

Chemokine and Cytokine Agents as Supplementary Agents:

In some embodiments the IL2R binding molecule is administered in combination with additional cytokines including but not limited to IL-7, IL-12, IL-15 and IL-18 including analogs and variants of each thereof.

Activation-induced Cell Death Inhibitors

In some embodiments the IL2R binding molecule is administered in combination with one or more supplementary agents that inhibit Activation-Induced Cell Death (AICD). AICD is a form of programmed cell death resulting from the interaction of Fas receptors (e.g., Fas, CD95) with Fas ligands (e.g., FasL, CD95 ligand), helps to maintain peripheral immune tolerance. The AICD effector cell expresses FasL, and apoptosis is induced in the

cell expressing the Fas receptor. Activation-induced cell death is a negative regulator of activated T lymphocytes resulting from repeated stimulation of their T-cell receptors. Examples of agents that inhibit AICD that may be used in combination with the IL2R binding molecules described herein include but are not limited to cyclosporin A (Shih, *et al.*, (1989) *Nature* **339**:625–626, IL-16 and analogs (including rhIL-16, Idziorek, *et al.*, (1998) *Clinical and Experimental Immunology* **112**:84-91), TGF β 1 (Genesteir, *et al.*, (1999) *J Exp Med* **189**(2): 231–239), and vitamin E (Li-Weber, *et al.*, (2002) *J Clin Investigation* **110**(5):681-690).

Physical Methods:

In some embodiments, the supplementary agent is a anti-neoplastic physical methods including but not limited to radiotherapy, cryotherapy, hyperthermic therapy, surgery, laser ablation, and proton therapy.

Dosage:

Dosage, toxicity and therapeutic efficacy of such subject IL2R binding molecules or nucleic acids compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with minimal acceptable toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of a subject IL2R binding molecule (i.e., an effective dosage) depends on the polypeptide selected. For instance, single dose amounts in the range of approximately 0.001 to 0.1 mg/kg of patient body weight can be administered; in some embodiments, about 0.005, 0.01, 0.05 mg/kg may be administered. In some embodiments, 600,000 IU/kg is administered (IU can be determined by a lymphocyte proliferation bioassay and is expressed in International Units (IU) as established by the World Health Organization 1st International Standard for Interleukin-2 (human)).

In some embodiments, the pharmaceutically acceptable forms of the IL2R binding molecules of the present disclosure are administered to a subject in accordance with a “low-dose” treatment protocol as described in Klatzman, *et al.* United States Patents Nos. 9,669,071 and 10,293,028B2 the entire teachings of which are herein incorporated by reference. Additional low dose protocols are described in Smith, K.A. (1993) Blood 81(6):1414-1423, He, et al., (2016) Nature Medicine 22(9): 991-993

Prophylactic Applications

In some embodiments where the IL10R binding molecule is used in prophylaxis of disease, the supplementary agent may be a vaccine. The IL10R binding molecule of the present invention may be administered to a subject in combination with vaccines as an adjuvant to enhance the immune response to the vaccine in accordance with the teaching of Doyle, et al United States Patent No 5,800,819 issued September 1, 1998. Examples of vaccines that may be combined with the IL10R binding molecule of the present invention include are HSV vaccines, *Bordetella pertussis*, *Escherichia coli* vaccines, pneumococcal vaccines including multivalent pneumococcal vaccines such as Prevnar® 13, diphtheria, tetanus and pertussis vaccines (including combination vaccines such as Pediatix®) and Pentacel®), varicella vaccines, Haemophilus influenzae type B vaccines, human papilloma virus vaccines such as Garasil®, polio vaccines, Leptospirosis vaccines, combination respiratory vaccine, Moraxella vaccines, and attenuated live or killed virus vaccine products such as bovine respiratory disease vaccine (RSV), multivalent human influenza vaccines such as Fluzone® and Quadravalent Fluzone®, feline leukemia vaccine, transmissible gastroenteritis vaccine, COVID-19 vaccine, and rabies vaccine.

PHARMACEUTICAL FORMULATIONS

[0197] In some embodiments, the subject IL2R binding molecule (and/or nucleic acids encoding the IL2R binding molecule or recombinant cells incorporating a nucleic acid sequence and modified to express the IL2R binding molecule) can be incorporated into compositions, including pharmaceutical compositions. Such compositions typically include the polypeptide or nucleic acid molecule and a pharmaceutically acceptable carrier. A pharmaceutical composition is formulated to be compatible with its intended route of administration and is compatible with the therapeutic use for which the IL2R binding molecule is to be administered to the subject in need of treatment or prophylaxis.

Carriers:

[0198] Carriers include a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants, e.g., sodium dodecyl sulfate. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS).

Buffers:

[0199] The term buffers includes buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as mono- and/or di-basic sodium phosphate, hydrochloric acid or sodium hydroxide (e.g., to a pH of about 7.2-7.8, e.g., 7.5).

Dispersions:

[0200] Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Preservatives:

[0201] The pharmaceutical formulations for parenteral administration to a subject should be sterile and should be fluid to facilitate easy syringability. It should be stable under the conditions of manufacture and storage and are preserved against the contamination. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. Sterile solutions can be prepared by incorporating the active compound in the required amount in an appropriate

solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

Tonicity Agents:

[0202] In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition.

Routes of Administration:

[0203] In some embodiments of the therapeutic methods of the present disclosure involve the administration of a pharmaceutical formulation comprising a IL2R binding molecule (and/or nucleic acids encoding the IL2R binding molecule or recombinantly modified host cells expressing the IL2R binding molecule) to a subject in need of treatment. The pharmaceutical formulation comprising a IL2R binding molecules of the present disclosure may be administered to a subject in need of treatment or prophylaxis by a variety of routes of administration, including parenteral administration, oral, topical, or inhalation routes.

Parenteral Administration:

[0204] In some embodiments, the methods of the present disclosure involve the parenteral administration of a pharmaceutical formulation comprising a IL2R binding molecule (and/or nucleic acids encoding the IL2R binding molecule or recombinantly modified host cells expressing the IL2R binding molecule) to a subject in need of treatment. Examples of parenteral routes of administration include, for example, intravenous, intradermal, subcutaneous, transdermal (topical), transmucosal, and rectal administration. Parenteral formulations comprise solutions or suspensions used for parenteral application can include vehicles the carriers and buffers. Pharmaceutical formulations for parenteral administration include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. In one embodiment, the formulation is provided in a prefilled syringe for parenteral administration.

Oral Administration:

In some embodiments, the methods of the present disclosure involve the oral administration of a pharmaceutical formulation comprising a IL2R binding molecule (and/or nucleic acids encoding the IL2R binding molecule or recombinantly modified host cells

expressing the IL2R binding molecule) to a subject in need of treatment. Oral compositions, if used, generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, e.g., gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel™, or corn starch; a lubricant such as magnesium stearate or Sterotes™; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

Inhalation Formulations:

[0205] In some embodiments, the methods of the present disclosure involve the inhaled administration of a pharmaceutical formulation comprising a IL2R binding molecule (and/or nucleic acids encoding the IL2R binding molecule or recombinantly modified host cells expressing the IL2R binding molecule) to a subject in need of treatment.. In the event of administration by inhalation, subject IL2R binding molecules, or the nucleic acids encoding them, are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer. Such methods include those described in U.S. Pat. No. 6,468,798.

Mucosal and Transdermal Formulations:

[0206] In some embodiments, the methods of the present disclosure involve the mucosal or transdermal administration of a pharmaceutical formulation comprising a IL2R binding molecule (and/or nucleic acids encoding the IL2R binding molecule or recombinantly modified host cells expressing the IL2R binding molecule) to a subject in need of treatment. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery. For transdermal administration,

the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art and may incorporate permeation enhancers such as ethanol or lanolin.

Extended Release and Depot Formulations:

[0207] In some embodiments of the method of the present disclosure, the IL2R binding molecule is administered to a subject in need of treatment in a formulation to provide extended release of the IL2R binding molecule agent. Examples of extended release formulations of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin. In one embodiment, the subject IL2R binding molecules or nucleic acids are prepared with carriers that will protect the IL2R binding molecules against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Such formulations can be prepared using standard techniques. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

Administration of Nucleic Acids Encoding the IL2R Binding Molecule:

[0208] In some embodiments of the method of the present disclosure, delivery of the the IL2R binding molecule to a subject in need of treatment is achieved by the administration of a nucleic acid encoding the IL2R binding molecule. Methods for the administration nucleic acid encoding the IL2R binding molecule to a subject is achieved by transfection or infection using methods known in the art, including but not limited to the methods described in McCaffrey et al. (Nature (2002) 418:6893), Xia et al. (Nature Biotechnol. (2002) 20:1006-1010), or Putnam (Am. J. Health Syst. Pharm. (1996) 53: 151-160 erratum at Am. J. Health Syst. Pharm. (1996) 53:325). In some embodiments, the IL2R binding molecule is administered to a subject by the administration of a pharmaceutically acceptable formulation of recombinant expression vector comprising a nucleic acid sequence encoding the IL2R binding molecule operably linked to one or more expression control sequences operable in a mammalian subject. In some embodiments, the expression control sequence may be selected that is operable in a limited range of cell types (or single cell type) to facilitate the selective

expression of the IL2R binding molecule in a particular target cell type. In one embodiment, the recombinant expression vector is a viral vector. In some embodiments, the recombinant vector is a recombinant viral vector. In some embodiments the recombinant viral vector is a recombinant adenoassociated virus (rAAV) or recombinant adenovirus (rAd), in particular a replication deficient adenovirus derived from human adenovirus serotypes 3 and/or 5. In some embodiments, the replication deficient adenovirus has one or more modifications to the E1 region which interfere with the ability of the virus to initiate the cell cycle and/or apoptotic pathways in a human cell. The replication deficient adenoviral vector may optionally comprise deletions in the E3 domain. In some embodiments the adenovirus is a replication competent adenovirus. In some embodiments the adenovirus is a replication competent recombinant virus engineered to selectively replicate in the target cell type.

[0209] In some embodiments, particularly for administration of IL2R binding molecules to the subject, particular for treatment of diseases of the intestinal tract or bacterial infections in a subject, the nucleic acid encoding the IL2R binding molecule may be delivered to the subject by the administration of a recombinantly modified bacteriophage vector encoding the IL2R binding molecule. As used herein, the terms “procaryotic virus,” “bacteriophage” and “phage” are used interchangeably hereinto describe any of a variety of bacterial viruses that infect and replicate within a bacterium. Bacteriophage selectively infect procaryotic cells, restricting the expression of the IL2R binding molecule to procaryotic cells in the subject while avoiding expression in mammalian cells. A wide variety of bacteriophages capable of selection a broad range of bacterial cells have been identified and characterized extensively in the scientific literature. In some embodiments, the phage is modified to remove adjacent motifs (PAM). Elimination of the of Cas9 sequences from the phage genome reduces ability of the Cas9 endonuclease of the target procaryotic cell to neutralize the invading phage encoding the IL2R binding molecule.

Administration of Recombinantly Modified Cells Expressing the IL2R Binding Molecule:

[0210] In some embodiments of the method of the present disclosure, delivery of the the IL2R binding molecule to a subject in need of treatment is achieved by the administration of recombinant host cells modified to express the IL2R binding molecule may be administered in the therapeutic and prophylactic applications described herein. In some embodiments, the recombinant host cells are mammalian cells, e.g., human cells.

[0211] In some embodiments, the nucleic acid sequence encoding the IL2R binding molecule (or vectors comprising same) may be maintained extrachromosomally in the recombinantly modified host cell for administration. In other embodiments, the nucleic acid sequence encoding the IL2R binding molecule may be incorporated into the genome of the host cell to be administered using at least one endonuclease to facilitate incorporation of a nucleic acid sequence into the genomic sequence of the cell. As used herein, the term “endonuclease” is used to refer to a wild-type or variant enzyme capable of catalyzing the cleavage of bonds between nucleic acids within a DNA or RNA molecule, preferably a DNA molecule. Endonucleases are referred to as “rare-cutting” endonucleases when such endonucleases have a polynucleotide recognition site greater than about 12 base pairs (bp) in length, more preferably of 14-55 bp. Rare-cutting endonucleases can be used for inactivating genes at a locus or to integrate transgenes by homologous recombination (HR) i.e. by inducing DNA double-strand breaks (DSBs) at a locus and insertion of exogenous DNA at this locus by gene repair mechanism. Examples of rare-cutting endonucleases include homing endonucleases (Grizot, et al (2009) *Nucleic Acids Research* 37(16):5405-5419), chimeric Zinc-Finger nucleases (ZFN) resulting from the fusion of engineered zinc-finger domains (Porteus M and Carroll D., *Gene targeting using zinc finger nucleases* (2005) *Nature Biotechnology* 23(3):967-973, a TALEN-nuclease, a Cas9 endonuclease from CRISPR system as or a modified restriction endonuclease to extended sequence specificity (Eisenschmidt, et al. 2005; 33(22): 7039--7047).

[0212] In some embodiments, particularly for administration of IL2R binding molecules to the intestinal tract, the IL2R binding molecule may be delivered to the subject by a recombinantly modified procaryotic cell (e.g., *Lactobacillus lacti*). The use of engineered procaryotic cells for the delivery of recombinant proteins to the intestinal tract are known in the art. See, e.g. Lin, et al. (2017) *Microb Cell Fact* 16:148. In some embodiments, the engineered bacterial cell expressing the IL2R binding molecule may be administered orally, typically in aqueous suspension, or rectally (e.g. enema).

Prophylactic Applications

[0213] In some embodiments where the IL2R binding molecule is used in prophylaxis of disease, the supplementary agent may be a vaccine. The IL2R binding molecule of the present invention may be administered to a subject in combination with vaccines as an adjuvant to enhance the immune response to the vaccine in accordance with the teaching of Doyle, et al

United States Patent No 5,800,819 issued September 1, 1998. Examples of vaccines that may be combined with the IL2R binding molecule of the present invention include are HSV vaccines, Bordetella pertussis, Escherichia coli vaccines, pneumococcal vaccines including multivalent pneumococcal vaccines such as Prevnar® 13, diphtheria, tetanus and pertussis vaccines (including combination vaccines such as Pediatix®) and Pentacel®), varicella vaccines, Haemophilus influenzae type B vaccines, human papilloma virus vaccines such as Garasil®, polio vaccines, Leptospirosis vaccines, combination respiratory vaccine, Moraxella vaccines, and attenuated live or killed virus vaccine products such as bovine respiratory disease vaccine (RSV), multivalent human influenza vaccines such as Fluzone® and Quadrivalent Fluzone®, feline leukemia vaccine, transmissible gastroenteritis vaccine, COVID-19 vaccine, and rabies vaccine.

[0214] Kits: The present disclosure also contemplates kits comprising pharmaceutical compositions IL2R binding molecules and a pharmaceutical composition thereof. The kits are generally in the form of a physical structure housing various components, as described below, and can be utilized, for example, in practicing the methods described above. A kit may comprise a IL2R binding molecule in the form of a pharmaceutical composition suitable for administration to a subject that is ready for use or in a form or requiring preparation for example, thawing, reconstitution or dilution prior to administration. When the IL2R binding molecule is in a form that needs to be reconstituted by a user, the kit may also comprise a sterile container providing a reconstitution medium comprising buffers, pharmaceutically acceptable excipients, and the like. A kit of the present disclosure can be designed for conditions necessary to properly maintain the components housed therein (e.g., refrigeration or freezing). A kit may further contain a label or packaging insert including identifying information for the components therein and instructions for their use. Each component of the kit can be enclosed within an individual container, and all of the various containers can be within a single package. Labels or inserts can include manufacturer information such as lot numbers and expiration dates. The label or packaging insert can be, e.g., integrated into the physical structure housing the components, contained separately within the physical structure, or affixed to a component of the kit (e.g., an ampule, syringe or vial). Labels or inserts may be provided in a physical form or a computer readable medium. In some embodiments, the actual instructions are not present in the kit, but rather the kit provides a means for obtaining the instructions from a remote source, e.g., via an internet site, including by secure access by providing a password (or scannable code such as a barcode or QR code on the container of the

IL2R binding molecule or kit comprising) in compliance with governmental regulations (e.g., HIPAA) are provided.

[0215] It is intended that every maximum numerical limitation given throughout this specification includes every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

[0216] No admission is made that any reference cited herein constitutes prior art. The discussion of the references states what their authors assert, and the inventors reserve the right to challenge the accuracy and pertinence of the cited documents. It will be clearly understood that, although a number of information sources, including scientific journal articles, patent documents, and textbooks, are referred to herein; this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

[0217] The discussion of the general methods given herein is intended for illustrative purposes only. Other alternative methods and alternatives will be apparent to those of skill in the art upon review of this disclosure.

EXAMPLES

Example 1 – V_HH Generation

[0218] Camels were acclimated at research facility for at least 7 days before immunization. Antigen was diluted with 1×PBS (antigen total about 1 mg). The quality of the antigen was assessed by SDS-PAGE to ensure purity (e.g., >80%). For the first time, 10 mL CFA (then followed 6 times using IFA) was added into mortar, then 10 mL antigen in 1×PBS was slowly added into the mortar with the pestle grinding. The antigen and CFA/IFA were ground until the component showed milky white color and appeared hard to disperse. Camels were injected with antigen emulsified in CFA subcutaneously at at least six sites on the body, injecting about 2 mL at each site (total of 10 mL per camel). A stronger immune response was generated by injecting more sites and in larger volumes. The immunization was conducted every week (7 days), for 7 times. The needle was inserted into the subcutaneous space for 10

to 15 seconds after each injection to avoid leakage of the emulsion. Alternatively, a light pull on the syringe plunger also prevented leakage. The blood sample was collected three days later after 7th immunization.

[0219] After immunization, the library was constructed. Briefly, RNA was extracted from blood and transcribed to cDNA. The VHH regions were obtained via two-step PCR, which fragment about 400 bp. The PCR outcomes and the vector of pMECS phagemid were digested with Pst I and Not I, subsequently, ligated to pMECS/Nb recombinant. After ligation, the products were transformed into Escherichia coli (E. coli) TG1 cells by electroporation. Then, the transformants were enriched in growth medium and planted on plates. Finally, the library size was estimated by counting the number of colonies.

[0220] Library biopanning was conducted to screen candidates against the antigens after library construction. Phage display technology was applied in this procedure. Positive colonies were identified by PE-ELISA.

Example 2 – Recombinant Production and Purification

[0221] Codon optimized DNA inserts were cloned into modified pcDNA3.4 (Genscript) for small scale expression in HEK293 cells in 24 well plates. The binding molecules were purified in substantial accordance with the following procedure. Using a Hamilton Star automated system, 96 x 4 mL of supernatants in 4 x 24-well blocks were re-arrayed into 4 x 96-well, 1 mL blocks. PhyNexus micropipette tips (Biotage, San Jose CA) holding 80 µL of Ni-Excel IMAC resin (Cytiva) are equilibrated wash buffer: PBS pH 7.4, 30 mM imidazole. PhyNexus tips were dipped and cycled through 14 cycles of 1 mL pipetting across all 4 x 96-well blocks. PhyNexus tips were washed in 2 x 1 mL blocks holding wash buffer. PhyNexus tips were eluted in 3 x 0.36 mL blocks holding elution buffer: PBS pH 7.4, 400 mM imidazole. PhyNexus tips were regenerated in 3 x 1 mL blocks of 0.5 M sodium hydroxide.

[0222] The purified protein eluates were quantified using a Biacore® T200 as in substantial accordance with the following procedure. 10 µL of the first 96 x 0.36 mL eluates were transferred to a Biacore® 96-well microplate and diluted to 60 µL in HBS-EP+ buffer (10 mM Hepes pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.05% Tween 20). Each of the 96 samples was injected on a CM5 series S chip previously functionalized with anti-histidine capture antibody (Cytiva): injection is performed for 18 seconds at 5 µL/min. Capture levels were recorded 60 seconds after buffer wash. A standard curve of known VHH concentrations (270, 90, 30, 10, 3.3, 1.1 µg/mL) was acquired in each of the 4 Biacore chip flow cells to

eliminate cell-to-cell surface variability. The 96 captures were interpolated against the standard curve using a non-linear model including specific and unspecific, one-site binding. Concentrations in the first elution block varied from 12 to 452 $\mu\text{g}/\text{mL}$ corresponding to a 4-149 μg . SDS-PAGE analysis of 5 randomly picked samples was performed to ensure molecular weight of eluates corresponded to expected values (~30 kDa).

[0223] The concentration of the proteins was normalized using the Hamilton Star automated system in substantial accordance with the following procedure. Concentration values are imported in an Excel spreadsheet where pipetting volumes were calculated to perform dilution to 50 $\mu\text{g}/\text{mL}$ in 0.22 mL. The spreadsheet was imported in a Hamilton Star method dedicated to performing dilution pipetting using the first elution block and elution buffer as diluent. The final, normalized plate was sterile filtered using 0.22 μm filter plates (Corning).

Example 3 – ELISA Studies for IL2Rb VHH

[0224] The single domain antibodies of the present disclosure were obtained from camels by immunization with an extracellular domain of a IL2Rb receptor. IL2Rb VHH molecules of the present disclosure of the present disclosure were generated in substantial accordance with the teaching of the Examples. Briefly, a camel was sequentially immunized with the ECD of the human IL2Rb and mouse IL2Rb over a period several weeks of by the subcutaneous an adjuvanted composition containing a recombinantly produced fusion proteins comprising the extracellular domain of the IL2Rb, the human IgG1 hinge domain and the human IgG1 heavy chain Fc. Following immunization, RNAs extracted from a blood sample of appropriate size VHH-hinge-CH2-CH3 species were transcribed to generate DNA sequences, digested to identify the approximately 400bp fragment comprising the nucleic acid sequence encoding the VHH domain was isolated. The isolated sequence was digested with restriction endonucleases to facilitate insertion into a phagemid vector for in frame with a sequence encoding a his-tag and transformed into E. coli to generate a phage library. Multiple rounds of biopanning of the phage library were conducted to identify VHHs that bound to the ECD of IL2Rb (human or mouse as appropriate). Individual phage clones were isolated for periplasmic extract ELISA (PE-ELISA) in a 96-well plate format and selective binding confirmed by colorimetric determination. The IL2Rb binding molecules that demonstrated specific binding to the IL2Rb antigen were isolated and sequenced and sequences analyzed to identify VHH sequences, CDRs and identify unique VHH clonotypes. As used herein, the term “clonotypes” refers a collection of binding molecules that originate from the same B-cell

progenitor cell, in particular collection of antigen binding molecules that belong to the same germline family, have the same CDR3 lengths, and have 70% or greater homology in CDR3 sequence. The VHH molecules demonstrating specific binding to the hIL2Rb ECD antigen (anti-human IL2Rb VHHs) and the CDRs isolated from such VHHs are provided in Table 6. The VHH molecules demonstrating specific binding to the mIL2Rb ECD antigen (anti-mouse IL2Rb VHHs) and the CDRs isolated from such VHHs are provided in Table 7. Nucleic acid sequences encoding the VHHs of Table 6 and 7 are provided in Tables 10 and 11, respectively.

[0225] To more fully characterize the binding properties and evaluate binding affinity of the VHH molecules generated in accordance with the foregoing, representative examples of each of the human VHH clonotypes were subjected to analysis by surface plasmon resonance in substantial accordance with the teaching of Example 5 herein. The results of these SPR studies are summarized in Table 6 below.

Example 4. Evaluation of Binding Affinity Via Surface Plasmon Resonance for IL2Rb VHH

[0226] A representative example from each hIL2Rb VHH clonotype generated as described above was selected for evaluation of binding via SPR as follows. Evaluation of binding affinity of the hIL2Rb binding molecules shown in Table 16 was conducted using surface plasmon resonance (SPR) in substantial accordance with the following procedure. All experiments were conducted in 10 mM Hepes, 150 mM NaCl, 0.05% (v/v) Polysorbate 20 (PS20) and 3 mM EDTA (HBS-EP+ buffer) on a Biacore T200 instrument equipped with a Protein A derivatized sensor chip (Cytiva). Mono-Fc VHH ligands were flowed at 5 μ L/min for variable time ranging from 18 to 300 seconds, reaching the capture loads listed in the tables below. Following ligand capture, injections of a 2-fold dilution series of the extracellular domain of the IL2Rb -receptor modified to incorporate a C-terminal poly-His sequence, typically comprising at least five concentrations between 1 μ M and 1 nM, were performed in either high performance or single cycle kinetics mode. Surface regeneration was achieved by flowing 10 mM glycine-HCl, pH 1.5 (60 seconds, 50 μ L/min). Buffer-subtracted sensograms were processed with Biacore T200 Evaluation Software and globally fit with a 1:1 Langmuir binding model (bulk shift set to zero) to extract kinetics and affinity constants (k_a , k_d , K_D). $R_{MAX} < 100$ RU indicates surface density compatible with kinetics analysis. Calculated R_{max} values were generated using the equation: $R_{max} = \text{Load (RU)} \times \text{valency of ligand} \times (\text{Molecular weight of analyte} / \text{Molecular weight of ligand})$. Surface activity was defined as the ratio of

experimental/calculated Rmax. The results of these binding affinity experiments are provided in Table 16.

Table 16. anti-hIL2Rb Mono-Fc VHHs binding to hIL2Rb-his							
Ligand	SEQ ID NO	k_{ON} (1/Ms)	k_{OFF} (1/s)	Affinity (nM)	Rmax (RU)	Load (RU)	R _d
hIL2Rb_VHH1	25	1.98E+07	1.99E-02	1	17.6	62.4	
hIL2Rb_VHH2	26	1.39E+05	2.24E-03	16	5.4	26.8	
hIL2Rb_VHH3	27	1.57E+05	6.99E-03	47	14.7	36.8	
hIL2Rb_VHH4	28	6.00E+05	2.05E-03	3.4	24.4	33.2	
hIL2Rb_VHH5	29	3.82E+06	1.54E-03	0.4	32.3	100	
hIL2Rb_VHH6	30	1.90E+07	2.29E-02	1.2	38.9	96.9	
hIL2Rb_VHH7	31	2.86E+06	3.17E-03	1.1	32.1	98.3	
hIL2Rb_VHH8	32	ND	ND	ND	~5	279	

Example 5 – ELISA Studies for IL2Rg VHH

The single domain antibodies of the present disclosure were obtained from camels by immunization with an extracellular domain of a IL2Rg receptor (CD132). IL2Rg VHH molecules of the present disclosure were generated in substantial accordance with the teaching of the Examples. Briefly, a camel was sequentially immunized with the ECD of the human IL2Rg and mouse IL2Rg over a period several weeks of by the subcutaneous an adjuvanted composition containing a recombinantly produced fusion proteins comprising the extracellular domain of the IL2Rg, the human IgG1 hinge domain and the human IgG1 heavy chain Fc. Following immunization, RNAs extracted from a blood sample of appropriate size VHH-hinge-CH2-CH3 species were transcribed to generate DNA

sequences, digested to identify the approximately 400bp fragment comprising the nucleic acid sequence encoding the VHH domain was isolated. The isolated sequence was digested with restriction endonucleases to facilitate insertion into a phagemid vector for in frame with a sequence encoding a his-tag and transformed into *E. coli* to generate a phage library. Multiple rounds of biopanning of the phage library were conducted to identify VHHs that bound to the ECD of IL2Rg (human or mouse as appropriate). Individual phage clones were isolated for periplasmic extract ELISA (PE-ELISA) in a 96-well plate format and selective binding confirmed by colorimetric determination. The IL2Rg binding molecules that demonstrated specific binding to the IL2Rg antigen were isolated and sequenced and sequences analyzed to identify VHH sequences, CDRs and identify unique VHH clonotypes. As used herein, the term “clonotypes” refers a collection of binding molecules that originate from the same B-cell progenitor cell, in particular collection of antigen binding molecules that belong to the same germline family, have the same CDR3 lengths, and have 70% or greater homology in CDR3 sequence. The VHH molecules demonstrating specific binding to the hIL2Rg ECD antigen (anti-human IL2Rg VHHs) and the CDRs isolated from such VHHs are provided in Table 8. The VHH molecules demonstrating specific binding to the mIL2Rg ECD antigen (anti-mouse IL2Rg VHHs) and the CDRs isolated from such VHHs are provided in Table 9. Nucleic acid sequences encoding the VHHs of Table 8 and 9 are provided in Tables 12 and 13, respectively.

Example 6. Evaluation of Binding Affinity Via Surface Plasmon Resonance for IL2Rg VHH

[0003] To more fully characterize the binding properties and evaluate binding affinity of the VHH molecules generated in accordance with the foregoing, representative examples of each of the human VHH clonotypes were subjected to analysis of by surface plasmon resonance in substantial accordance with the teaching of the examples herein. The results of these SPR studies are summarized in Table 16 below.

Table 17: anti-hIL2Rg Mono-Fc VHHs binding to hIL2Rg-his (Antigen: Sino Biological, Catalog# 10555)							
Ligand	SEQ ID NO	k_{ON} (1/Ms)	k_{OFF} (1/s)	Affinity (nM)	Rmax (RU)	Load (RU)	C Rmax (R
hIL- 2Rg_VHH8	306	3.66E+05	8.12E- 04	2.2	41.6	49.3	1

hIL- 2Rg_VHH15	313	9.68E+04	2.51E- 03	26	47.1	84	6
hIL- 2Rg_VHH19	317	2.85E+06	6.93E- 03	2.4	7	28	1
hIL- 2Rg_VHH20	318	1.92E+05	2.70E- 03	14.1	227	103	47
hIL- 2Rg_VHH21	319	6.56E+04	1.13E- 03	17.2	14.6	57.5	24
hIL- 2Rg_VHH22	320	5.54E+05	3.54E- 03	6.4	39.6	47.8	1

[0004] As illustrated by the data presented in Table 17, the hIL2Rg binding molecules generated in accordance with the teaching of present disclosure exhibit specific binding and provided a range of affinities to the the extracellular domain of hIL2Rg.

Example 7 Evaluation of Binding Affinity Via Surface Plasmon Resonance for IL2IL2Rg VHH

Murine Dimer Constructs

Additional experiments were conducted with murine dimer constructs. All experiments were conducted in 10 mM Hepes, 150 mM NaCl, 0.05% (v/v) Polysorbate 20 (PS20) and 3 mM EDTA (HBS-EP+ buffer) on a Biacore T200 instrument equipped with Protein A or CAP biotin chips (Cytiva). For experiments on Protein A chips, Fc-fused ligands were flowed at 5 μ L/min for variable time ranging from 18 to 300 seconds, reaching the capture loads listed in the tables below.

Following ligand capture, injections of a 2-fold dilution series of analyte typically comprising at least five concentrations between 1 μ M and 1 nM were performed in either high performance or single cycle kinetics mode. Surface regeneration was achieved by flowing 10 mM glycine-HCl, pH 1.5 (60 seconds, 50 μ L/min). Buffer-subtracted sensograms were processed with Biacore T200 Evaluation Software and globally fit with a 1:1 Langmuir binding

model (bulk shift set to zero) to extract kinetics and affinity constants (k_a , k_d , KD). $R_{MAX} < 100$ RU indicates surface density compatible with kinetics analysis.

[0001] Experiments on CAP chips were performed as described above with an additional capture step of Biotin CAPture reagent (10 seconds, 40 uL/min) performed prior to capture of biotinylated ligands.

[0002] Calculated R_{max} were generated using the equation $R_{max} = \text{Load (RU)} \times \text{valency of ligand} \times (\text{Molecular weight of analyte} / \text{Molecular weight of ligand})$. Surface activity was defined as the ratio experimental/calculated R_{max} . See tables below for sample information and experimental results.

[0003] Results for Anti-mIL2Rb/mIL2Rg dual VHHs binding to mIL2Rb-Fc were as follows:

Analyte	Ligand	k_{ON} (1/Ms)	k_{OFF} (1/s)	Affinity (nM)	R_{max} (RU)	Load (RU)	alc. R_{max} (RU)	Surface Activity
DR 870-his	mIL2Rb-Fc (SinoBiological cat#50792)	4.0E+04	1.6E-03	39	13.9	181	169	8%
DR 871-his		<5.2E+05	9.9E-04	>1.9	18.4	175	164	11%
DR 873-his		5.2E+04	1.6E-03	31	16.6	172	160	10%

[0004] Results for Anti-mIL2Rb/mIL2Rg dual VHHs binding to mIL2Rg-Fc (Sino Biological, catalog#50087) were as follows:

Analyte	Ligand	k_{ON} (1/Ms)	k_{OFF} (1/s)	Affinity (nM)	R_{max} (RU)	Load (RU)	alc. R_{max} (RU)	Surface Activity
DR 870-his	mIL2Rg-Fc (SinoBiological cat#50087)	3.2E+06	1.6E-03	0.49	68.5	198	148	46%
DR 871-his		2.8E+06	<1E-05	<0.01	87.8	200	149	59%
DR 873-his		6.1E+06	4.4E-04	0.072	80.9	195	146	56%

Table 3: Anti hIL10Ra/hIL10Rb dual VHH binding to human and cynomolgus IL10Ra and IL10Rb

Analyte	Ligand	k_{ON} (1/Ms)	k_{OFF} (1/s)	Affinity (nM)	Rmax (RU)	Load (RU)
hIL10Ra_VHH17 Q1E N29Q- hIL10Rb_VHH16-OPEG lot#P210301BJ2	hIL10Ra- Fc (R&D Systems cat #9044)	2.1E+06	4.7E- 04	0.23	51.2	127
	cIL10Ra- Fc (lot#P210115SV6)	2.5E+06	6.6E- 04	0.27	63.1	146
	hIL10Rb- Fc (SinoBiological cat#10945)	7.7E+05	1.6E- 03	2.05	52.1	116
	cIL10Rb- Fc (lot# P210115SV7)	7.5E+05	1.5E- 03	2.01	46.9	89.6

Table 4: Anti-hIL10Ra/hIL10Rb dual VHH binding to hIL10Rb-his (Sino Biological cat#10945-H08H)

Analyte	Ligand	k_{ON} (1/Ms)	k_{OFF} (1/s)	Affinity (nM)	Rmax (RU)	Load (RU)
hIL10Rb- his (Sino Biological cat#10945)	hIL10Ra_VHH17 -hIL10Rb_VHH16 biotin	1.6E+05	4.5E- 04	2.9	30	62
	hIL10Rb_VHH12 -hIL10Ra_VHH8 biotin	4.4E+05	6.8E- 04	1.5	32	53
	hIL10Rb_VHH1 -hIL10Ra_VHH9 biotin	6.6E+05	1.8E- 03	2.7	31	90
	hIL10Ra_VHH12 -hIL10Rb_VHH27 biotin	1.6E+06	3.0E- 03	1.9	100	84

Example xx. Evaluation of Activity of IL-2 VHH dimers on NKL cells

[0005] The IL2 VHH dimers were evaluated for activity in NKL cells (Robertson, et al (1996) Experimental Hematology 24(3):406-15). NKL cells are an IL-2 dependent human cell line that expresses IL-2R β and IL-2R γ chains and can respond to IL-2 by phosphorylation of STAT5 and proliferation.

[0006] NKL were contacted with purified VHH dimers to examine induction of STAT5 phosphorylation as follows: Cells were seeded in growth medium consisting of RPMI 1640 (ThermoFisher), 10 percent fetal bovine serum (ThermoFisher), 1 percent penicillin/streptomycin (ThermoFisher), 1 percent glutamax (ThermoFisher) at 0.5 million cells per ml. After two days of culture, cells were seeded into 96-well plates (Falcon) at 100 thousand cells per well in 90 μ l DPBS prewarmed at 37 degrees centigrade. Ten μ l of each of the 120 purified VHH dimers in DPBS at 300 nM was added to the cells and plates were transferred to a humidified incubator (ThermoFisher) and incubated at 37 degrees centigrade, 5 percent carbon dioxide for 20 minutes.

[0007] Plates were removed from the incubator and 100 μ l 2x Complete Lysis buffer (Tris Lysis Buffer, Protease Inhibitor Solution, Phosphatase Inhibitor I, Phosphatase Inhibitor II) was added according to manufacturer's instructions (MSD Phospho-STAT Panel K15202D). Plates were incubated on ice for 15 minutes and centrifuged for 5 minutes at 600 x g and Lysates were transferred to a new 96 well plate.

[0008] The level of phospho-STAT5 induction in the lysate was measured using the MSD multi-spot assay system with the Phospho-STAT panel kit (K15202D) according to manufacturer's instructions. MSD 96 well assay plates were washed 3 times with 1x Tris wash buffer and 150 μ l Blocker A solution was added to each well. Plates were incubated on an orbital shaker (VWR Scientific) for 60 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. Cell lysates (25 μ l) were added to the plate. Plates were incubated on an orbital shaker (VWR Scientific) for 60 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. Detection antibody solution (25 μ l) was added to the plate. Plates were incubated on an orbital shaker (VWR Scientific) for 60 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. 150 μ l 1x Read Buffer T was added to each well and emitted light intensity was read in luminescence units on a MSD Quickplex SQ120 instrument.

[0009] For measurement of proliferation, NKL were contacted with purified VHH dimers as follows: Cells were seeded in growth medium consisting of RPMI 1640 (ThermoFisher), 10 percent fetal bovine serum (ThermoFisher), 1 percent penicillin/streptomycin (ThermoFisher), 1 percent glutamax (ThermoFisher) at 0.5 million cells per ml. After two days of culture, cells were seeded into 96-well plates (Falcon) at 25 thousand cells per well in 90 μ l growth medium. Ten μ l of each of the 120 purified VHH dimers in DPBS at 300 nM was added to the cells and plates were transferred to a humidified incubator (ThermoFisher) and incubated at 37 degrees centigrade, 5 percent carbon dioxide for 72 hrs.

[0010] Plates were removed from the incubator and kept at room temperature for 30 minutes. Cells were lysed by adding 100 μ l per well of Celltiterglo (Promega). Cell lysates were mixed on an orbital shaker (VWR Scientific) for two minutes at 200 rpm then held at room temperature for 10 minutes. Luminescence for NKL lysates were read as counts per second in an Envision 2103 Multilabel Plate Reader (Perkin Elmer).

[0011] To compare the effect of each IL-2 VHH dimer upon pSTAT5 induction and NKL cell proliferation, luminescence values from pSTAT and celltiterglo measurements were compared to those obtained for control cells treated with growth medium alone and control cells treated with human IL-2 at 100 pM. IL-2 VHH dimers were identified that induced higher luminescence signals for pSTAT5 induction and cell proliferation than media control but lower than IL-2 at the concentrations used. The data from these experiments is presented in Table x.

Table x. Activity of VHH dimers on NKL cell pSTAT5 induction and proliferation.			
VHH SEQ ID	N-terminal VHH- C-terminal VHH	pSTAT5 (LU)	Proliferation (LU)
DR632	DR229-DR214	595	116506
DR633	DR229-DR217	870	138124
DR634	DR229-DR583	1189	136398
DR635	DR229-DR584	6264	178418
DR636	DR229-DR585	912	115410

Table x. Activity of VHH dimers on NKL cell pSTAT5 induction and proliferation.			
VHH SEQ ID	N-terminal VHH- C-terminal VHH	pSTAT5 (LU)	Proliferation (LU)
DR637	DR229-DR586	850	122216
DR638	DR229-DR587	4495	148994
DR639	DR229-DR588	3077	177470
DR640	DR229-DR589	1145	137282
DR641	DR229-DR590	697	132522
DR642	DR230-DR214	3212	147492
DR643	DR230-DR217	5300	177402
DR644	DR230-DR583	1034	132806
DR645	DR230-DR584	2968	152106
DR646	DR230-DR585	1095	124118
DR647	DR230-DR586	5234	176110
DR648	DR230-DR587	3966	154232
DR649	DR230-DR588	579	135958
DR650	DR230-DR589	861	138590
DR651	DR230-DR590	742	140664
DR652	DR231-DR214	2626	168884
DR653	DR231-DR217	475	127516
DR654	DR231-DR583	923	144488
DR655	DR231-DR584	968	126880
DR656	DR231-DR585	1366	121026
DR657	DR231-DR586	1041	146186
DR658	DR231-DR587	683	105546
DR659	DR231-DR588	1037	152634
DR660	DR231-DR589	781	126740
DR661	DR231-DR590	888	136314
DR662	DR232-DR214	861	112768

Table x. Activity of VHH dimers on NKL cell pSTAT5 induction and proliferation.			
VHH SEQ ID	N-terminal VHH- C-terminal VHH	pSTAT5 (LU)	Proliferation (LU)
DR663	DR232-DR217	1055	139844
DR664	DR232-DR583	967	119004
DR665	DR232-DR584	1105	143704
DR666	DR232-DR585	1188	130088
DR667	DR232-DR586	1110	136678
DR668	DR232-DR587	944	103200
DR669	DR232-DR588	931	147228
DR670	DR232-DR589	1245	122194
DR671	DR232-DR590	1599	163078
DR672	DR233-DR214	1587	115320
DR673	DR233-DR217	1653	150560
DR674	DR233-DR583	3347	137342
DR675	DR233-DR584	3561	167770
DR676	DR233-DR585	1409	169444
DR677	DR233-DR586	845	135714
DR678	DR233-DR587	6138	184958
DR679	DR233-DR588	1810	156864
DR680	DR233-DR589	2073	122214
DR681	DR233-DR590	1374	153052
DR682	DR234-DR214	1055	110310
DR683	DR234-DR217	1006	140478
DR684	DR234-DR583	725	107838
DR685	DR234-DR584	1070	139478
DR686	DR234-DR585	868	120032
DR687	DR234-DR586	1173	128462
DR688	DR234-DR587	997	136754

Table x. Activity of VHH dimers on NKL cell pSTAT5 induction and proliferation.			
VHH SEQ ID	N-terminal VHH- C-terminal VHH	pSTAT5 (LU)	Proliferation (LU)
DR689	DR234-DR588	1227	149798
DR690	DR234-DR589	1280	126096
DR691	DR234-DR590	917	139710
DR692	DR214-DR229	898	108572
DR693	DR214-DR230	917	130734
DR694	DR214-DR231	984	113610
DR695	DR214-DR232	1262	137054
DR696	DR214-DR233	1603	96674
DR697	DR214-DR234	758	132076
DR698	DR217-DR229	1051	124264
DR699	DR217-DR230	837	141240
DR700	DR217-DR231	881	174410
DR701	DR217-DR232	2092	163990
DR702	DR217-DR233	1078	136290
DR703	DR217-DR234	734	140046
DR704	DR583-DR229	722	118478
DR705	DR583-DR230	1150	136916
DR706	DR583-DR231	752	113692
DR707	DR583-DR232	1119	130934
DR708	DR583-DR233	772	100954
DR709	DR583-DR234	1070	138480
DR710	DR584-DR229	812	113468
DR711	DR584-DR230	1091	134004
DR712	DR584-DR231	992	133920
DR713	DR584-DR232	1044	149190
DR714	DR584-DR233	923	116526

Table x. Activity of VHH dimers on NKL cell pSTAT5 induction and proliferation.			
VHH SEQ ID	N-terminal VHH- C-terminal VHH	pSTAT5 (LU)	Proliferation (LU)
DR715	DR584-DR234	1003	136656
DR716	DR585-DR229	2639	156810
DR717	DR585-DR230	2103	158976
DR718	DR585-DR231	6348	167952
DR719	DR585-DR232	1957	158644
DR720	DR585-DR233	1999	145766
DR721	DR585-DR234	2778	168984
DR722	DR586-DR229	4231	151054
DR723	DR586-DR230	881	125438
DR724	DR586-DR231	5638	180326
DR725	DR586-DR232	910	126750
DR726	DR586-DR233	1132	125440
DR727	DR586-DR234	701	127484
DR728	DR587-DR229	859	104160
DR729	DR587-DR230	948	124594
DR730	DR587-DR231	662	102252
DR731	DR587-DR232	1055	127720
DR732	DR587-DR233	862	108350
DR733	DR587-DR234	771	133262
DR734	DR588-DR229	800	119826
DR735	DR588-DR230	1090	141022
DR736	DR588-DR231	1406	145904
DR737	DR588-DR232	932	136648
DR738	DR588-DR233	847	104204
DR739	DR588-DR234	1020	137990
DR740	DR589-DR229	947	131632

Table x. Activity of VHH dimers on NKL cell pSTAT5 induction and proliferation.			
VHH SEQ ID	N-terminal VHH- C-terminal VHH	pSTAT5 (LU)	Proliferation (LU)
DR741	DR589-DR230	808	144776
DR742	DR589-DR231	1013	135948
DR743	DR589-DR232	938	138268
DR744	DR589-DR233	807	136268
DR745	DR589-DR234	638	132428
DR746	DR590-DR229	1304	132472
DR747	DR590-DR230	1622	157594
DR748	DR590-DR231	890	132586
DR749	DR590-DR232	432	117108
DR750	DR590-DR233	1041	142346
DR751	DR590-DR234	605	114290
Media	n/a	1194	147254
hIL-2	n/a	7571	205850

Example xx. Evaluation of Activity of IL-2 VHH dimers on Primary NK cells.

[0012] The IL2 VHH dimers were evaluated for activity in Primary NK cells isolated from PBMC. Primary NK cells express IL-2R β and IL-2R γ chains and can respond to IL-2 by phosphorylation of STAT5, proliferation and the production of IFN- γ .

[0013] PBMC were isolated from human Buffy Coats or Leucocyte Reduction System Chambers (LRSC) using the Custom Sedimentation Kit (Miltenyi, #130-126-357) and Custom Buffy Coat/LRSC PBMC Isolation kits (Miltenyi, 130-126-448) using protocol Cust5 on an autoMACS Pro Separator (Miltenyi) according to manufacturer's instructions. Purified PBMC were counted on a Vi-cell XR (Beckman Coulter) or Vi-cell Blue (Beckman Coulter) cell viability analyzer.

[0014] NK cells were isolated from human PBMC using CD56 microbeads (Miltenyi, 130-050-401) on an autoMACS Pro Separator (Miltenyi) with protocol possel according to

manufacturer's instructions. Purified NK cells were counted on a Vi-cell XR (Beckman Coulter) or Vi-cell Blue (Beckman Coulter) cell viability analyzer.

[0015] NK cells were contacted with purified VHH dimers to examine induction of STAT5 phosphorylation as follows: Cells were seeded into 96-well plates (Falcon) at 100 thousand cells per well in 95 μ l DPBS prewarmed at 37 degrees centigrade. Five μ l of each of the 120 purified VHH dimers in DPBS at 300 nM was added to the cells and plates were transferred to a humidified incubator (ThermoFisher) and incubated at 37 degrees centigrade, 5 percent carbon dioxide for 20 minutes.

[0016] Plates were removed from the incubator and 100 μ l 2x Complete Lysis buffer (Tris Lysis Buffer, Protease Inhibitor Solution, Phosphatase Inhibitor I, Phosphatase Inhibitor II) was added according to manufacturer's instructions (MSD Phospho-STAT Panel K15202D). Plates were incubated on ice for 15 minutes and centrifuged for 5 minutes at 600 x g and Lysates were transferred to a new 96 well plate.

[0017] The level of phospho-STAT5 induction in the lysate was measured using the MSD multi-spot assay system with the Phospho-STAT panel kit (K15202D) according to manufacturer's instructions. MSD 96 well assay plates were washed 3 times with 1x Tris wash buffer and 150 μ l Blocker A solution was added to each well. Plates were incubated on an orbital shaker (VWR Scientific) for 60 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. Cell lysates (25 μ l) were added to the plate. Plates were incubated on an orbital shaker (VWR Scientific) for 60 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. Detection antibody solution (25 μ l) was added to the plate. Plates were incubated on an orbital shaker (VWR Scientific) for 60 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. 150 μ l 1x Read Buffer T was added to each well and emitted light intensity was read in luminescence units on a MSD Quickplex SQ120 instrument.

[0018] For measurement of proliferation, NK were contacted with purified VHH dimers as follows: Cells were seeded into 96-well plates (Falcon) at 100 thousand cells per well in 190 μ l in growth medium consisting of Yssel's medium (Iscove's modified Dulbecco's Medium (ThermoFisher), 0.25% w/v percent human albumin (Sigma), 1 percent penicillin/streptomycin (ThermoFisher), 1 percent ITS-X Insulin, Transferrin, Selenium (Gibco), 30 mg/L Transferrin (Roche), 2 mg/L Palmitic Acid (Sigma), 1 percent LA-OA-Albumin Linoleic Acid, Oleic Acid (Sigma), 1 percent human serum (Gemini) (Yssel et al (1984) J Immunol Methods 72: 219 – 227). Ten μ l of each of the 120 purified VHH dimers in

DPBS at 300 nM was added to the cells and plates were transferred to a humidified incubator (ThermoFisher) and incubated at 37 degrees centigrade, 5 percent carbon dioxide for 72 hrs.

[0019] Plates were removed from the incubator and kept at room temperature for 30 minutes.

One hundred microliter of the cell culture supernatants was transferred to a new 96 well plate for measurement of IFN- γ levels.

[0020] Cells were lysed by adding 100 μ l per well of Celltiterglo (Promega). Cell lysates were mixed on an orbital shaker (VWR Scientific) for two minutes at 200 rpm then held at room temperature for 10 minutes. Luminescence for NK cell lysates were read as counts per second in an Envision 2103 Multilabel Plate Reader (Perkin Elmer).

[0021] The level of IFN- γ in the supernatants was measured using the MSD multi-spot assay system with the V-PLEX human IFN- γ kit (K151QOD-4) according to manufacturer's instructions. MSD 96 well assay plates were washed 3 times with 1x Tris wash buffer and 50 μ l of culture supernatants diluted 100 fold in diluent 2 were added to each well. Plates were incubated on an orbital shaker (VWR Scientific) for 120 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. Detection antibody solution (25 μ l) was added to the plate. Plates were incubated on an orbital shaker (VWR Scientific) for 120 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. 150 μ l 2 x Read Buffer T was added to each well and emitted light intensity was read in luminescence units on a MSD Quickplex SQ120 instrument.

[0022] To compare the effect of each IL-2 VHH dimer upon pSTAT5 induction, NK cell proliferation and IFN- γ production, luminescence values from pSTAT, celltiterglo and IFN- γ measurements were compared to those obtained for control cells treated with growth medium alone and control cells treated with human IL-2 at 100 pM. IL-2 VHH dimers were identified that induced higher luminescence signals for pSTAT5 induction, cell proliferation and IFN- γ production than media control but lower than IL-2 at the concentrations used. The data from these experiments is presented in Table x2.

Table x2. Activity of VHH dimers on NK cell pSTAT5 induction, proliferation and IFN- γ production.				
VHH SEQ ID	VHH DR1 DR2 ID	pSTAT5 (LU)	Proliferation (LU)	IFN- γ Production (LU)
DR632	DR229-DR214	574	59374	144618

Table x2. Activity of VHH dimers on NK cell pSTAT5 induction, proliferation and IFN- γ production.				
VHH SEQ ID	VHH DR1 DR2 ID	pSTAT5 (LU)	Proliferation (LU)	IFN- γ Production (LU)
DR633	DR229-DR217	339	84476	80516
DR634	DR229-DR583	2165	129450	1883810
DR635	DR229-DR584	15080	158792	1911538
DR636	DR229-DR585	3220	66196	36726
DR637	DR229-DR586	280	85292	142020
DR638	DR229-DR587	24366	133116	1907171
DR639	DR229-DR588	3561	140702	1128817
DR640	DR229-DR589	1756	140980	1137944
DR641	DR229-DR590	195	50668	36793
DR642	DR230-DR214	13823	132720	1849412
DR643	DR230-DR217	24065	156030	1850211
DR644	DR230-DR583	556	135388	795501
DR645	DR230-DR584	7741	150146	1960761
DR646	DR230-DR585	1789	72526	129630
DR647	DR230-DR586	21564	155762	1921088
DR648	DR230-DR587	13437	144822	1845712
DR649	DR230-DR588	236	64048	61590
DR650	DR230-DR589	349	47462	23788
DR651	DR230-DR590	338	49600	13376
DR652	DR231-DR214	12830	171010	1901886
DR653	DR231-DR217	223	57866	73604
DR654	DR231-DR583	220	49196	38744
DR655	DR231-DR584	2826	82024	641257
DR656	DR231-DR585	6038	127580	1901425
DR657	DR231-DR586	213	57212	133136
DR658	DR231-DR587	202	46432	83387
DR659	DR231-DR588	214	78720	218983
DR660	DR231-DR589	204	50996	31698

Table x2. Activity of VHH dimers on NK cell pSTAT5 induction, proliferation and IFN- γ production.				
VHH SEQ ID	VHH DR1 DR2 ID	pSTAT5 (LU)	Proliferation (LU)	IFN- γ Production (LU)
DR661	DR231-DR590	192	48910	12924
DR662	DR232-DR214	224	53038	127725
DR663	DR232-DR217	546	118242	787105
DR664	DR232-DR583	1126	91608	431059
DR665	DR232-DR584	221	45064	17316
DR666	DR232-DR585	255	49560	55300
DR667	DR232-DR586	604	117570	658694
DR668	DR232-DR587	277	60456	57260
DR669	DR232-DR588	456	59204	23328
DR670	DR232-DR589	400	81900	215999
DR671	DR232-DR590	1875	138056	1279164
DR672	DR233-DR214	1358	120064	1690293
DR673	DR233-DR217	3071	147252	1195243
DR674	DR233-DR583	5420	123220	1520704
DR675	DR233-DR584	8803	146160	1922798
DR676	DR233-DR585	580	55708	44387
DR677	DR233-DR586	836	107368	451720
DR678	DR233-DR587	16689	160706	1877120
DR679	DR233-DR588	5952	151432	1840663
DR680	DR233-DR589	5639	122932	1574319
DR681	DR233-DR590	1228	83802	79218
DR682	DR234-DR214	1175	94996	266392
DR683	DR234-DR217	480	82018	66068
DR684	DR234-DR583	214	46514	9713
DR685	DR234-DR584	881	97470	451812
DR686	DR234-DR585	246	56082	79541
DR687	DR234-DR586	289	63300	122522
DR688	DR234-DR587	2121	99810	1299512

Table x2. Activity of VHH dimers on NK cell pSTAT5 induction, proliferation and IFN- γ production.				
VHH SEQ ID	VHH DR1 DR2 ID	pSTAT5 (LU)	Proliferation (LU)	IFN- γ Production (LU)
DR689	DR234-DR588	3040	115836	1546871
DR690	DR234-DR589	1389	108534	851698
DR691	DR234-DR590	262	52358	33530
DR692	DR214-DR229	586	80168	341600
DR693	DR214-DR230	336	44468	32214
DR694	DR214-DR231	214	45548	13781
DR695	DR214-DR232	467	57448	50327
DR696	DR214-DR233	356	62564	20592
DR697	DR214-DR234	515	73146	128229
DR698	DR217-DR229	242	65260	373385
DR699	DR217-DR230	307	68008	108166
DR700	DR217-DR231	802	76818	68805
DR701	DR217-DR232	10020	152808	1716480
DR702	DR217-DR233	905	77978	114876
DR703	DR217-DR234	419	86778	390416
DR704	DR583-DR229	242	35080	18481
DR705	DR583-DR230	203	46406	26339
DR706	DR583-DR231	211	34682	13676
DR707	DR583-DR232	193	38418	20801
DR708	DR583-DR233	212	40002	28936
DR709	DR583-DR234	203	47088	25068
DR710	DR584-DR229	195	38422	71711
DR711	DR584-DR230	182	36094	16215
DR712	DR584-DR231	206	42562	17139
DR713	DR584-DR232	201	44564	10805
DR714	DR584-DR233	203	31896	17361
DR715	DR584-DR234	192	37140	30424
DR716	DR585-DR229	3207	135888	1415371

Table x2. Activity of VHH dimers on NK cell pSTAT5 induction, proliferation and IFN- γ production.				
VHH SEQ ID	VHH DR1 DR2 ID	pSTAT5 (LU)	Proliferation (LU)	IFN- γ Production (LU)
DR717	DR585-DR230	2034	125778	1860610
DR718	DR585-DR231	12281	115728	1665366
DR719	DR585-DR232	5818	131414	1334193
DR720	DR585-DR233	2254	136050	995884
DR721	DR585-DR234	10913	137718	1937087
DR722	DR586-DR229	7654	148710	1704185
DR723	DR586-DR230	293	74564	119540
DR724	DR586-DR231	10577	165338	1928068
DR725	DR586-DR232	442	55684	38001
DR726	DR586-DR233	184	36126	54648
DR727	DR586-DR234	195	38650	15553
DR728	DR587-DR229	205	40442	60701
DR729	DR587-DR230	221	42958	54437
DR730	DR587-DR231	210	43068	30615
DR731	DR587-DR232	217	47702	49737
DR732	DR587-DR233	233	42812	596023
DR733	DR587-DR234	221	48304	562814
DR734	DR588-DR229	594	113408	641028
DR735	DR588-DR230	528	119102	757479
DR736	DR588-DR231	1909	138570	1455626
DR737	DR588-DR232	360	84578	308300
DR738	DR588-DR233	266	48734	29645
DR739	DR588-DR234	580	108850	279562
DR740	DR589-DR229	312	115378	517465
DR741	DR589-DR230	647	113032	181177
DR742	DR589-DR231	330	58200	102522
DR743	DR589-DR232	318	47630	12119
DR744	DR589-DR233	801	96038	709867

Table x2. Activity of VHH dimers on NK cell pSTAT5 induction, proliferation and IFN- γ production.				
VHH SEQ ID	VHH DR1 DR2 ID	pSTAT5 (LU)	Proliferation (LU)	IFN- γ Production (LU)
DR745	DR589-DR234	206	47518	552283
DR746	DR590-DR229	375	110814	638377
DR747	DR590-DR230	809	124594	713175
DR748	DR590-DR231	474	129464	407832
DR749	DR590-DR232	231	59380	100488
DR750	DR590-DR233	275	62470	32777
DR751	DR590-DR234	189	40034	30496
Media	n/a	705	37693	16672
hIL-2	n/a	50261	132827	1793477
LU: Luminescence Units. n/a : not applicable				

Example xx. Evaluation of Activity of IL-2 VHH dimers on Primary CD8 positive T cells Blasts.

[0023] The IL2 VHH dimers were evaluated for activity in Primary CD8 T cells isolated from activated PBMC. Primary CD8 positive T cells blasts express IL-2R β and IL-2R γ chains and can respond to IL-2 by phosphorylation of STAT5, proliferation and the production of IFN- γ .

[0024] PBMC were isolated from human Buffy Coats or Leucocyte Reduction System Chambers (LRSC) using the Custom Sedimentation Kit (Miltenyi, #130-126-357) and Custom Buffy Coat/LRSC PBMC Isolation kits (Miltenyi, 130-126-448) using protocol Cust5 on an autoMACS Pro Separator (Miltenyi) according to manufacturer's instructions. Purified PBMC were counted on a Vi-cell XR (Beckman Coulter) or Vi-cell Blue (Beckman Coulter) cell viability analyzer.

[0025] PBMC were cultured on growth medium consisting of Yssel's medium (Iscove's modified Dulbecco's Medium (ThermoFisher), 0.25% w/v percent human albumin (Sigma), 1 percent penicillin/streptomycin (ThermoFisher), 1 percent ITS-X Insulin, Transferrin, Selenium (Gibco), 30 mg/L Transferrin (Roche), 2 mg/L Palmitic Acid (Sigma), 1 percent LA-OA-Albumin Linoleic Acid, Oleic Acid (Sigma), 1 percent human serum (Gemini) (Yssel et al (1984) J Immunol Methods 72: 219 – 227) at 1 million cells per

mL with 1µg/mL anti-CD3 mAb OKT3 (BioXcell) and 1µg/mL anti-CD28 mAb CD28.2 (BioXcell) in 100 mL in a T150 cell culture flask (Falcon) at 37 degrees centigrade, 5 percent carbon dioxide for 5 days.

[0026] Primary CD8 positive T cell blasts were isolated from activated human PBMC using CD8 microbeads (Miltenyi, 130-045-201) on an autoMACS Pro Separator (Miltenyi) with protocol possel according to manufacturer's instructions. Purified primary CD8 T cell blasts were counted on a Vi-cell XR (Beckman Coulter) or Vi-cell Blue (Beckman Coulter) cell viability analyzer.

[0027] Purified primary CD8 T cell blasts were contacted with purified VHH dimers to examine induction of STAT5 phosphorylation as follows: Cells were seeded into 96-well plates (Falcon) at 100 thousand cells per well in 95 µl DPBS prewarmed at 37 degrees centigrade. Five µl of each of the 120 purified VHH dimers in DPBS at 300 nM was added to the cells and plates were transferred to a humidified incubator (ThermoFisher) and incubated at 37 degrees centigrade, 5 percent carbon dioxide for 20 minutes.

[0028] Plates were removed from the incubator and 100 µl 2x Complete Lysis buffer (Tris Lysis Buffer, Protease Inhibitor Solution, Phosphatase Inhibitor I, Phosphatase Inhibitor II) was added according to manufacturer's instructions (MSD Phospho-STAT Panel K15202D). Plates were incubated on ice for 15 minutes and centrifuged for 5 minutes at 600 x g and Lysates were transferred to a new 96 well plate.

[0029] The level of phospho-STAT5 induction in the lysate was measured using the MSD multi-spot assay system with the Phospho-STAT panel kit (K15202D) according to manufacturer's instructions. MSD 96 well assay plates were washed 3 times with 1x Tris wash buffer and 150 µl Blocker A solution was added to each well. Plates were incubated on an orbital shaker (VWR Scientific) for 60 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. Cell lysates (25 µl) were added to the plate. Plates were incubated on an orbital shaker (VWR Scientific) for 60 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. Detection antibody solution (25 µl) was added to the plate. Plates were incubated on an orbital shaker (VWR Scientific) for 60 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. 150 µl 1x Read Buffer T was added to each well and emitted light intensity was read in luminescence units on a MSD Quickplex SQ120 instrument.

[0030] For measurement of proliferation, purified primary CD8 T cell blasts were contacted with purified VHH dimers as follows: Cells were seeded into 96-well plates (Falcon)

at 100 thousand cells per well in 190 μ l in growth medium consisting of Yssel's medium. Ten μ l of each of the 120 purified VHH dimers in DPBS at 300 nM was added to the cells and plates were transferred to a humidified incubator (ThermoFisher) and incubated at 37 degrees centigrade, 5 percent carbon dioxide for 72 hrs.

[0031] Plates were removed from the incubator and kept at room temperature for 30 minutes.

One hundred microliter of the cell culture supernatants was transferred to a new 96 well plate for measurement of IFN- γ levels.

[0032] Cells were lysed by adding 100 μ l per well of Celltiterglo (Promega). Cell lysates were mixed on an orbital shaker (VWR Scientific) for two minutes at 200 rpm then held at room temperature for 10 minutes. Luminescence for primary CD8 T cell blast lysates were read as counts per second in an Envision 2103 Multilabel Plate Reader (Perkin Elmer).

[0033] The level of IFN- γ in the supernatants was measured using the MSD multi-spot assay system with the V-PLEX human IFN- γ kit (K151QOD-4) according to manufacturer's instructions. MSD 96 well assay plates were washed 3 times with 1x Tris wash buffer and 50 μ l of culture supernatants diluted 10 fold in diluent 2 were added to each well. Plates were incubated on an orbital shaker (VWR Scientific) for 120 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. Detection antibody solution (25 μ l) was added to the plate. Plates were incubated on an orbital shaker (VWR Scientific) for 120 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. 150 μ l 2 x Read Buffer T was added to each well and emitted light intensity was read in luminescence units on a MSD Quickplex SQ120 instrument.

[0034] To compare the effect of each IL-2 VHH dimer upon pSTAT5 induction, primary CD8 T cell blast proliferation and IFN- γ production, luminescence values from pSTAT, celltiterglo and IFN- γ measurements were compared to those obtained for control cells treated with growth medium alone and control cells treated with human IL-2 at 100 pM. IL-2 VHH dimers were identified that induced higher luminescence signals for pSTAT5 induction, cell proliferation and IFN- γ production than media control but lower than IL-2 at the concentrations used. The data from these experiments is presented in Table x3.

Table x3. Activity of VHH dimers on primary CD8 T cell blasts pSTAT5 induction, proliferation and IFN- γ production.				
VHH SEQ ID	VHH DR1 DR2 ID	pSTAT5 (LU)	Proliferation (LU)	IFN- γ Production (LU)
DR632	DR229-DR214	1975	74694	357
DR633	DR229-DR217	2802	97528	400
DR634	DR229-DR583	7119	169082	3050
DR635	DR229-DR584	69738	199810	6050
DR636	DR229-DR585	4305	96650	364
DR637	DR229-DR586	3152	96020	407
DR638	DR229-DR587	67754	196596	4429
DR639	DR229-DR588	29763	186708	2653
DR640	DR229-DR589	15645	166848	1413
DR641	DR229-DR590	2566	59040	176
DR642	DR230-DR214	37103	169256	2125
DR643	DR230-DR217	83861	199372	5021
DR644	DR230-DR583	5757	150086	1077
DR645	DR230-DR584	46494	234368	5761
DR646	DR230-DR585	11691	100966	305
DR647	DR230-DR586	88561	207848	4615
DR648	DR230-DR587	55433	193526	3168
DR649	DR230-DR588	2353	89156	388
DR650	DR230-DR589	4356	59650	146
DR651	DR230-DR590	4380	60920	157
DR652	DR231-DR214	62461	214638	5290
DR653	DR231-DR217	2241	68142	356
DR654	DR231-DR583	3512	64188	131
DR655	DR231-DR584	10601	96790	480
DR656	DR231-DR585	31725	149116	2033
DR657	DR231-DR586	5973	68418	193
DR658	DR231-DR587	2572	50934	232

Table x3. Activity of VHH dimers on primary CD8 T cell blasts pSTAT5 induction, proliferation and IFN- γ production.				
VHH SEQ ID	VHH DR1 DR2 ID	pSTAT5 (LU)	Proliferation (LU)	IFN- γ Production (LU)
DR659	DR231-DR588	2262	78852	281
DR660	DR231-DR589	4159	70632	202
DR661	DR231-DR590	4637	61506	186
DR662	DR232-DR214	3144	58280	243
DR663	DR232-DR217	3041	127604	929
DR664	DR232-DR583	10939	99388	378
DR665	DR232-DR584	4927	60210	137
DR666	DR232-DR585	2936	57460	161
DR667	DR232-DR586	3221	123146	979
DR668	DR232-DR587	4857	77298	196
DR669	DR232-DR588	7147	78226	194
DR670	DR232-DR589	3756	90720	349
DR671	DR232-DR590	6860	142454	1306
DR672	DR233-DR214	19482	170126	1188
DR673	DR233-DR217	15296	188860	2153
DR674	DR233-DR583	29097	175798	2296
DR675	DR233-DR584	16694	182374	4191
DR676	DR233-DR585	6983	102448	241
DR677	DR233-DR586	5021	112872	686
DR678	DR233-DR587	71073	196456	5977
DR679	DR233-DR588	9245	172512	4357
DR680	DR233-DR589	35467	140116	3008
DR681	DR233-DR590	14232	110932	411
DR682	DR234-DR214	6798	113696	840
DR683	DR234-DR217	6461	87860	332
DR684	DR234-DR583	3136	48908	166
DR685	DR234-DR584	11098	112190	672

Table x3. Activity of VHH dimers on primary CD8 T cell blasts pSTAT5 induction, proliferation and IFN- γ production.				
VHH SEQ ID	VHH DR1 DR2 ID	pSTAT5 (LU)	Proliferation (LU)	IFN- γ Production (LU)
DR686	DR234-DR585	5015	68192	277
DR687	DR234-DR586	5052	76202	230
DR688	DR234-DR587	20746	113120	1705
DR689	DR234-DR588	29909	153806	933
DR690	DR234-DR589	8260	106106	565
DR691	DR234-DR590	3327	63728	159
DR692	DR214-DR229	7796	81058	283
DR693	DR214-DR230	5864	74190	202
DR694	DR214-DR231	4098	53506	133
DR695	DR214-DR232	4808	73792	184
DR696	DR214-DR233	4670	75228	310
DR697	DR214-DR234	4911	76072	217
DR698	DR217-DR229	4409	59082	172
DR699	DR217-DR230	3101	67000	232
DR700	DR217-DR231	6717	72854	210
DR701	DR217-DR232	40130	151010	2879
DR702	DR217-DR233	6388	83172	208
DR703	DR217-DR234	2112	73744	291
DR704	DR583-DR229	3828	47968	192
DR705	DR583-DR230	3977	53554	199
DR706	DR583-DR231	2996	43828	265
DR707	DR583-DR232	4123	51940	255
DR708	DR583-DR233	4414	50450	166
DR709	DR583-DR234	3552	52870	168
DR710	DR584-DR229	4380	41228	231
DR711	DR584-DR230	3083	45988	231
DR712	DR584-DR231	4739	47854	160

Table x3. Activity of VHH dimers on primary CD8 T cell blasts pSTAT5 induction, proliferation and IFN- γ production.				
VHH SEQ ID	VHH DR1 DR2 ID	pSTAT5 (LU)	Proliferation (LU)	IFN- γ Production (LU)
DR713	DR584-DR232	4168	50962	164
DR714	DR584-DR233	3885	44376	154
DR715	DR584-DR234	3414	49586	228
DR716	DR585-DR229	47906	161498	2271
DR717	DR585-DR230	19829	163070	2292
DR718	DR585-DR231	99121	176680	7211
DR719	DR585-DR232	41612	145996	1674
DR720	DR585-DR233	29633	144204	1251
DR721	DR585-DR234	48028	167206	4122
DR722	DR586-DR229	68270	171340	4087
DR723	DR586-DR230	2669	75522	468
DR724	DR586-DR231	78186	211052	4012
DR725	DR586-DR232	2018	61316	210
DR726	DR586-DR233	3643	49710	227
DR727	DR586-DR234	555	40706	211
DR728	DR587-DR229	3292	34304	251
DR729	DR587-DR230	3154	43390	226
DR730	DR587-DR231	2908	44146	203
DR731	DR587-DR232	1983	46354	206
DR732	DR587-DR233	2854	51346	390
DR733	DR587-DR234	2417	53770	294
DR734	DR588-DR229	4609	91694	676
DR735	DR588-DR230	2445	81412	856
DR736	DR588-DR231	9338	109598	1810
DR737	DR588-DR232	2946	70134	545
DR738	DR588-DR233	2876	55678	218
DR739	DR588-DR234	2011	94516	710

Table x3. Activity of VHH dimers on primary CD8 T cell blasts pSTAT5 induction, proliferation and IFN- γ production.				
VHH SEQ ID	VHH DR1 DR2 ID	pSTAT5 (LU)	Proliferation (LU)	IFN- γ Production (LU)
DR740	DR589-DR229	3880	81458	613
DR741	DR589-DR230	3493	88854	593
DR742	DR589-DR231	2713	56044	297
DR743	DR589-DR232	1579	48310	166
DR744	DR589-DR233	3969	75144	622
DR745	DR589-DR234	1741	46104	283
DR746	DR590-DR229	2869	91978	577
DR747	DR590-DR230	2890	128022	1905
DR748	DR590-DR231	4161	94978	962
DR749	DR590-DR232	1252	47614	366
DR750	DR590-DR233	2182	65174	275
DR751	DR590-DR234	955	41294	220
Media	/a	5263	46428	232
IL-2	/a	130278	143593	8956
LU: Luminescence Units.				
/a : not applicable				

Example xx. Evaluation of Activity of IL-2 VHH dimers on human CD4 positive T cell clone 3F8.

[0035] The IL2 VHH dimers were evaluated for activity in CD4 positive human T cell clone 3F8 cells. The CD4 positive T cell clone 3F8 was generated by activation of PBMC of a healthy donor with the EBV transformed B cell line JY in two successive rounds of Mixed Leukocyte Reactions followed by single cell cloning by limited dilution as described (Yssel and Spits (2002) Current Protocols in Immunology 7.19.1 – 7.19.12). The CD4 positive T cell clone 3F8 expresses IL-2R β and IL-2R γ chains and proliferates in response to IL-2.

[0036] For measurement of proliferation, 3F8 cells were contacted with purified VHH dimers as follows: Cells were grown in growth medium consisting of Yssel's medium (Iscove's modified Dulbecco's Medium (ThermoFisher), 0.25% w/v percent human albumin (Sigma), 1

percent penicillin/streptomycin (ThermoFisher), 1 percent ITS-X Insulin, Transferrin, Selenium (Gibco), 30 mg/L Transferrin (Roche), 2 mg/L Palmitic Acid (Sigma), 1 percent LA-OA-Albumin Linoleic Acid, Oleic Acid (Sigma), 1 percent human serum (Gemini) (Yssel et al (1984) J Immunol Methods 72: 219 – 227) at 0.2 million cells per ml with 50 Gy irradiated JY cells at 0.1 million cells per well and 40 Gy irradiated allogeneic PBMC at 1 million cells per mL. After ten days of culture and expansion with human IL-2 at 100 pM, cells were washed and seeded into black, clear bottom 96 well plates (Costar) at 50 thousand cells per well in 90 µl growth medium. Ten µl of each of the 120 purified VHH dimers in DPBS at 300 nM was added to the cells and plates were transferred to a humidified incubator (ThermoFisher) and incubated at 37 degrees centigrade, 5 percent carbon dioxide for 72 hrs.

[0037] Plates were removed from the incubator and cells were lysed by adding 100 µl per well of Celltiterglo (Promega). Cell lysates were mixed on an orbital shaker (VWR Scientific) for two minutes at 200 rpm then held at room temperature for 10 minutes. Luminescence for 3F8 cell lysates were read as counts per second in an Envision 2103 Multilabel Plate Reader (Perkin Elmer).

[0038] To compare the effect of each IL-2 VHH dimer upon 3F8 cell proliferation, luminescence values from celltiterglo measurements were compared to those obtained for control cells treated with growth medium alone and control cells treated with human IL-2 at 100 pM. IL-2 VHH dimers were identified that induced higher luminescence signals for 3F8 cell proliferation than media control but lower than IL-2 at the concentrations used. The data from these experiments is presented in Table x4.

Table x4. Activity of VHH dimers on human CD4 positive T cell clone 3F8		
VHH SEQ ID	VHH DR1 DR2 ID	Proliferation (LU)
DR632	DR229-DR214	33888
DR633	DR229-DR217	41194
DR634	DR229-DR583	66462
DR635	DR229-DR584	76938
DR636	DR229-DR585	44314

Table x4. Activity of VHH dimers on human CD4 positive T cell clone 3F8		
VHH SEQ ID	VHH DR1 DR2 ID	Proliferation (LU)
DR637	DR229-DR586	46870
DR638	DR229-DR587	87848
DR639	DR229-DR588	71832
DR640	DR229-DR589	64522
DR641	DR229-DR590	43324
DR642	DR230-DR214	84204
DR643	DR230-DR217	79866
DR644	DR230-DR583	57066
DR645	DR230-DR584	72772
DR646	DR230-DR585	47376
DR647	DR230-DR586	73828
DR648	DR230-DR587	72194
DR649	DR230-DR588	48562
DR650	DR230-DR589	45092
DR651	DR230-DR590	41826
DR652	DR231-DR214	71060
DR653	DR231-DR217	31888
DR654	DR231-DR583	37502
DR655	DR231-DR584	41534
DR656	DR231-DR585	78076
DR657	DR231-DR586	53584
DR658	DR231-DR587	40568
DR659	DR231-DR588	52996
DR660	DR231-DR589	41636
DR661	DR231-DR590	41330
DR662	DR232-DR214	49936
DR663	DR232-DR217	71038
DR664	DR232-DR583	69286

Table x4. Activity of VHH dimers on human CD4 positive T cell clone 3F8		
VHH SEQ ID	VHH DR1 DR2 ID	Proliferation (LU)
DR665	DR232-DR584	41762
DR666	DR232-DR585	50210
DR667	DR232-DR586	74854
DR668	DR232-DR587	51260
DR669	DR232-DR588	52574
DR670	DR232-DR589	62090
DR671	DR232-DR590	71850
DR672	DR233-DR214	63810
DR673	DR233-DR217	66648
DR674	DR233-DR583	78250
DR675	DR233-DR584	79716
DR676	DR233-DR585	47408
DR677	DR233-DR586	60714
DR678	DR233-DR587	84816
DR679	DR233-DR588	70776
DR680	DR233-DR589	72106
DR681	DR233-DR590	71270
DR682	DR234-DR214	59464
DR683	DR234-DR217	62038
DR684	DR234-DR583	40188
DR685	DR234-DR584	62736
DR686	DR234-DR585	48414
DR687	DR234-DR586	59830
DR688	DR234-DR587	71834
DR689	DR234-DR588	75204
DR690	DR234-DR589	75612
DR691	DR234-DR590	48538
DR692	DR214-DR229	52982

Table x4. Activity of VHH dimers on human CD4 positive T cell clone 3F8		
VHH SEQ ID	VHH DR1 DR2 ID	Proliferation (LU)
DR693	DR214-DR230	57408
DR694	DR214-DR231	41318
DR695	DR214-DR232	63280
DR696	DR214-DR233	59748
DR697	DR214-DR234	50344
DR698	DR217-DR229	46812
DR699	DR217-DR230	55404
DR700	DR217-DR231	47242
DR701	DR217-DR232	62282
DR702	DR217-DR233	62942
DR703	DR217-DR234	58558
DR704	DR583-DR229	36402
DR705	DR583-DR230	41940
DR706	DR583-DR231	36666
DR707	DR583-DR232	38206
DR708	DR583-DR233	32604
DR709	DR583-DR234	37410
DR710	DR584-DR229	35512
DR711	DR584-DR230	37716
DR712	DR584-DR231	37102
DR713	DR584-DR232	40972
DR714	DR584-DR233	27114
DR715	DR584-DR234	39740
DR716	DR585-DR229	81372
DR717	DR585-DR230	72588
DR718	DR585-DR231	80658
DR719	DR585-DR232	70762
DR720	DR585-DR233	88944

Table x4. Activity of VHH dimers on human CD4 positive T cell clone 3F8		
VHH SEQ ID	VHH DR1 DR2 ID	Proliferation (LU)
DR721	DR585-DR234	72802
DR722	DR586-DR229	74538
DR723	DR586-DR230	50868
DR724	DR586-DR231	70232
DR725	DR586-DR232	54800
DR726	DR586-DR233	35400
DR727	DR586-DR234	40006
DR728	DR587-DR229	35520
DR729	DR587-DR230	52430
DR730	DR587-DR231	34202
DR731	DR587-DR232	42902
DR732	DR587-DR233	44550
DR733	DR587-DR234	49704
DR734	DR588-DR229	38080
DR735	DR588-DR230	55892
DR736	DR588-DR231	87242
DR737	DR588-DR232	65336
DR738	DR588-DR233	40950
DR739	DR588-DR234	94516
DR740	DR589-DR229	81458
DR741	DR589-DR230	88854
DR742	DR589-DR231	56044
DR743	DR589-DR232	48310
DR744	DR589-DR233	75144
DR745	DR589-DR234	46104
DR746	DR590-DR229	91978
DR747	DR590-DR230	128022
DR748	DR590-DR231	94978

Table x4. Activity of VHH dimers on human CD4 positive T cell clone 3F8		
VHH SEQ ID	VHH DR1 DR2 ID	Proliferation (LU)
DR749	DR590-DR232	47614
DR750	DR590-DR233	65174
DR751	DR590-DR234	41294
Media	/a	46428
IL-2	/a	143593

Example xx. Evaluation of Activity of IL-2 VHH dimers on non-activated PBMC.

[0039] The IL2 VHH dimers were evaluated for activity in non-activated PBMC. Several cell types including NK cells, CD4 T cells, CD8 T cell, regulatory T cells and NKT cells express IL-2R β and IL-2R γ chains and can respond to IL-2 by proliferation and the production of IFN- γ .

[0040] PBMC were isolated from human Buffy Coats or Leucocyte Reduction System Chambers (LRSC) using the Custom Sedimentation Kit (Miltenyi, #130-126-357) and Custom Buffy Coat/LRSC PBMC Isolation kits (Miltenyi, 130-126-448) using protocol Cust5 on an autoMACS Pro Separator (Miltenyi) according to manufacturer's instructions. Purified PBMC were counted on a Vi-cell XR (Beckman Coulter) or Vi-cell Blue (Beckman Coulter) cell viability analyzer.

[0041] PBMC from 2 different donors were contacted with purified VHH dimers to examine proliferation and the production of IFN- γ as follows: Cells were seeded into 24-well plates (Corning) at 1 million cells per well in 1 mL growth medium consisting of Yssel's medium (Iscove's modified Dulbecco's Medium (ThermoFisher), 0.25% w/v percent human albumin (Sigma), 1 percent penicillin/streptomycin (ThermoFisher), 1 percent ITS-X Insulin, Transferrin, Selenium (Gibco), 30 mg/L Transferrin (Roche), 2 mg/L Palmitic Acid (Sigma), 1 percent LA-OA-Albumin Linoleic Acid, Oleic Acid (Sigma), 1 percent human serum (Gemini) (Yssel et al (1984) J Immunol Methods 72: 219 – 227). Twenty five μ l of each of the 120 purified VHH dimers in DPBS at 300 nM was added to the cells and plates were transferred to a humidified incubator (ThermoFisher) and incubated at 37 degrees centigrade, 5 percent carbon dioxide for 168 hrs.

[0042] Plates were removed from the incubator and 100 µl of the cell culture supernatants was transferred to a new 96 well plate for measurement of IFN-γ levels.

[0043] To examine proliferation in these cultures, cells were harvested from wells that still contained viable cells upon visual inspection and were phenotyped for CD3, CD4, CD8, CD25 and CD56 expression. Cells were washed in PBS and incubated in PBS with 1/1000 dilution of fix viability dye ef506 for 15 min on ice and quenched in FACS buffer consisting of PBS, 2 mM EDTA, 0.5% BSA. Cells were washed and stained with CD56-BV421, CD25-PE, CD3-BB515, CD4-BV786 and CD8-APC-Cy7 antibody-conjugates (All Biolegend) according to manufacturer's recommendation for 30 min on ice. Cells were washed, fixed with 0.1 % paraformaldehyde and analyzed on an Aurora Flow Cytometer (Cytex) with SpectroFlo software.

[0044] The level of IFN-γ in the supernatants was measured using the MSD multi-spot assay system with the V-PLEX human IFN-γ kit (K151QOD-4) according to manufacturer's instructions. MSD 96 well assay plates were washed 3 times with 1x Tris wash buffer and 50 µl of culture supernatants diluted 100 fold in diluent 2 were added to each well. Plates were incubated on an orbital shaker (VWR Scientific) for 120 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. Detection antibody solution (25 µl) was added to the plate. Plates were incubated on an orbital shaker (VWR Scientific) for 120 minutes at room temperature and washed 3 times with 1 x Tris wash buffer. 150 µl 2 x Read Buffer T was added to each well and emitted light intensity was read in luminescence units on a MSD Quickplex SQ120 instrument.

[0045] To compare the effect of each IL-2 VHH dimer upon IFN-γ production, luminescence values from IFN-γ measurements were compared to those obtained for control cells treated with growth medium alone and control cells treated with human IL-2 at 100 pM. IL-2 VHH dimers were identified that induced higher luminescence signals for IFN-γ production than media control. IL-2 VHH dimers that induced survival and proliferation of PBMC subpopulations were identified and some showed a bias in activity towards T cells. The data from these experiments is presented in Table x5 and Table x6.

Table x5. Activity of VHH dimers on PBMC IFN-γ production and phenotype Donor 1.				
VHH SEQ ID	VHH DR1 DR2 ID	IFN-γ Production (LU)	T cells (% CD3 positive)	NK cells (% CD56 positive)
DR632	DR229-DR214	135		

Table x5. Activity of VHH dimers on PBMC IFN- γ production and phenotype Donor 1.				
VHH SEQ ID	VHH DR1 DR2 ID	IFN- γ Production (LU)	T cells (% CD3 positive)	NK cells (% CD56 positive)
DR633	DR229-DR217	104		
DR634	DR229-DR583	16798	48.49	35.09
DR635	DR229-DR584	186103	47.77	50.35
DR636	DR229-DR585	2396	96.58	2.03
DR637	DR229-DR586	212		
DR638	DR229-DR587	399282	41.98	55.82
DR639	DR229-DR588	5160	50.32	47.22
DR640	DR229-DR589	786		
DR641	DR229-DR590	94		
DR642	DR230-DR214	9540	42.72	55.39
DR643	DR230-DR217	43321	46.6	51.51
DR644	DR230-DR583	14668	62.91	33.69
DR645	DR230-DR584	1462066	36.3	60.92
DR646	DR230-DR585	258		
DR647	DR230-DR586	50480	46	51.99
DR648	DR230-DR587	70954	42.48	55.24
DR649	DR230-DR588	301		
DR650	DR230-DR589	85		
DR651	DR230-DR590	76		
DR652	DR231-DR214	167926	44.52	53.45
DR653	DR231-DR217	633		
DR654	DR231-DR583	275		
DR655	DR231-DR584	18389	74.95	21.18
DR656	DR231-DR585	552204	38.8	59.01
DR657	DR231-DR586	87		
DR658	DR231-DR587	97		
DR659	DR231-DR588	83		
DR660	DR231-DR589	87		

Table x5. Activity of VHH dimers on PBMC IFN- γ production and phenotype Donor 1.				
VHH SEQ ID	VHH DR1 DR2 ID	IFN- γ Production (LU)	T cells (% CD3 positive)	NK cells (% CD56 positive)
DR661	DR231-DR590	82		
DR662	DR232-DR214	94		
DR663	DR232-DR217	283		
DR664	DR232-DR583	113		
DR665	DR232-DR584	79		
DR666	DR232-DR585	161		
DR667	DR232-DR586	239		
DR668	DR232-DR587	81		
DR669	DR232-DR588	247		
DR670	DR232-DR589	90		
DR671	DR232-DR590	526		
DR672	DR233-DR214	1087		
DR673	DR233-DR217	8738	41.99	55.81
DR674	DR233-DR583	2842	38.7	59.4
DR675	DR233-DR584	32239	44.85	53.27
DR676	DR233-DR585	83		
DR677	DR233-DR586	298		
DR678	DR233-DR587	32319	46.56	51.48
DR679	DR233-DR588	121672	36.13	61.88
DR680	DR233-DR589	11020	46.18	52.08
DR681	DR233-DR590	159		
DR682	DR234-DR214	3571	59.92	36.44
DR683	DR234-DR217	94		
DR684	DR234-DR583	82		
DR685	DR234-DR584	516		
DR686	DR234-DR585	168		
DR687	DR234-DR586	85		
DR688	DR234-DR587	2812	39.99	57.85

Table x5. Activity of VHH dimers on PBMC IFN- γ production and phenotype Donor 1.				
VHH SEQ ID	VHH DR1 DR2 ID	IFN- γ Production (LU)	T cells (% CD3 positive)	NK cells (% CD56 positive)
DR689	DR234-DR588	890		
DR690	DR234-DR589	246		
DR691	DR234-DR590	85		
DR692	DR214-DR229	88		
DR693	DR214-DR230	74		
DR694	DR214-DR231	76		
DR695	DR214-DR232	828		
DR696	DR214-DR233	111		
DR697	DR214-DR234	132		
DR698	DR217-DR229	78		
DR699	DR217-DR230	104		
DR700	DR217-DR231	96		
DR701	DR217-DR232	2570	57.26	40.3
DR702	DR217-DR233	90		
DR703	DR217-DR234	162		
DR704	DR583-DR229	85		
DR705	DR583-DR230	83		
DR706	DR583-DR231	81		
DR707	DR583-DR232	80		
DR708	DR583-DR233	77		
DR709	DR583-DR234	82		
DR710	DR584-DR229	67		
DR711	DR584-DR230	181		
DR712	DR584-DR231	94		
DR713	DR584-DR232	117		
DR714	DR584-DR233	1932	92.95	3.96
DR715	DR584-DR234	76		
DR716	DR585-DR229	5178	36.89	61.04

Table x5. Activity of VHH dimers on PBMC IFN- γ production and phenotype Donor 1.				
VHH SEQ ID	VHH DR1 DR2 ID	IFN- γ Production (LU)	T cells (% CD3 positive)	NK cells (% CD56 positive)
DR717	DR585-DR230	14665	48.32	49.33
DR718	DR585-DR231	79834	40.28	58.21
DR719	DR585-DR232	20846	38.28	59.66
DR720	DR585-DR233	734		
DR721	DR585-DR234	68144	40.48	57.36
DR722	DR586-DR229	5762	45.21	53
DR723	DR586-DR230	120		
DR724	DR586-DR231	12094	46.7	51.26
DR725	DR586-DR232	170		
DR726	DR586-DR233	79		
DR727	DR586-DR234	79		
DR728	DR587-DR229	92		
DR729	DR587-DR230	78		
DR730	DR587-DR231	93		
DR731	DR587-DR232	90		
DR732	DR587-DR233	503		
DR733	DR587-DR234	81		
DR734	DR588-DR229	878		
DR735	DR588-DR230	630		
DR736	DR588-DR231	136028	41.38	56.82
DR737	DR588-DR232	567		
DR738	DR588-DR233	224		
DR739	DR588-DR234	1454		
DR740	DR589-DR229	1272	78.22	17.89
DR741	DR589-DR230	2216	79.76	17
DR742	DR589-DR231	296		
DR743	DR589-DR232	98		
DR744	DR589-DR233	1099	85.55	10.34

Table x5. Activity of VHH dimers on PBMC IFN- γ production and phenotype Donor 1.				
VHH SEQ ID	VHH DR1 DR2 ID	IFN- γ Production (LU)	T cells (% CD3 positive)	NK cells (% CD56 positive)
DR745	DR589-DR234	91		
DR746	DR590-DR229	868		
DR747	DR590-DR230	3385	41.18	56.19
DR748	DR590-DR231	834		
DR749	DR590-DR232	90		
DR750	DR590-DR233	115		
DR751	DR590-DR234	98		
Media	n/a	235		
hIL-2	n/a	338863	63.24	34.52
LU: Luminescence Units. n/a : not applicable				

Table x6. Activity of VHH dimers on PBMC IFN- γ production and phenotype Donor 2.				
VHH SEQ ID	VHH DR1 DR2 ID	IFN- γ Production (LU)	T cells (% CD3 positive)	NK cells (% CD56 positive)
DR632	DR229-DR214	117		
DR633	DR229-DR217	163		
DR634	DR229-DR583	5188	62.22	35.73
DR635	DR229-DR584	11871	51.63	46.16
DR636	DR229-DR585	127	97.46	0.3
DR637	DR229-DR586	126		
DR638	DR229-DR587	40748	50.69	47.15
DR639	DR229-DR588	2237	58.85	39.54
DR640	DR229-DR589	3128		
DR641	DR229-DR590	74		
DR642	DR230-DR214	1043	58.95	39.26
DR643	DR230-DR217	12027	50.19	47.74

Table x6. Activity of VHH dimers on PBMC IFN- γ production and phenotype Donor 2.				
VHH SEQ ID	VHH DR1 DR2 ID	IFN- γ Production (LU)	T cells (% CD3 positive)	NK cells (% CD56 positive)
DR644	DR230-DR583	1395	77.48	20
DR645	DR230-DR584	147962	56.88	41.48
DR646	DR230-DR585	105		
DR647	DR230-DR586	24426	51.6	46.43
DR648	DR230-DR587	16661	53.06	45.12
DR649	DR230-DR588	107		
DR650	DR230-DR589	86		
DR651	DR230-DR590	101		
DR652	DR231-DR214	21818	49.74	47.99
DR653	DR231-DR217	265		
DR654	DR231-DR583	81		
DR655	DR231-DR584	8336	81.11	15.93
DR656	DR231-DR585	165479	55.4	42.95
DR657	DR231-DR586	81		
DR658	DR231-DR587	101		
DR659	DR231-DR588	85		
DR660	DR231-DR589	85		
DR661	DR231-DR590	77		
DR662	DR232-DR214	77		
DR663	DR232-DR217	232		
DR664	DR232-DR583	101		
DR665	DR232-DR584	82		
DR666	DR232-DR585	87		
DR667	DR232-DR586	180		
DR668	DR232-DR587	89		
DR669	DR232-DR588	82		
DR670	DR232-DR589	84		
DR671	DR232-DR590	250		

Table x6. Activity of VHH dimers on PBMC IFN- γ production and phenotype Donor 2.				
VHH SEQ ID	VHH DR1 DR2 ID	IFN- γ Production (LU)	T cells (% CD3 positive)	NK cells (% CD56 positive)
DR672	DR233-DR214	323		
DR673	DR233-DR217	1977	65.3	32.57
DR674	DR233-DR583	2565	61.54	36.29
DR675	DR233-DR584	41300	42.86	55.03
DR676	DR233-DR585	82		
DR677	DR233-DR586	242		
DR678	DR233-DR587	18981	53.53	44.54
DR679	DR233-DR588	5117	57.75	40.55
DR680	DR233-DR589	2370	63.81	34.23
DR681	DR233-DR590	154		
DR682	DR234-DR214	1462	68.15	29.23
DR683	DR234-DR217	145		
DR684	DR234-DR583	109		
DR685	DR234-DR584	840		
DR686	DR234-DR585	89		
DR687	DR234-DR586	79		
DR688	DR234-DR587	653	58.97	38.6
DR689	DR234-DR588	332		
DR690	DR234-DR589	132		
DR691	DR234-DR590	73		
DR692	DR214-DR229	101		
DR693	DR214-DR230	88		
DR694	DR214-DR231	76		
DR695	DR214-DR232	78		
DR696	DR214-DR233	92		
DR697	DR214-DR234	117		
DR698	DR217-DR229	82		
DR699	DR217-DR230	173		

Table x6. Activity of VHH dimers on PBMC IFN- γ production and phenotype Donor 2.				
VHH SEQ ID	VHH DR1 DR2 ID	IFN- γ Production (LU)	T cells (% CD3 positive)	NK cells (% CD56 positive)
DR700	DR217-DR231	100		
DR701	DR217-DR232	1227	63.05	35.35
DR702	DR217-DR233	96		
DR703	DR217-DR234	1143		
DR704	DR583-DR229	185		
DR705	DR583-DR230	81		
DR706	DR583-DR231	84		
DR707	DR583-DR232	96		
DR708	DR583-DR233	83		
DR709	DR583-DR234	84		
DR710	DR584-DR229	72		
DR711	DR584-DR230	75		
DR712	DR584-DR231	75		
DR713	DR584-DR232	91		
DR714	DR584-DR233	63	95.35	2.33
DR715	DR584-DR234	228		
DR716	DR585-DR229	1273	55.37	42.69
DR717	DR585-DR230	26060	45.59	51.88
DR718	DR585-DR231	38220	46.94	50.75
DR719	DR585-DR232	1850	88.89	6.13
DR720	DR585-DR233	766		
DR721	DR585-DR234	24693	48.32	49.32
DR722	DR586-DR229	5851	52.03	46.36
DR723	DR586-DR230	173		
DR724	DR586-DR231	3693	57.36	40.62
DR725	DR586-DR232	93		
DR726	DR586-DR233	85		
DR727	DR586-DR234	91		

Table x6. Activity of VHH dimers on PBMC IFN- γ production and phenotype Donor 2.				
VHH SEQ ID	VHH DR1 DR2 ID	IFN- γ Production (LU)	T cells (% CD3 positive)	NK cells (% CD56 positive)
DR728	DR587-DR229	340		
DR729	DR587-DR230	95		
DR730	DR587-DR231	237		
DR731	DR587-DR232	99		
DR732	DR587-DR233	113		
DR733	DR587-DR234	142		
DR734	DR588-DR229	4151		
DR735	DR588-DR230	625		
DR736	DR588-DR231	2595	55.41	42.05
DR737	DR588-DR232	923		
DR738	DR588-DR233	104		
DR739	DR588-DR234	830		
DR740	DR589-DR229	873	86.55	6.04
DR741	DR589-DR230	629	84.17	9.79
DR742	DR589-DR231	198		
DR743	DR589-DR232	82		
DR744	DR589-DR233	625	82.76	8.27
DR745	DR589-DR234	81		
DR746	DR590-DR229	212		
DR747	DR590-DR230	2272	68.05	29.44
DR748	DR590-DR231	1050		
DR749	DR590-DR232	386		
DR750	DR590-DR233	138		
DR751	DR590-DR234	88		
Media	n/a	226		
HL-2	n/a	174981	64.09	33.48
LU: Luminescence Units. n/a : not applicable				

[0005] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, sequence accession numbers, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

TABLES

Table 2 - anti-human IL2Rb sdAb CDRs

Name of VHH	CDR 1	SEQ ID NO:	CDR 2	SEQ ID NO:	CDR 3	SEQ ID NO:
hIL2Rb_VHH-1	YTYDTS DMS	25	DIDSGDWAA YADAVKG	33	SYWKW GKL NNF	41
hIL2Rb_VHH-2	FTFSNYWIF	26	TSNTGGDTT KYADSVKG	34	GRCARSG	42
hIL2Rb_VHH-3	FRFSNYGMS	27	YINGDGSRT HYADSVKG	35	GLSRDGWSL SAAS	43
hIL2Rb_VHH-4	YTTYSFN YM G	28	VIYTGGGSTL YADSVKG	36	DDQRFASPL YAYFGY	44
hIL2Rb_VHH-5	DTKSIRCMG	29	AIDREGFAT YADSVYD	37	QNMCRVVR GAMTGVDY	45
hIL2Rb_VHH-6	YTASRYCMA	30	AIHPGGGTT YYADSVKG	38	GSLWVPFGD RCAANY	46
hIL2Rb_VHH-7	YEYCRIHMT	31	SIGSDGRKTY ANSVTG	39	EYLYGLGCP DGSAY	47
hIL2Rb_VHH-8	YTYSSYYCM G	32	AIDSDGSTSY ADSVKG	40	SYEVVDCYP SGYGQDY	48

Table 3 – murine IL2Rb sdAb CDRs

Name	CDR	SE O ID NO:	CDR	SEQ ID NO:	CDR	SE O ID NO:
DR857	DMS	FTFSLY 49	SAKG GINSGGYSTYYAA	102	RGLTSPYVIPN I	125
DR1448	PLG	KSFSDY 50	TVKG HISWSGKLTYYRS	103	MKLFNYGGRY CVLKPLTMYQQ	126
DR1449	AIG	RSFSGY 51	DSVKG VVSWRGSSTYYA	104	VPSGRSWYG RNRY	127
DR1450	RMG	RSINYY 52	SVKG AIKWGGDGVYAD	105	MPLSSWSRG GYLEV	128
DR1451	NYAMY	RFSWG 53	SAKG AIGRNSMATYYRD	106	KFMVADGWS RQYDY	129
DR1452	MG	RTFRRF 54	DSVKG AINWPGGGTYYG	107	TRKYNLYKFA D	130
DR1453	SMG	RIFNTY 55	SVKG AIRWSGGTYYTD	108	RVRLSNTALL QRY	131
DR1454	PIG	RTFGDY 56	DSVKG SISWGGSRQYYT	109	RVRLSNTALL QRY	132
DR1455	AMG	RTFNSY 57	SVKG VITWNSGRTYYAD	110	APWAHNRE	133
DR1456	YMS	LTFRTY 58	VKG VISWIGSTTLYADS	111	NFLREGKREP RY	134
DR1457	SMG	RIFNTY 59	SVKG AIRWSGGTYYTD	112	RVFARRY	135
DR1458	AMG	RTLSTY 60	DSVKG AIRWASGRTYYG	113	RSRPYLYNGD FGY	136
DR1459	MV	RTISTYA 61	SVKG VISRSGDRTYYAD	114	GGYTGIETITA	137
DR1460	AVY	SIFNNN 62	KG LITIGGRTGYADSV	115	GLKFGNFYYS KTAYDY	138
DR1461	SMG	RIFNTY 63	SVKG AIRWSGGTYYTD	116	VPSGRSWYG RNRY	139
DR1462	AMG	RTFGYV 64	DSVKG SINWSGGSTAYA	117	STRFYATME QGSYDY	140
DR1463	AIG	RSFRSY 65	SLKD AISYDGRRTYYGR	118	HRSGTMFARY GMDY	141
DR1464	AMG	RTFSSY 66	SVKG AISRSGGYTSYAD	119	LIAPFFYGMD Y	142
DR1465	TMG	PTFTSY 67	VKG VISKGGRTYYADS	120	ORVGATSKYE YDY	143
DR1466	WMY	FTFSTD 68	SVKG LINTDGTSTSYTK	121	GRTYWFYAM DY	144
DR1467	MG	RISNYA 69	SVKG VITRSGGSTYYAD	122	RRSQKLVTFG AEYPW	145
DR1468	AMG	RTGTHY 70	SVKG LILWNGEFTTYKD	123	RVFARRY	146
DR1469	WMY	FTFSNY 71	SVKG HINTNGGNTYYRH	124	ANSOVGLGYY GMDY	147
DR1470	AVY	SIFNNN 72	KG LITIGGRTGYADSV	125	RPGYWSSSY DY	148
DR1471	AMA	RAPASY 73	DSVKG AINWSGRRTYYA	126	YLSGTYY	149

DR1472	FMT	LASSSF	74 SVKG	TISWTGRTSYAA	127 PIH	YPRTLVRNRE	150
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Table 4 – human IL2Rg sdAb CDRs

Name	CDR	SE O ID NO:	CDR	SEQ ID NO:	CDR	SE O ID NO:
VHH-1	hIL2Rg SDMG	FTFDD 151	TISSDGGSTYYA DSVKG	174	DFMIAIQAP GAGC	197
VHH-2	hIL2Rg YPMT	FSFSS 152	TIASDGGSTAY AASVEG	175	GYGDGTPA	198
VHH-3	hIL2Rg REMNI	FTFDD 153	TISSDGGSTYYA DSVKG	176	DFMIAIQAP GAGC	199
VHH-4	hIL2Rg SDMG	FTFDD 154	TISSDGNITYYT DSVKG	177	EPRGYYSN YGGRRRECNY	200
VHH-5	hIL2Rg YPMT	FSFSS 155	TIASDGGSTAY AASVEG	178	GYGDGTPA	201
VHH-6	hIL2Rg AHMS	FTFSN 156	SIYSGGSTWY ADSVKG	179	NRLHYYSN DDSL	202
VHH-7	hIL2Rg REMNI	FTFDD 157	TISSDGGSTYYA DSVKG	180	DFMIAIQAP GAGC	203
VHH-8	hIL2Rg YCMG	YTFSS 158	ALGGGSTYYA DSVKG	181	AWVACLEF GGSWYDLARYKH	204
VHH-9	hIL2Rg SDMG	FTFDD 159	TISSDGGSTYYA DSVKG	182	EPRGYYSN YGGRRRECNY	205
VHH-10	hIL2Rg AYIG	SIYSS 160	GIYTRDGSTA YADSVKG	183	GRRTKSYV YIERPEEYNY	206
VHH-11	hIL2Rg AHMS	FTFSS 161	SIYSGGGTFYA DSVKG	184	NRLHYYSN DDSL	207
VHH-12	hIL2Rg AHMS	FTFSN 162	SIYSGGSTWY ADSVKG	185	NRLHYYSN DDSL	208
VHH-13	hIL2Rg SDMG	FIFDD 163	TISSDGGSTYYA DSVKG	186	EPRGYYSN YGGRRRECNY	209
VHH-14	hIL2Rg SDMG	FTADD 164	TISSDGGSTYYA DSVKG	187	EPRGYYSN YGGRRRECNY	210
VHH-15	hIL2Rg AHMS	FTFSS 165	SIYSGGGTFYA DSVKG	188	NRLHYYSN DDSL	211
VHH-16	hIL2Rg AHMS	FTFSN 166	SIYSGGSTWY ADSVKG	189	NRLHYYSN DDSL	212
VHH-17	hIL2Rg AHMS	FTFSN 167	SIYSGGSTWY ADSVKG	190	NRLHYYSN DDSL	213
VHH-18	hIL2Rg YPMT	FTFSS 168	TIASDGGSTAY AASVEG	191	GYGDGTPA	214
VHH-19	hIL2Rg REMNI	FTFDD 169	TISSDGGSTYYA DSVKG	192	DFMIAIQAP GAGC	215
VHH-20	hIL2Rg SDMG	FTFDD 170	TISSDGGSTYYA DSVKG	193	EPRGYYSN YGGRRRECNY	216
VHH-21	hIL2Rg MG	YTSC 171	TIYTRGRSIYY ADSVKG	194	GGYSWSAG CEFNY	217
VHH-22	hIL2Rg YPMT	FSFSS 172	TIASDGGSTAY AASVEG	195	GYGDGTPA	218
VHH-23	hIL2Rg YPMT	FSFSS 173	TIASDGGSTAY AASVEG	196	GYGDGTPA	219

Table 5 – mouse IL2Rg sdAb CDRs

me	Na	CDR 1	S	CDR 2	S	CDR 3	SEQ
			EQ ID NO:		EQ ID NO:		ID NO:
R604	D	YGYN	220	VIYTGGS	235	SVYACLR	250
	YIG			DTYYADSVKG		GGHDEYAH	
L2Rg_VH	ml	STYAN	221	AIYSGGGS	236	ASAVKGD	251
H2	YLMG			TYADSVKG		KGDIVVVVTGTQR	
						MEYDY	
L2Rg_VH	ml	FTFDE	222	IISDDNT	237	RRRRPVY	252
H3	SVMS			YYDSVKG		DSDYELRPRPLCG	
						DFGV	
L2Rg_VH	ml	LPFDE	223	SISSDGT	238	GVHRQFG	253
H4	DDMG			YYADSVKG		GSSSCGDAFYGM	
						DY	
L2Rg_VH	ml	DVYG	224	VGYSVVT	239	DGNLWRG	254
H5	RNSMA			TTYADSVKG		LRPSEYTY	
L2Rg_VH	ml	FPYSR	225	AIEPDGS	240	DERCFYL	255
H6	YCMG			SYADSVKG		KDYDLRRPAQYR	
						Y	
L2Rg_VH	ml	FTFDE	226	VITSDDNP	241	RSRQPVYS	256
H7	SDMG			YYDSVKG		RDYELRPRPLCGD	
						FGV	
L2Rg_VH	ml	FTFDD	227	TISDDGS	242	EGALGSK	257
H8	FDMG			YYADSVKG		TNCGWVGNGFY	
L2Rg_VH	ml	FTFDD	228	TISDDGS	243	EGALGSK	258
H9	FDMG			YYADSVKG		TNCGWVGNGFY	
L2Rg_VH	ml	FTFDD	229	TISDDGS	244	EGALGSK	259
H10	FDMG			YYADSVKG		TNCGWVGNGFY	
L2Rg_VH	ml	FTFSD	230	TISDDGS	245	EGALGSK	260
H11	RDMG			YYADSVKG		TNCGWVGNGFY	
L2Rg_VH	ml	YGYN	231	VIYIGGSD	246	RYCVGSV	261
H12	YIG			TYADSVKG		YACLRGGHDEYA	
						H	
L2Rg_VH	ml	YGYN	232	VIYTGGS	247	RYCVGSV	262
H13	YIG			DTYYADSVKG		YACLRGGHDEYA	
						H	
L2Rg_VH	ml	FTFDD	233	TISDDGS	248	EGALGSK	263
H14	FDMG			YYANSVKG		TNCGWVGNGFY	
L2Rg_VH	ml	FTFDD	234	TISDDGS	249	EGALGSK	264
H15	FDMG			YYADSVKG		MNCGWVGNGFY	

Table 6- human anti-IL2Rb VHH Amino Acid Sequences

Name	VHH Sequence	SEQ ID NO.
hIL2 Rb_VHH-1	QVQLQESGGGSVQAGGSLRLSC VSGGYTYDTS ~~DM~~ SWYRQAPGKEREFS ~~D~~ IDSGDWAAYADAVKGRFTISRDNAAK TVYLQMNSLEPEDTAMYYCKASYWKW GKLNNFWGPGTQVTVSS	265
hIL2 Rb_VHH-2	QVQLQESGGGLVQPGGSLRLSCV ASGFTFSNYWIFWVRQAAGKGLEWLST SNTGGDTTKYADSVKGRFTISRDSAANT EYLQMNSLKPEDTAVYYCETGRCARSG GYQGTQVTVSS	266
hIL2 Rb_VHH-3	QVQLQESGGGLVQPGGSLKLSCA ASGFRFSNYGMSWVRQAPGEGLEWVSY INGDGSRT ~~HY~~ ADSVKGRFTISRDNAAKNT LYLQLNSLKTEDTAMYYCEKGLSRDGW SLSAASRGQGTQVTVSS	267
hIL2 Rb_VHH-4	QVQLQESGGGSVQTGGSLRLSCA VSGYTTYSFNYMGWFRQAPGKEREGVA VIYTGGGSTLYADSVKGRFTISQDNAKN TVYLQMNSLKPEDTAMYYCAADDORF ASPLYAYFGYWGQGTQVTVSS	268
hIL2 Rb_VHH-5	QVQLQESGGGSVQVGGSLRLSC ATSGDTKSIRCMGWFRQTPGKEREGIAA IDREGFATYADSVYDRFTIAQDNAQNTL YLEMNALKPEDTAMYYCAAQNMCRVV RGAMTGVDYWGKGTQVTVSS	269
hIL2 Rb_VHH-6	QVQLQESGGGSVQAGGSLRLSC AASEYTASRYCMAWFRQAPGKEREGVA AIHPGGGT ~~TY~~ ADSVKGRFSISQDSADN TLYLQMNSLKPEDTAMYYCAAGSLWVP FGDRCAANYWGQGTQVTVSS	270
hIL2 Rb_VHH-7	QVQLQESGGGSVQAGGSLRLSC AASGYEYCR ~~I~~ HMTWYRQGP ~~G~~ KEREFS SIGSDGRKTYANSVTGRFTISRDNANHT VYLQMNSLSPEDTAMYYCKTEYLYGLG CPDGSAYWGQGTQVTVSS	271
hIL2 Rb_VHH-8	QVQLQESGGGSVQVGGSLKLSC AASGYTYSSYYCMGWFRQAPGKEREGV AAIDSDGSTSYADSVKGRFTISQDDAKN TLYLQMNSLKPEDTAMYYCAASYEVVD CYP ~~S~~ GYGODYWGKGTQVTVSS	272

Table 7- murine anti-IL2Rb VHH Amino Acid Sequences

Name	VHH AA Sequence (CDRs Underlined)	VHH SEQ ID
DR857	QVQLQESGGGLVQPGGSLRLSC AASGFTFSLYDMSWVRQAPGKGLEW VSGINSGGYSTYYAASAKGRFTISRDN AKNTLYLQLSSVKTEDTAMYYCAQRG LTSPYVIPNIRLQGTQVTSS	273
DR1448	EVQLVESGGRLVQAGDSLRLSC VASGKSFS DYPLGWFRQAPGKAREYV AHISWSGKLTYYRSTVKGRFTISRDN ENKLYLQMNALKPEDTAVYYCAAMK LFNYGGRYCVLKPLTMYQQWSQGTQ VTSS	274
DR1449	EVQLVESGGGLVQAGGSLRLSC AASGRSFSGYAIGWFRQAPGKEREFVA VVS WRGSSTYYADSVKGRFTISRDN KGTVYLQMNSLKPEDTAAYYCAAVPS GRSWYGRNRYWGQGTQVTSS	275
DR1450	EVQLVESGGGLVQAGGSLRLSC VISGRSINY YRMGWFRQAPGNRRQFV AAIKWGGDGVYADSVKGRFTISRDN KNTVYLQMDSLKPEDTGTYCAKMPL SSWSRGGYLEVWGQGTTLVTSS	276
DR1451	EVQLVESGGGLVQAGDSLRLSC AASERFSWGNYAMYWFRQAPGKERE FVAAIGRNSMATYYRDSAKGRFVISRD NAKNTLYLEMNALKPEDTARYYCAA KFMVADGWSRQYDYWGQGTTLVTSS	277
DR1452	EVQLVESGGGLVQAGGALRLSC AASGRTFRRFMGWFRQAPGKEREFVA AINWPGGGTYYGDSVKGRFTISRDN KNTVYLQMNSLKPEDTANYYCAATR KYNLYKFADWGQGTQVTSS	278
DR1453	EVQLVESGGRLVQAGDSLRLSC VASGRIFNTYSMGWFRQVPGKERDFV AAIRWSGGTTYTDSVKGRFTISRDN KNTVYLQMNALKPEDTAVYYCWVRV RLSNTALLQRYWGQGTTLVTSS	279
DR1454	EVQLVESGGGLVQAGGSLRLFC ASSERTFGDYPIGWFRQAPGKEREFVA SISWGGSRQYYTDSVKGRFTITRDNDK NTVYLQMNALKPEDTAVYYCWVRV LSNTALLQRYWGQGTTLVTSS	280
DR1455	EVQLVESGGGLVQTGGSLRLSC AASGRTFNSYAMGWFRQSPGKEREFV AVITWNSGRTTYADSVKGRFTISRDN	281

	KNTVYLQMNSLKPEDTAVYYCNSAP WAHNREWGQGTQVTVSS	
DR1456	EVQLVESGGGLVQAGGSLRLSC AASGLTFRYYMSWFRQAPGKEREFV GVISWIGSTTLYADSVKGRFSISRDN KNTVYLQMNNLKPEDTAVYYCAANF LREGKREPRYWGGGTQVTVSS	282
DR1457	EVQLVESGGRLVQAGDSLRLSC VASGRIFNTYSMGWFRQVPGKERDFV AAIRWSGGTTYTDSVKGRFTISRDN KNTVYLQMNSLKPEDTAVYYCYLRVF ARRYWGQGTQVTVSS	283
DR1458	EVQLVESGGGLVQAGGSLRLSC AASGRTLSTYAMGWFRQAPGKEREFV AAIRWASGRTTYGDSVKGRFTISRDSA KNTVYLQMNSLKPEDTAVYYCAARSR PYLNYGDFGYWGQGTQVTVSS	284
DR1459	EVQLVESGGGLVQAGGSLRLSC AASGRTISTYAMVWFRQASGKEREFV GVISRSGDRTTYADSVKGRFTISRDN GNIVRLQLNSLKPEDTAVYYCARGGY TGIEITARGRGTQVTVSS	285
DR1460	EVQLVESGGGLVQAGDSLRLSC AAPESIFNNNAVYWYRQFPGKEREFV GLITIGGRTGYADSVKGRFTISRDN NVAFLQMDSLKPEDTAVYYCATGLKF GFNFYSKTAYDYWGQGTQVTVSS	286
DR1461	EVQLVESGGRLVQAGDSLRLSC VASGRIFNTYSMGWFRQVPGKERDFV AAIRWSGGTTYTDSVKGRFTISRDN KNTVYLQMKDLKPQDTAVYYCAAVP SGRSWYGRNRYWGQGTQVTVSS	287
DR1462	EVQLVESGGGLVQAGGSLRLSC VSSGRTFGYVAMGWFRQAPGKEREFV ASINWSGGSTAYADSVKGRFTISRDN KNTVYLQMNSLKPEDTAVYYCAGSTR FYIATMEQGSYDYWGQGTQVTVSS	288
DR1463	EVQLVESGGSVVQPGDSLRLAC TASGRSFRSYAIGWFRQASGKERVFA AISYDGRRTYYGRSLKDRFTISRDN KNTVYLQMNSLKPEDTAVYYCATHRSG TMFARYGMDYWGGGTQVTVSS	289
DR1464	EVQLVESGGGLVQAGGSLRLSC AASGRTFSSYAMGWFRQAPGKEREFV TAISRSGGYTSYADSVKGRFTISRDN KNTVYLQMNSLKPEDTAVYYCAKLIA PFYYGMDYWTKGTQVTVSS	290
DR1465	EVQLVESGGGLMQAGGALRLS CTASGPTFTSYTMGWFRQSPGKRREFV	291

	AVISKGGRTYYADSVKGRFTISRDN NTFYLMSSSLKPEDTAVYYCAGQRVG ATSKYEYDYWGQGTQVTVSS	
DR1466	EVQLVESGGGLVRAGGSLRLSC AASGFTFSTDWYMWVRRAPGKGLEW VSLINTDGTSTSYTKSVKGRFTVSRDN AKNTLYLQMNSLKPEDTALYYCARGR TYWIFYAMDYWGKGTQVTVSS	292
DR1467	EVQLVESGGGLVQAGDSLRLSC AASGRISNYAMGWFRQAPGKEREFVA VITRSGGSTYYADSVKGRFTISRDNK NTIDLQMNRLKPEDTAVYYCAVRRSQ KLVTFGAEYPWWGQGTQVTVSS	293
DR1468	EVQLVESGGGLVQAGGSLRLSC TTSGRTGTHYAMGWFRQAPGKEREFV SLILWNGEFTTYKDSVKGRFTISRDK ENTVYLMNSLKPEDTAVYYCYLRVF ARRYWGQGTQVTVSS	294
DR1469	EVQLVESGGGLVQPGGSLRLSC EVSGFTFSNYWYMWIRQAPGKGLEW VSHINTNGGNTYYRHSVKGRFTISRDN AKNTLYLQMNLKSEDVAVYYCAKA NSDVGLGYYGMDYWGKGTQVTVSS	295
DR1470	EVQLVESGGGSVQPGGSLRLSC AAPESIFNNNAVYWYRQFPGEREYV GLITIGGRTGYADSVKGRFTISRDNAN NVAFLQMDNLKPEDTAVYYCAARPG YWSSSYDYWGQGTQVTVSS	296
DR1471	EVQLVESGGGLVQAGGSLRLSC VFSGRAPASYAMAWFRQAVGNREFV AAINWSGRRTYADSVKGRFTISKDN AQNTAYLQMTNLEPEDTATYYCNAYL SGTYWYGQGTQVTVSS	297
DR1472	EVQLVESGGGLVRAGDSLRLSC AVSGLASSFFMTWFRQGQGEREFV ATISWTGRTSYYAASVKGRFTVSRDN AKNTVYLMNSLNSEDVAVYFCAAYP RTLVRNREPIHWGQGTQVTVSS	298

Table 8– human anti-IL2Rg VHH Amino Acid Sequences

Nam e	VHH Sequence (CDRs are underlined)	SEQ ID NO:
hIL2 Rg _VHH-1	QVQLQESGGGSVQAGGSLRLSCAASGE <u>TFDDSDMGWYRQAPGNECDLVSTISSDGSTY</u> <u>YADSVKGRFTISQDNAKNTVYLQMDSVKPED</u> <u>TAVYYCAADFMIAIQAPGAGCWGQGTQVTVS</u> S	299
hIL2 Rg _VHH-2	QVQLQESGGGSVPAGGSLKLSCAASGE <u>SFSSYPMTWARQAPGKGLEWVSTIASDGGST</u> <u>AYAASVEGRFTISRDNASTLYLQLNSLKTED</u> <u>TAMYYCTKGYGDGTPAPGQGTQVTVSS</u>	300
hIL2 Rg _VHH-3	QVQLQESGGGSVQTGGSLRLSCTASGE <u>TFDDREMNVYRQAPGNECELVSTISSDGSTYY</u> <u>ADSVKGRFTISQDNAKNTVYLQMDSVKPEDT</u> <u>AVYYCAADFMIAIQAPGAGCWGQGTQVTVSS</u>	301
hIL2 Rg _VHH-4	QVQLQESGGGSVQAGGSLRLSCTASGE <u>TFDDSDMGWYRQAPGNECELVSTISSDGNTY</u> <u>YTDSVKGRFTISQDNAKNTVYLQMNSLGPED</u> <u>TAVYYCAAEPGRGYYSNYGGRRECNYWGQGT</u> QVTVSS	302
hIL2 Rg _VHH-5	QVQLQESGGGSVQAGGSLRLSCAASGE <u>SFSSYPMTWARQAPGKGLEWVSTIASDGGST</u> <u>AYAASVEGRFTISRDNASTLYLQLNSLKTED</u> <u>TAMYYCTKGYGDGTPAPGQGTQVTVSS</u>	303
hIL2 Rg _VHH-6	QVQLQESGGGAVQAGGSLRLSCAASGE <u>TFSNAHMSWVRQAPGKGREWISSIYSGGSTW</u> <u>YADSVKGRFTISRDNASKNTLYLQLNSLKTEDT</u> <u>AMYYCAENRLHYSDDDSLRGQGTQVTVSS</u>	304
hIL2 Rg _VHH-7	QVQLQESGGGLVQPGGSLRLSCAASGE <u>TFDDREMNVYRQAPGNECELVSTISSDGSTYY</u> <u>ADSVKGRFTISQDNAKNTVYLQMDSVKPEDT</u> <u>AVYYCAADFMIAIQAPGAGCWGQGTQVTVSS</u>	305
hIL2 Rg _VHH-8	QVQLQESGGGSVQAGGSLRLSCVASGY <u>TFSSYCMGWFRQAPGKEREGVAALGGGSTYY</u> <u>ADSVKGRFTISQDNAKNTLYLQMNSLKPEDT</u> <u>AMYYCAA~~AW~~VACLEFGGSWYDLARYKHWG</u> QGTQVTVSS	306
hIL2 Rg _VHH-9	QVQLQESGGGSVQAGGSLRLSCTASGE <u>TFDDSDMGWYRQAPGGCELVSTISSDGSTYY</u> <u>ADSVKGRFTISQDNAKNTVYLQMNSLKPEDT</u> <u>AVYYCAAEPGRGYYSNYGGRRECNYWGQGTQ</u> VTVSS	307
hIL2 Rg _VHH-10	QVQLQESGGGSVQAGGSLRLSCAASGS <u>IYSSAYIGWFRQAPGKKREGVAGIYTRDGSTA</u> <u>YADSVKGRFTISQDSAKKTVYLQMNSLKPEDT</u>	308

	AMYYCAAGRRTKSYVYIFRPEEYNYWGQGT QVTVSS	
hIL2 Rg_VHH- 11	QVQLQESGGGSVQAGGSLRLSCAASGE TFSSAHMSWVRQAPGKGREWIASIYSGGGTFY ADSVKGRFTISRDNANTLYLQLNSLKTEDTA MYCATNRLHYYSDDDSLRLGQGTQVTVSS	309
hIL2 Rg_VHH- 12	QVQLQESGGGSVQAGGSLRLSCAASGE TFSNAHMSWVRQAPGKGREWISSIYSGGSTW YADSVKGRFTISRDNANTLYLQLNSLKTEDT AMYYCAENRLHYYSDDDSLRLGQGTQVTVSS	310
hIL2 Rg_VHH- 13	QVQLQESGGGSVQAGGSLRLSCTASRFI FDDSDMGWYRQAPGNECELVSTISSDGSTYY ADSVKGRFTISRDNANTVYLQMNSLKPEDT AVYYCAAEPRGYYSNYGGRRRECNYWGQGTQ VTVSS	311
hIL2 Rg_VHH- 14	QVQLQESGGGSVQAGGSLRLSCTVSGE TADDSMGWYRQPGNECELVSTISSDGSTYY ADSVKGRFTISQDNANTVYLQMNSLKPEDT AVYYCAAEPRGYYSNYGGRRRECNYWGQGTQ VTVSS	312
hIL2 Rg_VHH- 15	QVQLQESGGGLVQPGGSLRLSCAASGE TFSSAHMSWVRQAPGKGREWIASIYSGGGTFY ADSVKGRFTISRDNANTLYLQLNSLKAEDTA MYCATNRLHYYSDDDSLRLGQGTQVTVSS	313
hIL2 Rg_VHH- 16	QVQLQESGGGLVQPGGSLRLSCVASGE TFSNAHMSWVRQAPGKGREWISSIYSGGSTW YADSVKGRFTISRDNANTLYLQLNSLKTEDT AMYYCAENRLHYYSDDDSLRLGQGTQVTVSS	314
hIL2 Rg_VHH- 17	QVQLQESGGGLVQPGGSLRLSCAASGE TFSNAHMSWVRQAPGKGREWISSIYSGGSTW YADSVKGRFTISRDNANTLYLQLNSLKTEDT AMYYCAENRLHYYSDDDSLRLGQGTQVTVSS	315
hIL2 Rg_VHH- 18	QVQLQESGGGLVQPGGSLRLSCAASGE TFSSYPMTWARQAPGKGLEWVSTIASDGGST AYAASVEGRFTISRDNASTLYLQLNSLKTED TAMYYCTKGYGDGTPAPGQGTQVTVSS	316
hIL2 Rg_VHH- 19	QVQLQESGGGSVQAGGSLRLSCTASGE TFDDREMNVYRQAPGNECELVSTISSDGSTYY ADSVKGRFTISQDNANTVYLQMDSVKPEDT AVYYCAADFMAIQAPGAGCWGQGTQVTVSS	317
hIL2 Rg_VHH- 20	QVQLQESGGGSVQAGGSLRLSCTASGE TFDDSDMGWYRQAPGNECELVSTISSDGSTYY ADSVKGRFTISQDNANTVYLQMNSLKPEDT AVYYCAAEPRGYYSNYGGRRRECNYWGQGTQ VTVSS	318

hIL2 Rg_VHH- 21	QVQLQESGGGSVQAGGSLRLSCVASGY <u>TSCMGWFRQAPGKEREAVATIIYTRGRSIYYA</u> <u>DSVKGRFTISQDNAKNTLYLQMNSLKPEDIAM</u> YSCAAGGYSWSAGCEFNYWGQGTQVTVSS	319
hIL2 Rg_VHH- 22	QVQLQESGGGLVQPGGSLRLSCTASGE <u>SFSSYPMTWARQAPGKGLEWVSTIASDGGST</u> <u>AYAASVEGRFTISRDNASTLYLQLNSLKTED</u> TAMYCYCTKGYGDGTPAPGQGTQVTVSS	320
hIL2 Rg_VHH- 23	QVQLQESGGGLVQPGGSLRLSCAASGE <u>SFSSYPMTWARQAPGKGLEWVSTIASDGGST</u> <u>AYAASVEGRFTISRDNASTLYLQLNSLKTED</u> TAMYCYCTKGYGDGTPAPGQGTQVTVSS	321

Table 9– murine anti-IL2Rg VHH Amino Acid Sequences

Na me	VHH AA Sequence (CDRs Underlined)	SEQ ID NO:
mI L2Rg_VH H1	QVQLQESGGGSVLGGSLRLSCVASGYGYNYIGWFRQTP GKEREGVAVIYTGGGDTYYADSVKGRFTASRDNAKSTLYLQMN SLEPEDTAMYYGVARYCVGSVYACLRGGHDEYAHWGQGTQVT VSS	322
mI L2Rg_VH H2	QVQLQESGGGSVQPGGSLRLSCAASGSTYANYLMGWFRQ APGKEREGVAAIYSGGGSTYYADSVKGRFTISQDNAKNTLYLQM NSLKPEDTAMYYCAAASAVKGDGDIVVVVTGTQORMEYDYWG HGTQVTVSS	323
mI L2Rg_VH H3	QVQLQESGGGSVQAGASLRLSCSVSGFTFDESVM ~~SWLRQ~~ GPGNECDAVAIISSDDNTYYDDSVKGRFTISEDNAKNTMVYLQMN SLKPEDTAVYYCAARRRRPVYDSYELRPRPLCGDFGVWGQGT QVTVSS	324
mI L2Rg_VH H4	QVQLQESGGGSVQAGGSLRLSCIGSGLPFDEDDMGWYRQ APGNECELVSSISSDGTAYYADSVKGRFTISRDNKNTVLLQMN LKPEDTAVYYCAAGVHROFGGSSSCGDAFYGMDYWGKGTQVT VSS	325
mI L2Rg_VH H5	QVQLQESGGGSVQAGGSLRLSCVASGDVYGRNSMAWFR QAPGKEREGVAVGYSVTTTYYADSVKGRFTISEDNDKNTVYLE MNSLKPEDTAMYYCAADGNLWRGLRPSEYTYWGQGTQVTVSS	326
mI L2Rg_VH H6	QVQLQESGGGSVQAGGSLRLSCATSGFPYSRYCMGWFRQ APGKEREGVAAIEPDGSTSYADSVKGRFTISQDNAVNTLYLQMN NLKPEDTAMYYCAADERCFYLKDYDLRRPAQYRYWGQGTQVT VSS	327
mI L2Rg_VH H7	QVQLQESGGGLVQPGGSLRLSCTVSGFTFDES ~~DMGWLRQ~~ NPGNECGVVSVITSDDNPYYDDSVKGRFTISEDNAKNTMVYLQM NSLKPEDTGYYCATRSROPVYSRDYELRPRPLCGDFGVWGQGT QVTVSS	328
mI L2Rg_VH H8	QVQLQESGGGSVQAGGSLRLSCTASGFTFDDFDMGWYRQ APGNECELVSTISDDGSTYYADSVKGRSSISRDNKNTVYLQMN RLKPEDTGYYCAAEGALGSKTNCGWVGNFGYWGQGTQVTVS S	329
mI L2Rg_VH H9	QVQLQESGGGSVQAGGSLRLSCAASGFTFDDFDMGWYR QAPGNECELVSTISDDGSTYYADSVKGRSSISRDNKNTVYLQM NSLKPEDTAVYYCAAEGALGSKTNCGWVGNFGYWGQGTQVTV SS	330
mI L2Rg_VH H10	QVQLQESGGGLVQPGGSLRLSCAASGFTFDDFDMGWYRQ APGNECELVSTISDDGSTYYADSVKGRSSISRDNKNTVYLQMN LKPEDTGYYCAAEGALGSKTNCGWVGNFGYWGQGTQVTVSS	331
mI L2Rg_VH H11	QVQLQESGGGLVQPGGSLRLS ~~CAASGFTFSDRDMGWYRQ~~ APGNECERVSTISDDGSTYYADSVKGRSSISRDNKNTVYLQMN SLKPEDTAVYYCAAEGALGSKTNCGWVGNFGYWGQGTQVTVS S	332

ml L2Rg_VH H12	QVQLQESGGGSVLAGGSLRLSCVASGYGYNIGWFRQTP GKEREGVAVIYIGGGDTYYADSVKGRFTASRDNAKSTLYLQMNS LEPEDTAMYYCVARYCVGSVYACLRGGHDEYAHWGQGTQVTV SS	333
ml L2Rg_VH H13	QVQLQESGGGSVLAGGSLRLSCVASGYGYNIGWFRQTP GKEREGVAVIYTGGGDTYYADSVKGRFTASRDNAKSTLYLQMN SLEPEDTAMYYCVARYCVGSVYACLRGGHDEYAHWGQGTQVT VSS	334
ml L2Rg_VH H14	QVQLQESGGGSVQAGGSLRLSCAASGFTFDDFDMGWYR QAPGNECELVSTISDDGSTYYANSVKGRSSISRDNAMVYLQM NSLKPEDTAVYYCAAEGALGSKTNCGWVGNFGYWGQGTQVTV SS	335
ml L2Rg_VH H15	QVQLQESGGGSVQAGGSLRLSCTASGFTFDDFDMGWYRQ APGNECELVSTISDDGSTYYADSVKGRSSISRDNAMNTVYLQMN RLKPEDTGVYYCAAEGALGSKMNCGWVGNFGYWGQGTQVTVS S	336

Table 10- IL2Rb sdAb VHH DNA SEQUENCE

Table 2. hIL10Ra VHH DNA Sequences		SE Q ID NO:
Name	Sequence	
hIL10Ra_ VHH1	CAGGTTTCAGCTTCAGGAGTCCGGTGGAGG CTCCATCCAGGCCGGGGGCTCTCTCCGCCTGTCTT GCGCCGCTTCCAGATACCTCTACAGTATCGACTA CATGGCTTGGTTTCGTCAGAGCCCAGGAAAAGAG CGGGAACCCGTGGCAGTAATCTACACTGCCTCAG GTGCCACATTTTACCCGACTCTGTCAAGGGCAG GTTCAACATCTCTCAGGATAATGCCAAGATGACA GTGTACTTGCAGATGAACTCCCTGAAATCTGAGG ATACCGCTATGTATTACTGTGCCGCAGTGCGCAA GACCGATTCTTACCTGTTTCGACGCTCAGAGTTTTA CCTACTGGGGCCAGGGCACTCAGGTCACCGTCAG CAGC	337
hIL10Ra_ VHH2	CAGGTGCAGTTGCAGGAGTCCGGCGGGGG TTCCGTGCAAGCAGGCGGATCTCTGCGCCTGTCC TGCGTGGCCTCTCGTTATTTGTATAGCACCAACTA CATGGCTTGGTTCCGTCAGTCCCCAGGCAAAGAG CGCGAAGCCGTAGCCGTAATCTATACGGCCTCTG GGGCAACACTCTATACCGACTCAGTGAAGGGACG CTTCACGATTTCCCAAGACAATGCAAAGATGACC GTGTACTTGCAGATGAACCGCCTGAAGAGCGAGG ACACGGCTATGTATTACTGCGCAGCCGTGCGCAA GACCGACTCCTACTTGTGTTGACGCTCAGTCCTTCA CTTATTGGGGCCAGGGTACACAGGTCACCGTGAG CAGT	338
hIL10Ra_ VHH3	CAAGTACAGCTCCAGGAGAGCGGCGGTGG ATCTATCCAAGCAGGGGGTAGCCTTAGGTTGTCC TGTGTGGCGTCCAGATACCTGTATAGCACGAACT ACATGGCATGGTTCAGACAGTCCCCAGGCAAGGA ACGCGAGGCAGTCGCCGTTATTTACACTGCATCT GGGGCCACCCTCTATACGGACAGCGTGAAGGGA AGGTTTACAATCTCCCAGGACAACGCGAAGATGA CCGTGTACCTTCAGATGAACCGCCTGAAGTCCGA GGACACCGCCATGTATTACTGTGCAGCGGTGCGC AAGACCGACAGCTATCTGTTTCGACGCGCAGTCAT TCACTTATTGGGGCCAAGGAACCCAAGTGACCGT CAGCTCA	339
hIL10Ra_ VHH4	CAGGTGCAGCTCCAAGAGTCCGGGGGAGG CTCTATCCAGGCGGGAGGCAGTCTGCGCTTGTCC TGCGCCGCAAGTCGTTATCTGTACTCCATTGATTA CATGGCATGGTTCCGCGCAGTCCCCAGGTAAGGAA CGTGAACCTGCCGCTGTGATCTACACCGCTTCTG GAGCAACCTTTTATCCTGATAGCGTTAAGGGTCG CTTCACCATCTCTCAGGATAACGCCAAAATGACA	340

	GTGTACCTCCAGATGAACAGCCTGAAGTCTGAGG ACACTGCCATGTACTATTGTGCGGCTGTGCGCAA GACCGACTCCTATCTGTTTGATGCACAGAGCTTT ACCTATTGGGGTCAGGGCACCCAGGTGACTGTGT CTAGC	
hIL10Ra_ VHH5	CAGGTCCAGTTGCAGGAGTCCGGTGGAGG TTCCATCCAGGCGGGTGGGTCCCTTCGTCTCTCCT GCGTGGCCTCTAAGTACCTGTATTCAACCAACTA CATGGCATGGTTCAGACAGTCTCCCGGCAAAGAG CGTGAGGCAGTGGCCGCGATCTATACAGCTTCTG GGGCCACCCTGTACTCTGATTCCAATAAGGGAAG GTTCACTATCTCACAGGATAACGCCAAAATGACC GTCTACCTTCAGATGAACAGCCTCAAGTCTGAAG ACACGGCAATGTATTACTGTGCAGCCGTGCGCAA AACTGGGAGCTACCTGTTTGACGCTCAGTCTTTC ACTTATTGGGGCCAGGGTACGCAGGTGACAGTCT CTTCT	341
hIL10Ra_ VHH6	CAGGTGCAACTCCAGGAGAGCGGAGGCG GTTCTGTTTCAGGCAGGAGGTTCCCTGAGACTGTC CTGTGCCGCGTCTCGCTTTACGTATTCATCCTACT GCATGGGATGGTTCAGACAAGCGCCGGGGAAAG AAAGGGAAGGCGTGGCCTCCATTGACTCCGACGG CTCAACTTCATACACTGATAGCGTGAAAGGCCGG TTCACCATCTCTAAGGACAACGCGAAGAACACCC TGTATCTCCAGATGAACAGCCTCAAGCCTGAGGA TACTGCCATGTACTATTGCGCACTCGACCTGATGT CTACTGTGGTCCCAGGCTTCTGCGGGTTCTTGCTC TCTGCTGGCATGGACTACTGGGGGAAGGGCACTC AGGTAACGGTTAGCTCC	342
hIL10Ra_ VHH7	CAGGTGCAGCTTCAGGAATCTGGCGGGGG CTCCGTGCAGGCCGGGGGCTCCCTCAGACTTTCC TGTGCCGTCTCCGGTTACACATTTAACAGTAACT GTATGGGCTGGTTCCGCCAGGCACAGGCAAGGA GAGGGAAGGTGTGGCCACAATCTATACTGGTGTT GGGAGTACGTAATATGCTGATTCCGTGAAAGGTC GCTTCACAATTTCCAGGACAACGCGAAGAACAC TGTGTACTTGCAGATGAATAGCCTGAAGCCTGAA GATACCGCAATGTATTACTGCGCTGCCGAGCCAC TCTCCCGCGTATATGGTGGAAGTTGCCCCACCCC CACTTTCGGTTACTGGGGCCAGGGCACTCAAGTG ACCGTGTCTCT	343
hIL10Ra_ VHH8	CAGGTTTCAGCTTCAGGAGTCTGGGGGCGG TTCAGTGCAGGCTGGCGGTTCTCTCCGCCTGTCCT GCGCTGCCAGCGGCTATACTTACAGCATGTACTG CATGGGCTGGTTCCGGCAAGCCCCCGGCAAAGAG CGTGAGGGCGTCGCTCAAATCAACAGCGACGGGT CAACCAGCTACGCCGATTCTGTCAAGGGCAGATT TACTATCAGCAAGGACAACGCCAAAAACACACT GTACCTCCAGATGAACTCTTTGAAGCCTGAGGAC ACCGCGATGTATTACTGCGCCGCTGACAGCCGCG	344

TGTACGGTGGCAGCTGGTATGAGAGGCTGTGCGG CCCGTACACCTACGAGTACAACCTATTGGGGACAG GGCACGCAGGTGACAGTTAGCTCC	
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Table 11- murine IL2Rb sdAb VHH DNA SEQUENCE

Name	DNA Sequence	SEQ ID NO
DR857 DNA	CAGGTGCAGTTGCAGGAGAGCGGGGGCGGTCTG GTCCAGCCGGGCGGGTCACTGCGCCTGTCTTGTGCCGCT TCAGGATTTACCTTTAGTTTGTACGACATGAGTTGGGTT AGGCAAGCGCCTGGCAAGGGTCTGGAGTGGGTGTCTGG CATCAACTCAGGAGGCTATAGCACCTATTACGCGGCCT CCGCCAAGGGCCGCTTCACCATCTCTAGGGATAACGCA AAGAACACTCTTTACCTCCAGCTCAGCTCTGTTAAGACT GAGGATACTGCCATGTATTACTGTGCCCAGCGCGGCCT CACCAGCCCGTATGTGATTCCGAACATTGCTTGCAGG GCACACAGGTGACTGTGTCCAGC	345
DR1448 DNA	GAGGTCCAACCTGGTGGAGAGCGGCGGAAGGCTG GTGCAGGCTGGCGACTCCCTGCGCTTGAGCTGTGTGGC AAGCGGAAAGTCCTTTTCCGATTACCCTCTCGGTTGGTT CCGTCAAGGCTCCTGGAAAAGCTAGGGAGTATGTGGCC ACATCTCTTGGAGCGGCAAACTGACTTACTATCGCTCA ACAGTGAAGGGCCGTTTACTATCAGCCGCGATAACGC TGAAAATAAACTGTACCTCCAGATGAACGCCCTGAAGC CCGAGGATACTGCCGTGTATTACTGTGCTGCCATGAAG TTGTTCAACTATGGCGGGCGTTACTGTGTTCTCAAGCCC CTGACAATGTACCAACAGTGGAGCCAGGGTACTCAGGT CACAGTTAGCTCC	346
DR1449 DNA	GAGGTCCAGCTCGTTGAGAGCGGCGGGGGCCTG GTGCAGGCCGGTGGCAGCCTCCGTCTCTCCTGTGCCGCT TCTGGCCGCGAGTTTCTCCGGGTATGCTATCGGGTGGTTC AGACAGGCACCAGGCAAGGAGCGCGAGTTTGTGCTGT CGTGAGCTGGCGGGTCTAGCACCTACTATGCCGACT CAGTCAAGGGCCGCTTCACAATTAGCAGGGACAACGCC AAGGGCACTGTATACCTCCAGATGAACTCCCTGAAGCC AGAGGATACCGCCGCGTATTACTGCGCTGCCGTGCCAT CTGGCCGCTCCTGGTACGGTAGGAACCGTTACTGGGGT CAGGGAACTCAGGTACCCGTGTCCTCA	347
DR1450 DNA	GAAGTGCAGCTCGTTGAAAGCGGCGGGGGCCTC GTGCAAGCTGGAGGCTCACTTCGCCTTTCTTGTGTATC AGTGGCCGCTCTATCAATTATTACCGGATGGGCTGGTTC CGCCAGGCCCTGGCAACCGCAGGCAATTCTGGCGGC TATCAAGTGGGGTGGCGACGGTGTGTACGCCGACTCCG TGAAGGGGCGCTTTACCATTAGTCGGGACAACACCAAG AACACCGTATACTTGCAGATGGACAGTCTGAAGCCCGA AGACACCGGAACATATTACTGCGCCAAAATGCCTCTTT CTAGCTGGTCCAGAGGTGGCTACCTTGAGGTGTGGGGT CAAGGCACGCTGGTGACCGTGTCTTCT	348
DR1451 DNA	GAAGTGCAACTCGTGGAAAGTGGAGGCGGTCTC GTCCAGGCCGGGGACAGCCTGCGTCTGTCTTGCGCCGC ATCCGAGCGTTTTTCTTGGGGCAACTATGCTATGTATTG	349

	GTTCAGGCAGGCCCCCTGGCAAGGAACGCGAGTTCGTGG CTGCCATTGGCCGCAACAGCATGGCCACGTATTACAGA GATAGCGCCAAGGGCCGCTTCGTGCATCAGCCGTGACAA CGCTAAGAACACCCTGTACCTGGAAATGAACGCCTTGA AGCCTGAAGATACTGCTAGGTACTATTGCGCCGCGAAG TTCATGGTGGCCGACGGCTGGAGCAGACAGTATGACTA CTGGGGCCAGGGCACTCTGGTAACGGTCTCCTCC	
DR1452 DNA	GAAGTTCAGCTTGTGGAAAGCGGCGGTGGGCTT GTCCAGGCTGGTGGAGCGCTGCGCCTCTCCTGCGCAGC GAGTGGCAGGACCTTCCGCGGTTTCATGGGTGGTTTCG CCAGGCCCCAGGGAAGGAGCGCGAGTTTGTGCTGCCA TCAACTGGCCTGGAGGTGGCACCTACTATGGCGATAGC GTGAAGGGCCGTTTCAACAATCTCCAGGGACAACGCCAA GAATACCGTCTACCTGCAAATGAACTCCCTGAAGCCGG AGGACACCGCGAAGTATTACTGCGCCGCAACCCGCAAG TACAACCTGTATAAATTCGCGGACTGGGGCCAGGGCAC CCAGGTGACAGTGTCTCT	350
DR1453 DNA	GAGGTCCAGCTCGTCGAGTCCGGCGGGCGGGCTG GTGCAGGCTGGCGACAGCCTTCGCCTGTCTGTGTGGC ATCCGGCAGAATCTTTAACACCTACTCAATGGGTTGGTT TAGGCAGGTTCCCGGAAAGGAGAGGGATTTCGTGGCTG CCATCAGATGGTCCGGTGGCACCACATATTACACTGAT TCTGTCAAGGGGCGCTTACCAATTAGTCCGCGATAACGC AAAAAACACCGTGTACCTGCAAATGAATAGCCTGAAGC CTGAGGACACCGCCGTATATTACTGTTGGGTGCGCGTTT GCCTGAGCAACACAGCCCTGCTTCAGCGCTACTGGGGT CAGGGAACCTTGGTTACCGTGTCAAGC	351
DR1454 DNA	GAAGTCCAGCTCGTGGAGTCCGGGGGAGGTCTG GTTCAAGCTGGGGGTTCTTTGCGCCTCTTTTGGCGTCC AGCGAGCGTACTTTCGGAGATTACCCAATCGGATGGTT CCGTCAAGCCCCAGGCAAGGAGCGCGAGTTTGTGCGGT CCATCAGCTGGGGTGGCTCACGTACGTAATACTGAC TCCGTAAAGGGCCGCTTACAGATTACAAGAGATAATGA TAAGAACACCGTGTATCTCCAGATGAACTCCCTCAAGC CCGAGGACACTGCTGTTTACTATTGCTGGGTGCGGGTG CGTCTGTCAAACACGGCACTGCTTCAGCGCTATTGGGG ACAGGGCACCCCTGGTCAACCGTCTCCTCA	352
DR1455 DNA	GAAGTCCAGCTGGTGGAGTCAAGCGGGGACTG GTGCAGACTGGGGGTAGTCTGCGCCTGAGCTGCGCAGC TTCAGGAAGAACCCTTCAACTCCTACGCTATGGGCTGGTT CAGACAGAGCCCAGGCAAAGAGCGGGAGTTTCGTGGCG GTGATTACGTGGAACCTTGCCCGCACGTAATACTGCTGA CAGTGTCAAAGGCAGATTTACCATCAGTAGGGATAACG CCAAGAACACAGTGTATCTCCAGATGAACTCTCTGAAG CCCGAGGATACTGCTGTGTATTACTGTAACAGCGCCCC CTGGGCTCACAATCGTGAGTGGGGGCAGGGGACCCTCG TTACCGTCAGCAGC	353
DR1456 DNA	GAGGTGCAGCTGGTGGAAATCTGGTGGAGGGCTG GTGCAGGCTGGCGGTTCCCTCCGTCTGTCTTGTGCGGCC TCAGGGCTGACCTTCAGGACCTACTATATGTCATGGTTC CGCCAAGCGCCCGGCAAGGAACGCGAGTTTCGTCCGAGT GATCTCTTGGATCGGCTCCACTACCCTCTACGCCGATTTC TGTGAAAGGTAGGTTTTCCATCTCACGCGATAATGCTA AGAACACCGTCTACCTCCAGATGAATAACTTGAAACCC	354

	GAGGACACCGCCGTCTACTATTGCGCGGCCAACTTCCT CAGAGAGGGAAAGCGCGAACCTCGGTATTGGGGACAA GGGACCCAGGTGACCGTTTCCTCC	
DR1457 DNA	GAGGTGCAGTTGGTTGAGTCTGGCGGAAGGCTC GTTCAAGCTGGTGACAGCCTGCGGCTGTCTTGGCTCGCT TCTGGACGCATCTTCAACACATATTCAATGGGCTGGTTC AGACAGGTGCCTGGCAAGGAGCGCGACTTCGTGGCAGC TATCCGTTGGAGCGGGGGCACTACGTATTACACCGATT CTGTGAAGGGGCGCTTCACAATCTCCAGGGATAATGCA AAGAACACCGTGTACCTTCAGATGAACAGCTTGAAGCC TGAAGATACCGCAGTGTACTATTGTTATCTGAGGGTGT CGCTCGGCGCTATTGGGGCCAGGGCACACAGGTGACAG TGTCTCC	355
DR1458 DNA	GAAGTGCAGCTGGTTCGAGAGCGGGGTGGACTT GTGCAGGCTGGTGGCTCCCTTAGGCTGAGCTGCGCCGC TTCCGGCAGAACTCTCTCTACCTATGCTATGGGTGGTT CCGTCAGGCCCCCGGCAAGGAGCGCGAGTTCGTGCGCG CCATCCGCTGGGCTTCTGGCCGTACTTATTACGGTGACA GCGTGAAGGGTCGGTTCACCATCTCTCGTGACAGTGCG AAAAATACCGTGTACCTCCAGATGAACTCCCTGAAGCC GGAGGACACGGCGGTTTATTACTGCGCGGCCAGGAGCA GGCCTTACCTGAACTACGGAGACTTTGGGTACTGGGGC CAGGGGACCCAGGTCAACCGTGTCTATCC	356
DR1459 DNA	GAAGTCCAGCTCGTGGAGTCTGGGGGTGGACTC GTACAAGCCGGGGGATCACTTCGCTTGTCTGCGCGGC TTCTGGCAGGACCATCTCAACTTACGCAATGGTTTGGTT CAGGCAAGCCTCTGGTAAGGAGCGTGAGTTTGTGGGCG TTATCTCCCGCAGTGGAGACCGCACTTACTATGCTGATT CTGTGAAGGGCAGATTCACTATCAGTCGCGATAATCTG GGCAACATTGTGCGTTTGCAGCTCAATTCACTTAAACCT GAAGACACAGCCGTTTATTACTGCGCACGCGGCGGATA TACCGGGATTGAGACAATTACGGCTCGGGGTGCGGGCA CATTGGTCAACCGTGTCCAGC	357
DR1460 DNA	GAGGTTCAAGCTCGTTGAGAGTGGTGGAGGCCTC GTGCAGACCGGGGATTCCCTTCGCCTTTCTGTGCAGCT CCAGAGTCCATCTTCAACAATAACGCCGTTTACTGGTAC AGGCAGTTCCTCGGCAAGGAGAGGGAGTATGTTGGTCT CATCACCATCGGTGGCAGGACCGGGTACGCGGACTCTG TGAAAGGCCGCTTTACCCTCTCCAGAGACAACGCCAAT AACGTGGCCTTTTTGCAGATGGATTCCCTCAAGCCCGA GGATACTGCTGTCTACTATTGTGCCACGGGCTTGAAGTT CGGCTTCAACTTCTACAGTAAGACTGCCTACGACTACTG GGGACAAGGGACCCAGGTGACCGTCAGCTCT	358
DR1461 DNA	GAGGTGCAGCTCGTGGAGTCTGGAGGTGCGCTG GTGCAGGCTGGCGATTCCCTGCGCCTGTCTGTGTGGCC TCTGGTCGCATTTTCAACACTTATTCTATGGGTGGTTC AGACAGGTTCTGGAAAGGAAAGAGACTTCGTGGCAGC CATTCGGTGGAGTGGTGGCACCACTTATTACACAGACT CCGTGAAGGGTCGCTTTACTATCTCTCGGGATAACGCC AAAAACACTGTCTACCTCCAGATGAAAGACCTGAAGCC CCAGGACACCGCCGTCTATTACTGTGCTGCCGTCCCTC TGGCCGCAGCTGGTACGGTCGCAACCGTACTGGGGCC AGGGCACTCTGGTGACCGTCAGCTCT	359

DR1462 DNA	GAGGTGCAGTTGGTGGAGAGCGGCGGTGGCCTG GTCCAGGCGGGCGGGTCCCTCCGCCTGAGTTGTGTGTCT TCAGGCCGGACCTTTGGATATGTCGCTATGGGTTGGTTC CGTCAAGCCCCAGGTAAGGAACGCGAGTTCGTGGCGAG CATTAACTGGAGCGGCGGGTCCACGGCCTATGCGGACT CCGTAAAGGGCCGGTTCACCTATCAGCCGCGACAACGCT AAGAATACCGTGTACTTGCAGATGAACAGCCTGAAGCC TGAGGATACAGCCGTGTATTACTGCGCTGGATCAACCC GCTTCTATATCGCGACGATGGAACAGGGCTCCTACGAT TACTGGGGCCAAGGTACTCAGGTGACCGTAAGCAGC	360
DR1463 DNA	GAGGTGCAACTGGTGGAAATCAGGAGGCTCCGTG GTCCAGCCAGGGGACAGCCTTCGTCTTGCCTGCACCGC CTCTGGTCGCAGTTTCAGGTCTTACGCGATTGGCTGGTT TAGGCAGGCATCCGGCAAGGAAAGGGTGTGTGTGGCTG CCATCTCTTATGACGGTAGGCGCACCTACTATGGGCGTT CATTGAAGGACCGTTTCACTATCTCTCGGGACAACGCT AAGAACACAGTGTACTTGCAGATGAACTCCCTCAAGCC CGAGGACACTGCCGTGTACTATTGCGCTACCCATCGCTC CGGTACAATGTTTCGCTCGGTATGGTATGGATTACTGGG GTAAGGGTACTTTGGTTACCGTGTCCAGC	361
DR1464 DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGTGGCCTG GTGCAAGCAGGCGGATCTCTGCGTCTGTCTTGTGCTGCG TCAGGCCGCACCTTCTCCTCTTATGCTATGGGGTGGTTT AGACAAGCTCCTGGAAAGGAGAGGGAGTTTGTGACTGC CATCTCCAGATCCGGTGGATACACTAGCTACGCCGATA GTGTTAAGGGCCGGTTCACCTATCTCTCGCGACAATGCC AAGAACACCGTGTATCTTCAGATGAACTCCCTGAAACC CGAGGACACCGCCGTCTACTATTGTGCGAAACTGATCG CTCCATTCTATTACGGCATGGATTACTGGACCAAGGGG ACCCAGGTGACAGTGTCTAGC	362
DR1465 DNA	GAAGTGCAGCTGGTGGAAAGCGGCGGAGGTCTG ATGCAGGCAGGTGGAGCCCTTAGGCTCTCTTGTACCGC CTCTGGGCCTACTTTTACCTCTTATACGATGGGCTGGTT CCGCCAATCTCCTGGCAAGCGTCGCGAGTTTGTGGCCG TCATCTCCAAAGGCGGCGGACCTATTACGCCGACTCC GTGAAGGGACGCTTCACTATTTCCCGCGACAACGCTAA GAATACCTTCTATCTCCAGATGTCTCTCTGAAGCCTGA GGACACAGCAGTGTATTACTGCGCCGGGCAGCGTGTGG GCGCGACTAGCAAGTATGAGTATGATTACTGGGGGCAG GGCACCCAAGTGACCGTGTCTATCC	363
DR1466 DNA	GAAGTGCAGCTGGTGGAGAGCGGAGGGGGTCTG GTACGCGCAGGTGGCTCCCTGAGGCTCTCCTGCGCTGC GTCCGGCTTCACTTTTAGTACCGACTGGATGTACTGGGT AAGACGCGCTCCAGGAAAGGGGCTGGAGTGGGTGTCCC TTATCAACACTGACGGGACTTCTACCTCCTATACTAAGT CTGTGAAGGGGCGCTTCACAGTCTCCCGCGATAATGCC AAGAACACCTTGTACCTTCAGATGAACTCCCTCAAGCC GGAGGACACAGCTCTGTATTACTGTGCACGCGGAAGAA CCTACTGGTTTACGCGATGGATTACTGGGGCAAGGGC ACCCAGGTGACCGTCTCATCT	364
DR1467 DNA	GAGGTCCAGTTGGTGGAAATCTGGAGGCGGACTG GTGCAGGCTGGAGACAGTCTGAGATTGTCTTGTGCCGC TTCTGGCCGGATCAGCAACTACGCAATGGGCTGGTTCC GGCAGGCACCCGGTAAAGAAAGGGAGTTCGTGCTGTCT	365

	ATCACCAGGAGCGGCGGAAGCACATACTATGCTGATAG TGTTAAGGGCCGCTTCACCATTTCCAGAGATAACGGCA AAAACACGATTGATCTTCAGATGAACAGACTGAAGCCT GAAGACACAGCAGTGTACTATTGTGCCGTGAGGCGCAG TCAAAAACCTGGTAACCTTTGGCGCTGAGTATCCTTGGTG GGGCCAGGGAACATTGGTGACTGTCAGCTCC	
DR1468 DNA	GAGGTGCAGTTGGTGGAGAGCGGCGGAGGCTTG GTTCAAGCTGGGGGCTCACTCAGGCTGTCTTGCACTACC TCTGGGCGTACAGGCACCCATTATGCGATGGGTTGGTTT AGGCAAGCGCCCGGCAAGGAACGCGAGTTCGTTAGTCT CATCCTGTGGAACGGCGAGTTTACGACCTATAAAGATT CTGTTAAGGGCCGCTTCACCATCTCCCGTGAGAAAGGC GAAAACACGGTCTACTTGCAAATGAACTCTCTGAAACC CGAGGATACTGCGGTGTATTACTGCTACCTGAGGGTGT TTGCTAGGCGCTACTGGGGCCAGGGAACCCAGGTGACC GTGTCCAGT	366
DR1469 DNA	GAAGTGCAGCTGGTGGAAAGTGGAGGCGGACTG GTGCAGCCAGGGGGCAGCCTCCGCCTTTCTTGTGAGGT GTCCGGCTTTACCTTCAGCAACTACTGGATGTACTGGAT TCGCCAAGCCCCTGGGAAGGGACTGGAGTGGGTGTCCC ACATTAACACCAACGGTGGCAACACTTATTACCGCCAT AGTGTTAAAGGTAGATTCACTATCAGCAGGGATAACGC TAAGAATAACCCTGTACTTGCAGATGAACGGCCTGAAGT CCGAGGACACCGCTGTGTATTACTGTGCCAAGGCTAAC TCCGATGTCCGGTTGGGTTATTACGGCATGGATTACTGG GGTAAGGGAACCTCAGGTACAGTGAGTTCT	367
DR1470 DNA	GAGGTGCAGCTGGTGGAAAGTGGCGGGGGCTCT GTTCAAGCCCGGCGGATCTCTGCGCCTGAGCTGTGCTGC ACCAGAGTCCATCTTCAACAATAACGCTGTTTACTGGTA TCGGCAATTTCCGGGCAAAGAAAGGGAGTACGTGGGCC TCATCACGATTGGTGGGCGCACTGGATACGCCGACTCT GTCAAGGGCCGCTTTACTATCAGTCGTGATAACGCCAA CAATGTTGCTTTTCTCCAGATGGATAACCTGAAGCCGG AAGATACTGCGGTATATTACTGTGCCGCTAGGCCTGGA TATTGGTCCAGTTCCTACGATTATTGGGGTCAGGGAACC CAAGTAACAGTGTCTCT	368
DR1471 DNA	GAAGTGCAGCTGGTGGAAAGCGGCGGTGGCCTC GTGCAGGCGGGCGGGTCCCTGAGACTGTCATGCGTCTT CTCTGGCCCGCGCCCGGCTAGTTATGCAATGGCTTGGTT TCGCCAGGCCGTGGGCAACGAGAGGGAGTTTGTGCTG CGATCAACTGGTCCGGCAGGCGCACTTACTATGCCGAC TCAGTGAAGGGCCGCTTCACTATTTCCAAGGACAATGC ACAGAACACCGCCTATCTCCAGATGACCAACTTGGAAC CAGAGGATACTGCCACGTATTACTGTAATGCTTACTTGA GCGGAACATATTACTGGGGCCAGGGCACCCAGGTGACC GTCTCTAGC	369
DR1472 DNA	GAGGTCCAGCTGGTTCGAGTCTGGCGGTGGCTTG GTCCGCGCTGGGGACTCACTGCGCCTGAGTTGTGCTGT GTCCGGCCTGGCCAGCTCCTCTTTCTTTATGACTTGGTT CCGCCAAGGGCAGGGCAAGGAGCGGGAATTTGTGGCC ACTATCAGTTGGACTGGCCGTACATCCTATTACGCTGCC AGCGTGAAAGGCCGCTTTACCGTTAGTCGGGACAATGC CAAGAATACCGTGTACCTTCAGATGAACTCTCTGAACT CTGAGGATACAGCAGTCTACTTCTGTGCAGCCTACCCG	370

CGTACACTGGTGCGTAATCGCGAGCCGATCCATTGGGG
TCAGGGAACCCAGGTGACTGTGTCCTCC

Table 12- anti-IL2Rg VHH DNA sequences

Name	Sequence	SEQ ID NO:
1 hIL2Rg _VHH-	CAGGTCCAGCTCCAGGAGAGC GGGGGCGGTTCTGTGCAAGCCGGAGG CTCATTGAGACTCTCATGCGCTGCAAG TGGTTTTACCTTCGATGACAGCGATAT GGGATGGTATCGTCAGGCTCCGGGCAA TGAGTGTGATCTGGTCTCCACTATCTCC TCTGATGGTTCCACATACTATGCTGAC TCTGTCAAGGGGCGCTTTACCATCTCC CAAGATAATGCCAAGAACACCGTGTAC CTTCAGATGGATTCAAGTTAAGCCCGAG GACACAGCCGTCTATTACTGCGCTGCG GATTTTATGATTGCCATCCAAGCTCCC GGAGCGGGATGCTGGGGCCAGGGAAC CCAGGTCACTGTGAGCAGT	371
2 hIL2Rg _VHH-	CAGGTGCAGTTGCAGGAGTCC GGCGGGGGTTCTGTGCCAGCGGGTGGG AGCCTCAAGCTCTCCTGTGCCGCTTCC GGCTTCTCATTTCTCTCTTACCCTATGA CCTGGGCACGCCAAGCGCCCGGCAAG GGACTGGAATGGGTGTCCACCATTTGCT TCCGATGGCGGTAGTACAGCCTACGCC GCGTCAGTGGAGGGTCCGTTTACGATC AGCCGGGACAACGCGAAGAGCACACT CTACCTCCAGCTGAACTCTCTGAAGAC CGAGGACACCGCCATGTACTATTGCAC AAAGGGCTACGGCGACGGCACCCCGG CACCCGGCCAGGGCACCCAGGTGACA GTCTCTTCC	372
3 hIL2Rg _VHH-	CAGGTGCAGTTGCAGGAAAGT GGTGGAGGGAGTGTGCAGACTGGGGG CTCTCTCCGCTCAGCTGCACAGCCTCT GGATTTACCTTCGATGATCGCGAGATG AACTGGTATCGCCAGGCTCCGGGAAAC GAGTGCGAACTGGTGTCTACAATCAGT TCTGACGGGTCCACCTATTACGCTGAT AGTGTCAAGGGCCGCTTCACTATCTCT CAGGACAACGCGAAGAACACCGTTTA CTTGCAGATGGATAGCGTGAAGCCTGA AGATACAGCGGTGTATTACTGCGCTGC CGACTTTATGATTGCCATCCAGGCACC GGGGGCGGGTGTGGGGACAGGGAA CTCAGGTGACTGTGTCTCTCC	373
4 hIL2Rg _VHH-	CAGGTTCAACTCCAAGAGAGT GGTGGCGGAAGCGTGCAGGCGGGCGG TTCTCTGCGTCTGAGTTGCACTGCCAG CGGATTTACCTTCGACGATTCCGACAT GGGATGGTACAGACAGGCCCTGGTA ACGAGTGCGAACTCGTGAGTACTATCA GCTCCGACGGCAACACCTATTACACCG ATTCTGTGAAGGGCAGGTTACCATCT CCCAGGACAACGCTAAGAACACTGTGT ACCTGCAAATGAATAGCCTGGGACCCG	374

		AGGACACAGCGGTCTATTACTGCGCGG CAGAGCCGCGCGGCTATTACAGCAACT ACGGCGGTAGACGCGAGTGCAACTACT GGGGGCAGGGACGCAAGTGACTGTC TCCTCC	
5	hIL2Rg _VHH-	CAAGTGCAGCTTCAGGAGTCC GGGGGTGGCAGCGTCCAGGCTGGGGG CAGCTTGCGCCTGTCTTGCGCTGCGTCT GGGTTCAGCTTTAGCTCCTACCCATG ACCTGGGCTAGACAGGCCCCCGCAA GGGGCTGGAGTGGGTGAGTACAATCG CCTCCGACGGAGGTAGTACGGCCTACG CAGCGTCCGTCGAGGGTCGCTTCACCA TCAGCCGGGATAACGCTAAGTCCACCC TGTAACCTTCAGCTCAATTCTCTCAAAA CGGAGGATACCGCCATGTACTATTGCA CCAAGGGATATGGCGACGGCACCCCA GCTCCTGGACAGGGCACACAGGTCACC GTTAGCTCC	375
6	hIL2Rg _VHH-	CAGGTCCAGCTTCAGGAGTCTG GCGGGGGCGCAGTACAGGCAGGGGGT TCTCTGCGTCTGTCTGCGCCGCGTCCG GCTTTACTTTTCAGCAACGCACACATGA GTTGGGTGCGCCAAGCGCCCCGCAAG GGCCGGGAATGGATCAGTAGCATCTAC AGTGGAGGCAGCACATGGTACGCCGA CTCTGTAAAGGGTCGTTTTACGATCTCT CGTGACAACCTCCAAGAACACTTTGTAC CTCCAGCTCAATTCTCTCAAGACCGAG GACACCGCGATGTACTATTGTGCCGAG AACAGGCTGCACTACTATTCCGACGAT GACTCTCTCAGGGGCCAGGGAACCTCAA GTTACCGTGTCCAGC	376
7	hIL2Rg _VHH-	CAAGTGCAGCTCCAAGAGAGT GGTGGCGGGCTGGTTTCAGCCAGGGGG CAGCTTGAGACTCTCTGCGCAGCTTC AGGCTTTACCTTCGATGACCGTGAGAT GAACTGGTATCGTCAGGCCCCAGGCAA CGAGTGTGAGCTGGTTAGCACGATTTT TTCCGACGGTTCCACCTATTACGCCGA CTCTGTGAAGGGACGTTTCACTATCTC CCAGGACAATGCCAAGAACAACCGTGT ACCTCCAGATGGACAGCGTGAAGCCG GAGGATACTGCTGTGTATTACTGCGCT GCCGACTTTATGATCGCCATCCAGGCC CCTGGCGCGGGTTGCTGGGGCCAGGGC ACTCAGGTGACCGTGTCTTCC	377
8	hIL2Rg _VHH-	CAAGTGCAACTGCAAGAGTCC GGCGGTGGATCTGTGCAGGCCGAGG CAGCCTGCGGCTGAGCTGTGTAGCTTC CGGGTATACCTTTAGCTCATACTGTAT GGGCTGGTTTCGTCAGGCCCCCGGTAA GGAGCGCGAGGGCGTGGCCGCTCTTGG TGGAGGCTCCACCTATTACGCCGATTC CGTGAAGGGCAGGTTTACTATCTCCCA GGACAACGCGAAGAATACGCTCTATCT CCAGATGAATAGCCTGAAGCCCCGAGG ATACAGCTATGTATTACTGTGCTGCCG	378

		CTTGGGTAGCCTGCCTGGAGTTCGGTG GCTCCTGGTACGATCTGGCACGGTACA AACATTGGGGGCAGGGCACCCAGGTC ACCGTGTCTAGC	
9	hIL2Rg_VHH-	CAGGTCCAGTTGCAGGAATCTG GGGGCGGTTCCGTACAAGCAGGTGGCT CCCTTCGGTTGAGCTGTACCGCATCCG GCTTTACTTTTCGACGATAGCGATATGG GCTGGTATCGTCAGGCCCCAGGGGGCG AGTGCGAGCTGGTTACAATCTCCTCTG ACGGCAGTACCTATTACGCAGACTCCG TCAAGGGCAGGTTCACTATCAGTCAGG ACAATGCAAAGAACACTGTGTATCTCC AGATGAACTCTCTGAAGCCAGAAGATA CTGCCGTGTATTACTGCGCTGCGGAAC CGAGAGGCTATTACTCTAATTATGGCG GGCGTCGGGAGTGTAATTATTGGGGAC AGGGAACCCAGGTGACCGTGTCTCTCC	379
10	hIL2Rg_VHH-	CAGGTGCAGCTCCAGGAGAGT GGCGGAGGCTCCGTGCAGGCTGGGGG CTCTCTGCGTCTGAGCTGTGCCGCAAG CGGTAGCATTACAGCTCTGCCTACAT CGGGTGGTTTCGTCAAGCGCCGGGCAA AAAGCGCGAAGGCGTGGCCGGAATCT ACACGCGCGATGGCTCCACCGCTTATG CTGACAGCGTTAAGGGACGTTTTACGA TCAGCCAGGACTCTGCCAAAAAGACTG TGTATCTCCAGATGAACTCCCTGAAAC CTGAGGACACAGCCATGTATTACTGCG CCGCTGGCCGCCGTACAAAGAGCTATG TTTACATCTTTGCCCCGAAGAGTACA ACTACTGGGGCCAGGGAACCCAAGTG ACTGTGTCCAGT	380
11	hIL2Rg_VHH-	CAGGTTTCAGTTGCAGGAGTCCG GCGGAGGCAGCGTGCAGGCCGAGGC TCCTTGCGCTTGCTCTGTGCGGCTTCTG GCTTCACCTTCTCATCTGCTCACATGAG TTGGGTGCGTCAGGCCCCAGGGAAAG GTGCGAGTGGATTGCCTCCATCTACA GCGGTGGGGGCACTTTTTATGCGGACA GCGTGAAGGGCCGCTTTACCATCAGCC GTGACAACGCTAAGAACACCCTGTATC TCCAACCTCAATTCCCTCAAGACCGAGG ATACAGCGATGTAATTATTGTGCAACCA ACCGCCTTCACTATTACTCCGACGATG ACAGCCTGCGCGGACAGGGGACCCAG GTGACGGTGTCCAGC	381
12	hIL2Rg_VHH-	CAGGTGCAACTCCAGGAAAGT GGCGGAGGCTCAGTGCAGGCAGGTGG CTCTCTCCGCTTTTCTGCGCTGCCAGC GGATTCACCTTCTCTAACGCTCACATG AGCTGGGTTTCGTCAAGGCTCCCGGCAA GGCCGTGAATGGATTAGCTCCATCTAT AGTGGCGGAAGTACTTGGTACGCAGAT AGCGTCAAGGGCCGCTTCACTATTAGT CGGGATAACTCCAAGAACACTCTGTAC CTCCAGCTGAACTCATTGAAAACCGAG GACACGGCTATGTACTATTGTGCTGAG AACAGGCTGCACTATTACTCCGACGAT	382

	GACTCTCTGAGGGGTCAGGGCACCCAG GTGACCGTCAGCTCC	
13	hIL2Rg_VHH- CAGGTCCAACCTCCAGGAGTCC GGCGGAGGCAGCGTGCAGGCTGGAGG CTCTCTCCGCCTGAGCTGCACAGCTTC CAGATTCACTCTCGATGACTCCGACAT GGGCTGGTATCGCCAGGCTCCAGGGAA CGAGTGCGAACTGGTGAGCACCATCTC ITCAGACGGTAGCACCTATTACGCCGA CAGTGTGAAGGGGCGCTTCACCATCTC CCGCGACAATGCTAAAAATACGGTGTA TCTCCAGATGAACTCCCTCAAACCGGA GGACACAGCTGTATATTACTGTGCTGC GGAACCACGGGGCTACTATAGCAACTA TGGTGGAAGGCGCGAGTGCAACTACTG GGGTCAGGGCACACAGGTGACGGTTTC CTCC	383
14	hIL2Rg_VHH- CAGGTGCAGCTCCAGGAGAGC GGCGGTGGCTCCGTGCAGGCTGGTGGC AGCCTGAAGCTGTCTGCACCGTGAGT GGCTTCACAGCCGACGATTCTGATATG GGCTGGTATCGCCAAGGCCCCGGCAAT GAGTGCGAGCTGGTAACCATTAGCTCA GACGGCTCTACATACTATGCCGATTCT GTTAAGGGCCGCTTTACTATCTCACAG GATAATGCCAAGAACACAGTGTACTTG CAGATGAACTCTCTGAAACCGGAAGAC ACAGCTGTGTATTACTGTGCTGCGGAG CCTAGAGGGTATTACAGCAATTACGGG GGCCGGAGAGAGTGTAACCTATTGGGG GCAGGGCACCCAAGTGACCGTTTCCTC C	384
15	hIL2Rg_VHH- CAGGTCCAGCTTCAGGAATCTG GGGGCGGTCTCGTGCAGCCCCGCGGGT CCCTGCGTCTGTCTTGTGCTGCGAGCG GCTTCACGTTCTCAAGTGCCACATGA GCTGGGTAAGGCAGGCACCGGGCAAG GGGCGCGAGTGGATTGCAAGCATCTAT TCAGGCGGGGGCACATTCTACGCCGAC AGCGTGAAGGGACGTTTTACAATCTCC AGAGATAACGCAAGAACAACCTCTCTAC CTCCAACCTCAACTCCTTGAAGGCGGAA GATACTGCAATGTATTACTGTGCTACT AACCGTCTTCATTATTACTCTGACGAT GACTCCCTGCGGGGGCAGGGTACACA GGTGACAGTGAGTTCC	385
16	hIL2Rg_VHH- CAGGTGCAGCTGCAAGAATCT GGTGAGGGCTGGTCCAGCCTGGGGG CTCCCTGCGCCTCTCATGTGTGCGATCT GGCTTCACCTTCAGCAACGCCACATG AGCTGGGTTTCGCCAAGCCCCTGGGAAG GGCCGGGAGTGGATCTCCAGTATCTAT TCCGGCGGAAGCACTTGGTATGCAGAC AGCGTCAAAGGACGGTTCACTATTTCT CGTGATAATTCTAAGAACACCCTGTAC CTTCAGCTGAACAGCCTGAAGACCGAG GACACTGCTATGTACTATTGTGCTGAG AATCGCCTGCATTACTATAGCGACGAT	386

		GACAGTCTGCGCGGACAGGGGACCCA GGTCACCGTGTCCTCT	
17	hIL2Rg _VHH-	CAGGTTTCAGTTGCAGGAATCA GGAGGCGGTCTGGTGCAGCCTGGGGG CTCTCTGCGTCTCTCCTGCGCCGCTTCC GGCTTCACATTCTCCAACGCCACATG AGCTGGGTCCGCCAGGCCCTGGGAAG GGCCGCGAGTGGATCTCCAGTATCTAC AGCGGGGGCTCCACTTGGTACGCAGAC AGCGTCAAAGGGAGGTTTACCATTAGC CGTGACAATTCTAAGAACACATTGTAT TTGCAGCTGAACTCTCTTAAAACCGAG GACACCGCCATGTACTATTGTGCTGAG AACAGGCTCCACTATTACTCAGACGAT GACTCACTTCGCGGGCAGGGAACCCAG GTCACCGTCTCCTCT	387
18	hIL2Rg _VHH-	CAAGTCCAGTCCAGGAAAGC GGCGGTGGCCTGGTGCAACCTGGCGGG TCTCTGCGCTTGTATGCGCTGCCTCCG GCTTCACCTTCTCATCTTACCCTATGAC CTGGGCGCGTCAGGCTCCCGGCAAGGG ATTGGAGTGGGTGTCTACTATTGCCTC CGACGGTGGCAGCACGGCCTACGCAG CGTCTGTAGAAGGACGCTTACAATTA GCAGAGACAACGCAAAATCTACTTTGT ACCTTCAGCTCAACAGCCTGAAGACCG AAGACACAGCTATGTATTACTGCACAA AAGGCTACGGGGACGGCACGCCAGCG CCTGGACAGGGGACACAGGTGACCGT ATCTTCT	388
19	hIL2Rg _VHH-	CAGGTGCAGTTGCAGGAATCA GGGGGTGGCTCTGTGCAGGCCGGGGG CTCCCTGCGTCTGTCTGTACTGCGAG CGGCTTCACCTTTGATGACCGCGAGAT GAACTGGTATCGCCAGGCTCCGGGGAA CGAGTGCGAACTCGTGTCTACAATTAG CTCCGATGGTTCAACATACTATGCTGA TTCTGTCAAAGGTCGCTTTACCATCTCA CAGGACAACGCCAAGAACACCGTCTA CCTCCAGATGGACTCTGTGAAGCCTGA AGATACCGCCGTATACTATTGCGCCGC TGACTTTATGATTGCCATTAGGCTCC GGGTGCTGGATGCTGGGGTCAGGGGA CTCAGGTGACCGTGTCTTCA	389
20	hIL2Rg _VHH-	CAAGTGCAGTTGCAGGAAAGC GGCGGTGGGTCCGTGCAAGCCGGAGG TTCTCTCCGCTGTCTTGCACTGCCTCA GGTTTTACCTTCGACGATTCCGATATG GGCTGGTACAGGCAGGCTCCCGGCAAT GAGTGCGAGCTGGTGTCTACGATCTCA AGTGATGGCTCCACCTACTATGCCGAT AGCGTAAAAGGAAGGTTTACTATTAGC CAGGATAACGCGAAGAACACGGTGTA CCTCCAGATGAACAGTCTCAAGCCGGA GGATACTGCCGTGTATTACTGTGCTGC CGAGCCGCGTGGCTATTACTCCAATA CGGTGGCAGACGTGAATGCAATTACTG GGGACAGGGTACTCAGGTTACCGTGTCTCT	390

21	hIL2Rg _VHH-	CAGGTTCAACTTCAGGAATCCG GGGGCGGTTCCGTGCAAGCCGGGGT AGCCTGCGTCTGTCTTGGCTGGCCAGC GGCTATACCTCCTGTATGGGTTGGTTTC GGCAGGCTCCTGGGAAGGAGCGCGAA GCCGTGGCGACCATCTACACACGGGGC CGCAGCATCTATTACGCTGACAGTGTG AAGGGCCGCTTCACCATCTCCCAGGAT AACGCCAAGAATACCCTGTATCTGCAA ATGAACTCCCTGAAGCCTGAGGACATC GCCATGTATTCTGCGCAGCTGGAGGG TACTCATGGTCCGCTGGGTGCGAGTTT AATTATTGGGGCCAAGGAACCCAGGTG ACCGTCTCCTCA	391
22	hIL2Rg _VHH-	CAAGTGCAGCTCCAGGAGTCT GGCGGGGGCCTGGTTACAGCTGGTGGG TCCCTGCGCCTGTCTTGACGGCTTCCG GCTTTAGCTTCTCCTCATATCCAATGAC CTGGGCACGCCAGGCTCCTGGTAAGGG CCTGGAGTGGGTCTCCACCATCGCCTC TGATGGTGGGTCAACTGCCTATGCTGC CTCCGTGAGGGTAGATTACAAATCAG CAGAGACAACGCCAAATCCACGCTGTA CCTGCAACTCAACTCCTTGAAGACCGA GGACACAGCTATGTATTACTGTACCAA AGGCTACGGCGACGGCACTCCTGCTCC CGGACAGGGGACCCAGGTGACTGTGTCTAGC	392
23	hIL2Rg _VHH-	CAGGTCCAACTTCAGGAAAGC GGGGGTGGACTGGTACAGCCAGGGGG CAGTCTGCGCCTGTCTGTGCCGCAAG CGGGTTTTCTTTCTCCAGTTACCCCATG ACCTGGGCTCGCCAAGCACCTGGAAAG GGAAGTGGAGTGGGTGTCTACTATTGCG TCAGATGGTGGGAGTACGGCTTACGCC GCGAGCGTGGAGGGTCTGTTTTACGATC AGTAGGGACAACGCCAAAAGCACTCT GTACCTCCAGCTTAACAGCCTGAAGAC CGAGGACACCGCCATGTATTACTGTAC CAAGGGCTACGGAGACGGCACCCCTG CGCCGGGGCAAGGCACCCAGGTGACC GTAAGTTCA	393

Table 13- murine anti-IL2Rg VHH DNA sequences

Name	DNA Sequence	SEQ ID NO:
mIL2Rg_V HH1	CAGGTGCAACTCCAGGAGTCCGGCGGGGGCT CCGTGCTGGCTGGCGGATCTTTGAGGCTGTCTTGCGT GGCTTCTGGCTATGGCTATAATTACATCGGCTGGTTC CGTCAGACACCCGGCAAGGAGCGCGAAGGGGTGGC GGTCATTTACACAGGGGGTGGGGACACTTATTACGC CGACTCCGTCAAGGGTAGGTTTACCGCTAGTCGCGAT AATGCCAAAAGTACGCTGTACCTGCAAATGAACAGC TTGGAGCCAGAGGACACCGCCATGTATTACGGAGTG GCTCGCTACTGTGTGGGCAGTGTGTACGCTTGCCCTGC GCGGAGGCCACGACGAGTACGCACACTGGGGCCAGG GAACCCAGGTGACAGTGTCTAGC	394
mIL2Rg_V HH2	CAGGTGCAGCTCCAGGAGTCTGGGGGTGGCA GCGTCCAGCCAGGTGGCTCATTGAGACTGTCTTGTGC TGCATCTGGCTCCACCTACGCTAATTACCTGATGGGA TGGTTCAGGCAGGCCCTGGTAAGGAGCGTGAGGGC GTGGCCGCTATCTATTCTGGCGGTGGGTCCACCTACT ATGCTGACTCCGTCAAGGGACGCTTCACTATTTCTCA AGACAATGCCAAGAACAACCTTTGTACTTGCAAATGAA CTCACTCAAACCTGAGGACACCGCGATGTACTATTGC GCAGCGGCATCCGCAGTGAAGGGAGACAAAGGGGA TATCGTGGTAGTTGTGACCGGCACCCAGCGTATGGA GTACGACTACTGGGGACATGGCACCCAGGTGACAGT TAGCTCC	395
mIL2Rg_V HH3	CAGGTACAGTTGCAGGAGAGTGTTGGGGGTT CCGTCCAGGCCGGTGCCTCTCTTCGCCTCAGTTGTAG CGTGAGCGGTTTCACGTTGACGAGTCAGTGATGTCC TGGTTGCGCCAGGGTCCCGGCAATGAGTGCGACGCG GTCGCTATTATCAGCTCCGATGACAACACCTATTACG ACGATAGCGTGAAAGGCCGCTTTACCATCTCCGAGG ACAACGCCAAAAACATGGTGTATCTGCAAATGAACT CACTGAAGCCGGAAGACACCGCAGTGTACTATTGCG CCGCGCGTCCGGCGCAGACCTGTGTACGATTCCGATTA TGAACCTCCGGCCACGTCCGCTGTGTGGCGATTTCGGC GTGTGGGGCCAGGGGACCCAGGTGACGGTCTCCTCC	396
mIL2Rg_V HH4	CAGGTGCAGCTCCAGGAATCTGGCGGGGGCT CTGTGCAGGCTGGTGGCTCCCTTCGCCTGTCTGTAT TGGCTCCGGTCTTCCTTTTCGACGAGGATGACATGGGC TGGTATCGCCAGGCCCTGGGAATGAGTGTGAATTG GTCAGCTCAATCTCCAGTGACGGCACCGCCTATTACG CCGATTCCGTCAAGGGACGCTTCACTATCTCCAGAGA CAACGCCAAGAACAACACTGTGCTGTTGCAGATGAACTC CCTGAAGCCCGAGGATACCGCTGTCTATTACTGCGCA GCCGGGGTCCACAGACAGTTCCGGCGGTTCCAGTTCTT GCGGCGACGCCTTCTACGGCATGGATTACTGGGGCA AGGGAACTCAGGTACAGTGTCTTCC	397
mIL2Rg_V HH5	CAGGTTTCAGCTTCAGGAGTCCGGCGGGGGCT CCGTACAGGCAGGGGGCTCACTGCGTCTTTCCTGTGT GGCGAGTGGCGACGTGTATGGCCGTAACAGCATGGC TTGGTTCCGGCAGGCACCTGGAAAGGAACGCGAGGG CGTTGCAGTTGGGTATTCCGTAGTGACAACCACTTAC TATGCCGACAGTGTGAAGGGCCGGTTTACGATCTCA	398

	GAGGACAACGATAAAAAACACAGTGTACCTGGAGATG AACTCCCTGAAGCCGGAAGACACTGCTATGTATTACT GCGCTGCCGATGGCAACCTGTGGCGCGGACTCAGGC CCTCCGAGTACACTTATTGGGGTCAGGGCACCCAGG TGACCGTTTCAAGT	
mL2Rg_V HH6	CAGGTCCAGCTTCAGGAGTCAGGTGGCGGTA GTGTCCAGGCAGGCGGTAGCCTGCGCCTTAGCTGTG CTACATCCGGCTTCCCTTACTCACGCTATTGTATGGG CTGGTTCAGGCAAGCTCCCGGTAAAGAGCGCGAGGG AGTGGCAGCCATCGAGCCTGACGGGAGCACATCTTA TGCTGACTCTGTAAAGGGGCGTTTCACCATCTCTCAG GACAACGCCGTTAATACTACTGTACTTGCAAATGAAT AACCTGAAGCCCGAGGACACAGCTATGTATTACTGC GCAGCCGACGAGCGTTGCTTCTATTTGAAGGACTATG ACCTCAGAAGGCCAGCCAGTACCGCTACTGGGGGC AGGGCACCCAGGTTACCGTGTCTATCT	399
mL2Rg_V HH7	CAGGTGCAGTTGCAGGAGAGTGGCGGTGGCC TCGTGCAGCCTGGCGGAAGCCTCCGTCTGAGCTGCA CTGTGTCCGGCTTCACTTTCGACGAGAGCGACATGGG CTGGCTGAGGCAGAACCTGGTAACGAGTGGCGCGT TGTGAGTGTATCACGTCTGATGACAACCCATACTAT GATGACAGCGTCAAGGGCCCGCTTACTATCTCCGAG GATAACGCCAAGAACATGGTGTACCTCCAGATGAAC TCACTGAAGCCCGAGGATACCGGCGTTTATTACTGTG CAACCAGGAGCCGTCAGCCTGTGTACTCACGCGATT ACGAGCTGCGGCCCGCCCCCTCTGTGGAGACTTTGG TGTGTGGGGCCAGGGCACCCAGGTGACTGTTTCCAG C	400
mL2Rg_V HH8	CAGGTGCAGTTGCAGGAGAGTGGAGGGGGCT CAGTGCAGGCTGGCGGGTCTTTCGCTCTGTCTTGAC CGCTCTGGCTTACCTTCGATGACTTCGATATGGGT TGGTATCGCCAGGCTCCAGGGAACGAGTGCGAATTG GTCAGCACTATCAGCGACGATGGCTCAACATATTAC GCCGACTCTGTGAAGGGACGGTCTAGCATTAGCCGG GACAACGCAAAGAACACCGTCTATCTCCAGATGAAC CGTTGAAGCCTGAGGATACCGGAGTCTATTACTGC GCCGCTGAGGGCGCGTTGGGCTCCAAGACTAATTGT GGCTGGGTGGGCAACTTCGGATATTGGGGCCAGGGA ACACAGGTTACCGTTTCCAGC	401
mL2Rg_V HH9	CAGGTGCAGTTGCAGGAGTCTGGAGGCGGTT CCGTTTCAGGCCGGGGGCTCTCTGCGCCTGTCTGCGC TGCTCCGGGTTTACATTTGACGATTTTCGATATGGGC TGGTATCGCCAGGCCCTGGCAACGAGTGCGAAGT GTGTCTACTATCTCCGATGACGGCTCAACCTACTATG CAGACTCCGTAAAGGGCAGATCCAGCATCTCCCGCG ACAATGCCAAAAACACTGTGTACCTCCAGATGAAC CCCTCAAGCCTGAGGATACGGCGGTGTACTATTGTGC TGCCGAGGGTGCGCTCGGTAGCAAGACTAATTGCGG CTGGGTGGGCAACTTCGGGTACTGGGGTCAGGGGAC CCAGGTAACCGTGTCTTCT	402
mL2Rg_V HH10	CAGGTGCAGTTGCAGGAAAGCGGTGGGGGCC TGGTGCAGCCCGGAGGCAGCCTGCGCTTGAGCTGCG CTGCCTCTGGCTTCACATTCGATGACTTCGATATGGG CTGGTATCGTCAAGCACCCGGAACGAGTGCGAGCT	403

	GGTGAGTACAATCAGTGATGACGGATCTACCTACTA TGCCGACAGCGTCAAGGGAAGATCCAGCATCAGTCG CGACAACGCCAAGAGCACCGTTTACCTCCAGATGAA CCGCCTCAAGCCTGAGGACACAGGAGTCTATTACTG TGCTGCGGAGGGGGCCTTGGGCAGCAAGACTAAGT TGGATGGGTGGGAAACTTCGGGTATTGGGGTCAGGG TACACAGGTCACAGTGTCTTCA	
mL2Rg_V HH11	CAAGTTCAGCTTCAGGAAAGTGGGGGCGGGC TGGTGACGCCAGGGGTTCCCTGAAGCTGAGCTGCG CTGCCTCTGGGTTTACATTCTCTGATCGCGACATGGG CTGGTATCGCCAAGCGCCGGGCAATGAATGCGAAAG AGTGAGTACTATTTCTGACGATGGTTCTACTTACTAT GCTGACTCCGTGAAGGGCCGTAGCTCCATTTCCAGG GACAACGCGAAGAACACCGTATACCTCCAGATGAAC TCTCTGAAGCCCGAGGACACCGCTGTGTATTACTGCG CTGCCGAGGGGGCTCTCGGCTCAAAGACCAACTGCG GATGGGTCCGTAACCTTCGGCTACTGGGGCCAGGGCA CCCAAGTGACAGTCTCCTCC	404
mL2Rg_V HH12	CAGGTCCAGTTGCAGGAGAGCGGGGGTGGAA GCGTCCTCGCCGAGGGAGCCTCCGTTTGAGCTGCGT CGCCTCAGGCTACGGCTACAATTACATCGGATGGTTC AGACAGACGCCTGGTAAAGAGCGGGAAGGCGTCGCC GTGATTTATATCGGTGGCGGAGACACCTATTACGCTG ACTCAGTGAAGGGGCGTTTCACCGCAAGCCGGGACA ACGCTAAGAGCACCTGTACCTCCAGATGAACTCTCT CGAACCTGAGGACACTGCAATGTATTACTGCGTGGC TCGTTACTGCGTCGGGAGTGTCTACGCCTGCCTGAGG GGCGGGCATGATGAGTATGCCCACTGGGGACAAGGA ACACAGGTGACTGTCTCCAGT	405
mL2Rg_V HH13	CAGGTTCAGCTCCAGGAGTCTGGTGGCGGTTTC CGTGCTGGCCGGGGGCTCTCTGCGCCTGTCTTGTTGTC GCCTCAGGGTACGGCTATAACTACATTGGCTGGTTCA GACAGACCCCTGGGAAAGAGCGGGAGGGTGTGGCTG TCATTTACACCGCGGAGGGCGACACCTACTATGCCG ATTCAGTTAAGGGCAGGTTTACCGCGAGCCGTGACA ACGCGAAGTCTACTCTGTACCTGCAAATGAACAGCC TGGAACCTGAGGATACTGCGATGTACTATTGTGTGGC CCGGTACTGCGTAGGCTCAGTGTATGCCTGCCTGCGC GGGGGTACGACGAGTACGCACACTGGGGACAGGG AACTCAGGTCACCGTGTCTAGC	406
mL2Rg_V HH14	CAGGTGCAACTCCAGGAGTCCGGCGGGGGCT CCGTCCAAGCTGGTGGCTCACTGAGGCTTAGCTGTGC TGCTCCGGCTTTACTTTTCGACGATTTTCGACATGGGT TGGTATCGCCAGGCTCCGGGCAATGAGTGCGAGCTG GTCTCTACCATTTCCGATGACGGCTCTACCTACTATG CCAACAGTGTTAAGGGTAGGTCTTCCATCTCCCGCGA CAACGCTAAGAATATGGTGTACTTGCAGATGAACTC TCTGAAGCCTGAGGACACTGCTGTCTACTATTGCGCT GCCGAAGGTGCCCTGGGCTCAAAGACTAATTGCGGC TGGGTCGGTAACCTTTGGCTACTGGGGTCAGGGGACT CAGGTGACCGTCAGCTCC	407
mL2Rg_V HH15	CAGGTCCAGTTGCAGGAAAGCGGCGGGGGCT CTGTTCAAGGCAGGCGGAAGCCTTCGTCTGTCTGTAC TGCCAGTGGTTTCACCTTTGATGACTTTGACATGGGC	408

TGGTATCGGCAAGCCCCGGAACGAGTGCGAGCTG GTATCCACCATTTCGATGACGGGTCCACGTACTATG CTGATAGCGTGAAGGGCAGGTCTTCATCAGCCGGG ACAACGCCAAGAACACAGTGTATTTGCAGATGAACC GCCTCAAGCCAGAAGACACCGGGGTATATTACTGTG CAGCGGAAGGTGCCCTGGGTAGCAAGATGAACTGCG GATGGGTGGGTAAATTTGGATACTGGGGCCAGGGCA CGCAGGTTACAGTGTCCAGC

Table 14. FDA Antineoplastic Disease Antibodies and Indications

Name	Tradename(s)	Target, format	Indication
[fam]- trastuzumab deruxtecan	Enhertu	HER2; Humanized IgG1 ADC	HER2 ⁺ breast cancer
Enfortumab vedotin	Padcev	Nectin-4; Human IgG1 ADC	Urothelial cancer
Polatuzumab vedotin	Polivy	CD79b; Humanized IgG1 ADC	Diffuse large B-cell lymphoma
Cemiplimab	Libtayo	PD-1; Human mAb	Cutaneous squamous cell carcinoma
Moxetumomab pasudotox	Lumoxiti	CD22; Murine IgG1 dsFv immunotoxin	Hairy cell leukemia
Mogamuizumab	Poteligeo	CCR4; Humanized IgG1	Cutaneous T cell lymphoma
Tildrakizumab	Ilumya	IL23p19; Humanized IgG1	Plaque psoriasis
Ibalizumab	Trogarzo	CD4; Humanized IgG4	HIV infection
Durvalumab	IMFINZI	PD-L1; Human IgG1	Bladder cancer
Inotuzumab ozogamicin	BESPONSA	CD22; Humanized IgG4, ADC	Hematological malignancy
Avelumab	Bavencio	PD-L1; Human IgG1	Merkel cell carcinoma
Atezolizumab	Tecentriq	PD-L1; Humanized IgG1	Bladder cancer
Olaratumab	Lartruvo	PDGFR α ; Human IgG1	Soft tissue sarcoma

Name	Tradename(s)	Target, format	Indication
Ixekizumab	Taltz	IL17a; Humanized IgG4	Psoriasis
Daratumumab	Darzalex	CD38; Human IgG1	Multiple myeloma
Elotuzumab	Empliciti	SLAMF7; Humanized IgG1	Multiple myeloma
Necitumumab	Portrazza	EGFR; Human IgG1	Non-small cell lung cancer
Dinutuximab	Unituxin	GD2; Chimeric IgG1	Neuroblastoma
Nivolumab	Opdivo	PD1; Human IgG4	Melanoma, non-small cell lung cancer
Blinatumomab	Blincyto	CD19, CD3; Murine bispecific tandem scFv	Acute lymphoblastic leukemia
Pembrolizumab	Keytruda	PD1; Humanized IgG4	Melanoma
Ramucirumab	Cyramza	VEGFR2; Human IgG1	Gastric cancer
Siltuximab	Sylvant	IL6; Chimeric IgG1	Castleman disease
Obinutuzumab	Gazyva	CD20; Humanized IgG1; Glycoengineered	Chronic lymphocytic leukemia
Ado-trastuzumab emtansine	Kadcyla	HER2; Humanized IgG1, ADC	Breast cancer
Pertuzumab	Perjeta	HER2; Humanized IgG1	Breast Cancer
Brentuximab vedotin	Adcetris	CD30; Chimeric IgG1, ADC	Hodgkin lymphoma, systemic

Name	Tradename(s)	Target, format	Indication
			anaplastic large cell lymphoma
Ipilimumab	Yervoy	CTLA-4; Human IgG1	Metastatic melanoma
Ofatumumab	Arzerra	CD20; Human IgG1	Chronic lymphocytic leukemia
Certolizumab pegol	Cimzia	TNF; Humanized Fab, pegylated	Crohn disease
Catumaxomab	Removab	EPCAM/CD3; Rat/mouse bispecific mAb	Malignant ascites
Panitumumab	Vectibix	EGFR; Human IgG2	Colorectal cancer
Bevacizumab	Avastin	VEGF; Humanized IgG1	Colorectal cancer
Cetuximab	Erbitux	EGFR; Chimeric IgG1	Colorectal cancer
Tositumomab- I131	Bexxar	CD20; Murine IgG2a	Non-Hodgkin lymphoma
Ibritumomab tiuxetan	Zevalin	CD20; Murine IgG1	Non-Hodgkin lymphoma
Gemtuzumab ozogamicin	Mylotarg	CD33; Humanized IgG4, ADC	Acute myeloid leukemia
Trastuzumab	Herceptin	HER2; Humanized IgG1	Breast cancer
Infliximab	Remicade	TNF; Chimeric IgG1	Crohn disease

Name	Tradename(s)	Target, format	Indication
Rituximab	MabThera, Rituxan	CD20; Chimeric IgG1	Non-Hodgkin lymphoma
Edrecolomab	Panorex	EpCAM; Murine IgG2a	Colorectal cancer

Table 15 FDA Immune Disease Antibodies and Indications		
Name	Target	Indication
belimumab	BLyS	Systemic lupus erythematosus
efalizumab	CD11a	Psoriasis
ocrelizumab	CD20	Multiple sclerosis
rituximab	CD20	Multiple sclerosis
basiliximab	CD25	Transplantation rejection
daclizumab	CD25	Transplantation rejection
muromonab	CD3	Transplantation rejection
alemtuzumab	CD52	Multiple sclerosis
omalizumab	IgE	Asthma
ustekinumab	IL12/IL23	Plaque psoriasis
brodalumab	IL17a	Psoriasis, psoriatic arthritis, ankylosing spondylitis
secukinumab	IL17a	Psoriasis, psoriatic arthritis, ankylosing spondylitis

ixekizu mab	IL17a	Psoriasis, psoriatic arthritis, ankylosing spondylitis
canaki numab	IL1 β	Cryopyrin-associated periodic syndrome, tumor necrosis factor receptor associated periodic syndrome, hyperimmunoglobulin D syndrome, mevalonate kinase deficiency, familial Mediterranean fever, rheumatoid arthritis
dupilu mab	IL4R α	Asthma, dermatitis
mepoli zumab	IL5	Asthma
reslizu mab	IL5	Asthma
tociliz umab	IL6R	Rheumatoid arthritis
vedoliz umab	Integrin- α 4 β 7	Ulcerative colitis, Crohn's disease
denosu mab	RANKL	Osteoporosis
certoli zumab	TNF α	Crohn's disease, rheumatoid arthritis
golimu mab	TNF α	Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis
adalim umab	TNF α	Rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, plaque psoriasis
infixi mab	TNF α	Crohn's disease, ulcerative colitis, rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, plaque psoriasis

ranibiz umab	VEGF -A	Neovascular age-related macular degeneration, macular edema
nataliz umab	VLA- 4	Multiple sclerosis, relapsing multiple sclerosis, Crohn's disease

WHAT IS CLAIMED IS:

1. An IL2Rb/IL2R γ binding molecule that specifically binds to IL2Rb subunit (IL2Rb) and IL2R γ subunit (IL2R γ),
 - wherein the binding molecule causes the multimerization of IL2Rb and IL2R γ when bound to IL2Rb and IL2R γ , and
 - wherein the binding molecule comprises a single-domain antibody (sdAb) that specifically binds to IL2Rb (an IL2Rb sdAb) and a sdAb that specifically binds to IL2R γ (an anti-IL2R γ sdAb).
2. The IL2Rb/IL2R γ binding molecule of claim 1, wherein the IL2Rb sdAb is a VHH antibody (an anti-IL2R γ sdAb) and/or the anti-IL2R γ sdAb is a VHH antibody (an anti-IL2R γ VHH antibody).
3. The IL2Rb/IL2R γ binding molecule of any one of claims 1 to 2, wherein the IL2Rb sdAb and the anti-IL2R γ sdAb are joined by a peptide linker.
4. The IL2Rb/IL2R γ binding molecule of claim 3, wherein the peptide linker comprises between 1 and 50 amino acids.
5. The IL2Rb/IL2R γ binding molecule of claim 4, wherein the peptide linker comprises a sequence of GGGS (SEQ ID NO: 11).
6. The IL2Rb/IL2R γ binding molecule of any one of claims 2 to 5, wherein the anti-IL2Rb sdAb comprises one or more CDRs in a row of Table 2 or Table 3, wherein each CDR independently comprises 0, 1, 2, or 3 amino acid changes relative to the sequence of Table 2 or Table 3.
7. The IL2Rb/IL2R γ binding molecule of any one of claims 2 to 6, wherein the anti-IL2R γ sdAb comprises one or more CDRs in a row of Table 4 or Table 5 wherein each CDR independently comprises 0, 1, 2, or 3 amino acid changes relative to the sequence of Table 4 or Table 5.

8. The IL2Rb/IL2R γ binding molecule of any one of claims 2 to 7, wherein the IL2Rb/IL2R γ binding molecule comprises

an anti- IL2Rb sdAb comprising a CDR1, a CDR2, and a CDR3 in a row of Table 2 or Table 3; and

an anti- IL2R γ sdAb comprising a CDR1, a CDR2, and a CDR3 in a row of Table 4 or Table 5.

9. The IL2Rb/IL2R γ binding molecule of any one of claims 1 to 8, wherein the binding molecule comprises an anti- IL2Rb sdAb linked to the N-terminus of a linker and an anti- IL2R γ sdAb linked to the C-terminus of the linker.

10. The IL2Rb/IL2R γ binding molecule of any one of claims 1 to 8, wherein the binding molecule comprises an anti- IL2R γ sdAb linked to the N-terminus of a linker and an IL2Rb sdAb linked to the C-terminus of the linker.

11. The IL2Rb/IL2R γ binding molecule of claims 9 or 10, wherein the IL2Rb sdAb comprises a sequence having at least 90% sequence identity to a sequence of Table 6 or Table 7.

12. The IL2Rb/IL2R γ binding molecule of claims 9 or 10, wherein the IL2Rb sdAb comprises a sequence of Table 6.

13. The IL2Rb/IL2R γ binding molecule of claims 9 or 10, wherein the anti-IL2R γ sdAb comprises a sequence having at least 90% sequence identity to a sequence of Table 8 or Table 9.

14. The IL2Rb/IL2R γ binding molecule of claims 9 or 10, wherein the anti-IL2R γ sdAb comprises a sequence of Table 8.

15. The IL2Rb/IL2R γ binding molecule of any one of claims 9 to 14, wherein each of the IL2Rb sdAbs comprises a sequence having at least 90% sequence identity to a sequence of Table 6 or Table 7, and each of the anti- IL2R γ sdAbs comprises a sequence having at least 90% sequence identity to a sequence of Table 8 or Table 9.

16. The IL2Rb/IL2R γ binding molecule of any one of claims 9 to 14, wherein each of the IL2Rb sdAbs comprises a sequence of Table 6 or Table 7, and each of the anti-IL2R γ sdAbs comprises a sequence having a sequence of Table 8 or Table 9.

17. An isolated nucleic acid encoding the IL2Rb/IL2R γ binding molecule of any one of claims 1 to 16.

18. The isolated nucleic acid of claim 17, wherein the isolated nucleic acid comprises a sequence having at least 90% sequence identity to a sequence of Table 10 or Table 11, and a sequence having at least 90% sequence identity to a sequence of Table 12 or Table 13.

19. An expression vector comprising the nucleic acid of claim 17 or 18.

20. An isolated host cell comprising the vector of claim 19.

21. A pharmaceutical composition comprising the IL2Rb/IL2R γ binding molecule of any one of claim 1 to 16 and a pharmaceutically acceptable carrier.

22. A method of treating an autoimmune or inflammatory disease, disorder, or condition or a viral infection in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an IL2Rb/IL2R γ binding molecule of any one of claims 1 to 16 or a pharmaceutical composition of claim 21.

23. The method of claim 23, further comprising administering one or more supplementary agents selected from the group consisting of a corticosteroid, a Janus kinase inhibitor, a calcineurin inhibitor, a mTor inhibitor, an IMDH inhibitor, a biologic, a vaccine, and a therapeutic antibody.

24. The method of claim 24, wherein the therapeutic antibody is an antibody that binds a protein selected from the group consisting of BLYS, CD11a, CD20, CD25, CD3, CD52, IgEIL12/IL23, IL17a, IL18, IL4R α , IL5, IL6R, integrin- α 4 β 7, RANKL, TNF α , VEGF-A, and VLA-4.

25. The method of any one of claims 22 to 24, wherein the disease, disorder, or condition is selected from viral infections, *heliobacter pylori* infection, HTLV, organ rejection, graft versus host disease, autoimmune thyroid disease, multiple sclerosis, allergy, asthma, neurodegenerative diseases including Alzheimer's disease, systemic lupus erythramatosis (SLE), autoinflammatory diseases, inflammatory bowel disease (IBD), Crohn's disease, diabetes, cartilage inflammation, arthritis, rheumatoid arthritis, juvenile arthritis, juvenile rheumatoid arthritis, juvenile rheumatoid arthritis, polyarticular juvenile rheumatoid arthritis, systemic onset juvenile rheumatoid arthritis, juvenile ankylosing spondylitis, juvenile enteropathic arthritis, juvenile reactive arthritis, juvenile Reiter's Syndrome, SEA Syndrome, juvenile dermatomyositis, juvenile psoriatic arthritis, juvenile scleroderma, juvenile systemic lupus erythematosus, juvenile vasculitis, pauciarticular rheumatoidarthritis, polyarticular rheumatoidarthritis, systemic onset rheumatoidarthritis, ankylosing spondylitis, enteropathic arthritis, reactive arthritis, Reiter's syndrome, SEA Syndrome, psoriasis, psoriatic arthritis, dermatitis (eczema), exfoliative dermatitis or atopic dermatitis, pityriasis rubra pilaris, pityriasis rosacea, parapsoriasis, pityriasis lichenoiders, lichen planus, lichen nitidus, ichthyosiform dermatosis, keratodermas, dermatosis, alopecia areata, pyoderma gangrenosum, vitiligo, pemphigoid, urticaria, prokeratosis, rheumatoid arthritis; seborrheic dermatitis, solar dermatitis; seborrheic keratosis, senile keratosis, actinic keratosis, photo-induced keratosis, keratosis follicularis; acne vulgaris; keloids; nevi; warts including vernuca, condyloma or condyloma acuminatum, and human papilloma viral (HPV) infections.

26. A method of treating a neoplastic disease, disorder or condition in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an IL2Rb/IL2R γ binding molecule of any one of claims 1 to 16 or a pharmaceutical composition of claim 21.

27. The method of claim 26, further comprising administering one or more supplementary agents selected from the group consisting of a chemotherapeutic, an immune checkpoint inhibitor, cell therapy, cytokine therapy, and a therapeutic antibody.

28. The method of claim 27, wherein the therapeutic antibody is an antibody that binds to at least one tumor antigen selected from the group consisting of HER2, nectin-4, CD79, CTLA4, CD22, CCR4, IL23p19, PDL1, IL17a, CD38, SLAMF7, CD20, CD30,

CD33, CD52, EpCam, CEA, fpA33, TAG-72, CAIX, PSMA, PSA, folate binding protein, GD2, GD3, IL6, GM2, Le^x, VEGF, VEGFR, VEGFR2, PDGFR α , EGFR, ERBB2, ERBB3, MET, IGF1R, EPHA3, TRAIL R1, TRAIL R2, RANKL RAP, tenascin, integrin α V β 3, and integrin α 4 β 1.

29. The method of any one of claims 26 to 28, wherein the neoplastic disease, disorder or condition is selected from the group consisting of breast cancers, sarcomas, leukemias, lymphomas, genitourinary cancers, gastrointestinal cancers, lung cancers, myelomas, pancreatic cancers, liver cancers, kidney cancers, endocrine cancers, skin cancers, and brain or central and peripheral nervous (CNS) system tumors, malignant tumors, benign tumors, gliomas, neuroblastomas, astrocytomas, myelodysplastic disorders, cervical carcinoma-in-situ, intestinal polypses, oral leukoplakias, histiocytoses, hyperproliferative scars, hemangiomas, hyperproliferative arterial stenosis, psoriasis, inflammatory arthritis, hyperkeratoses, and papulosquamous eruptions.

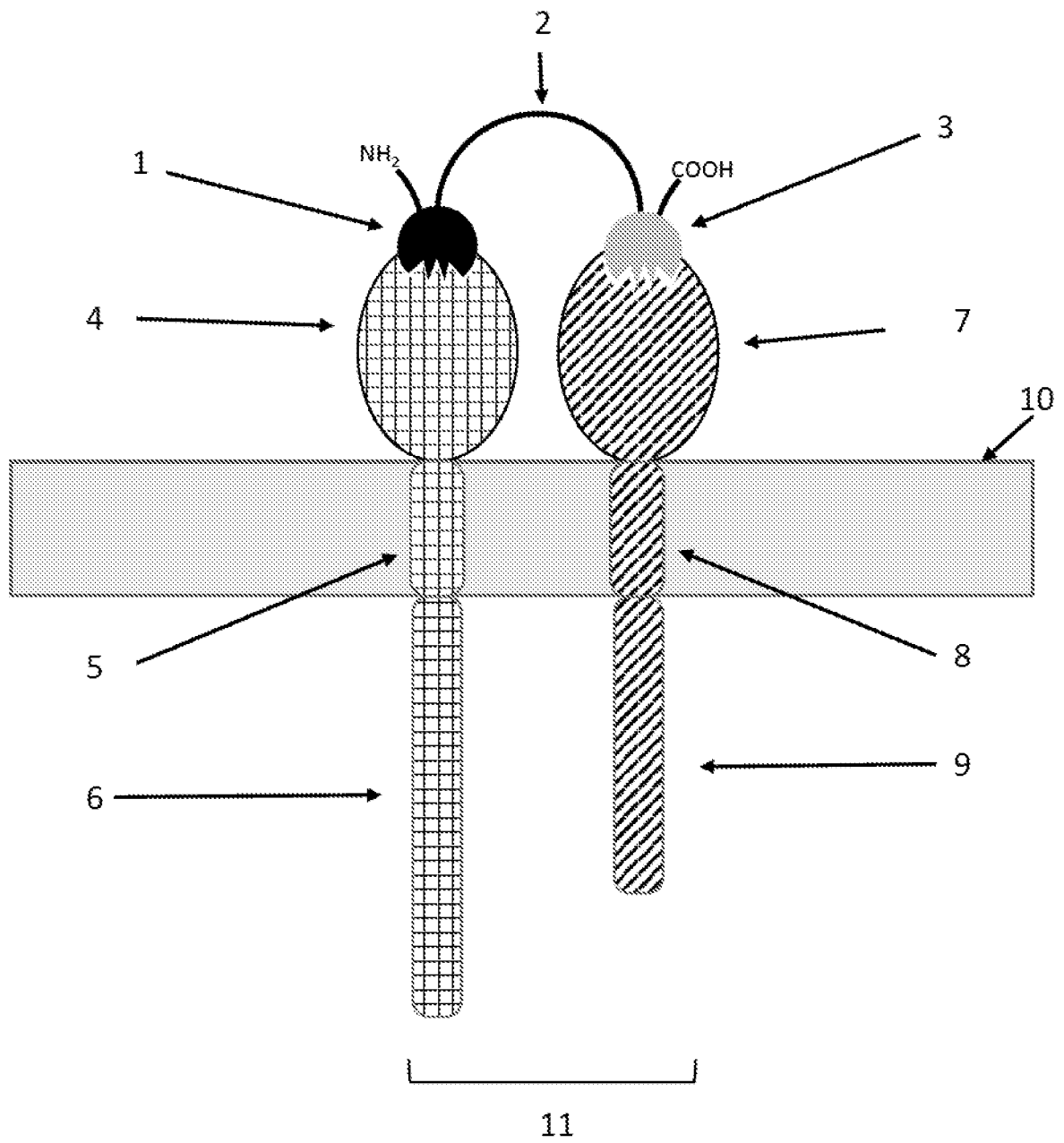


Figure 1

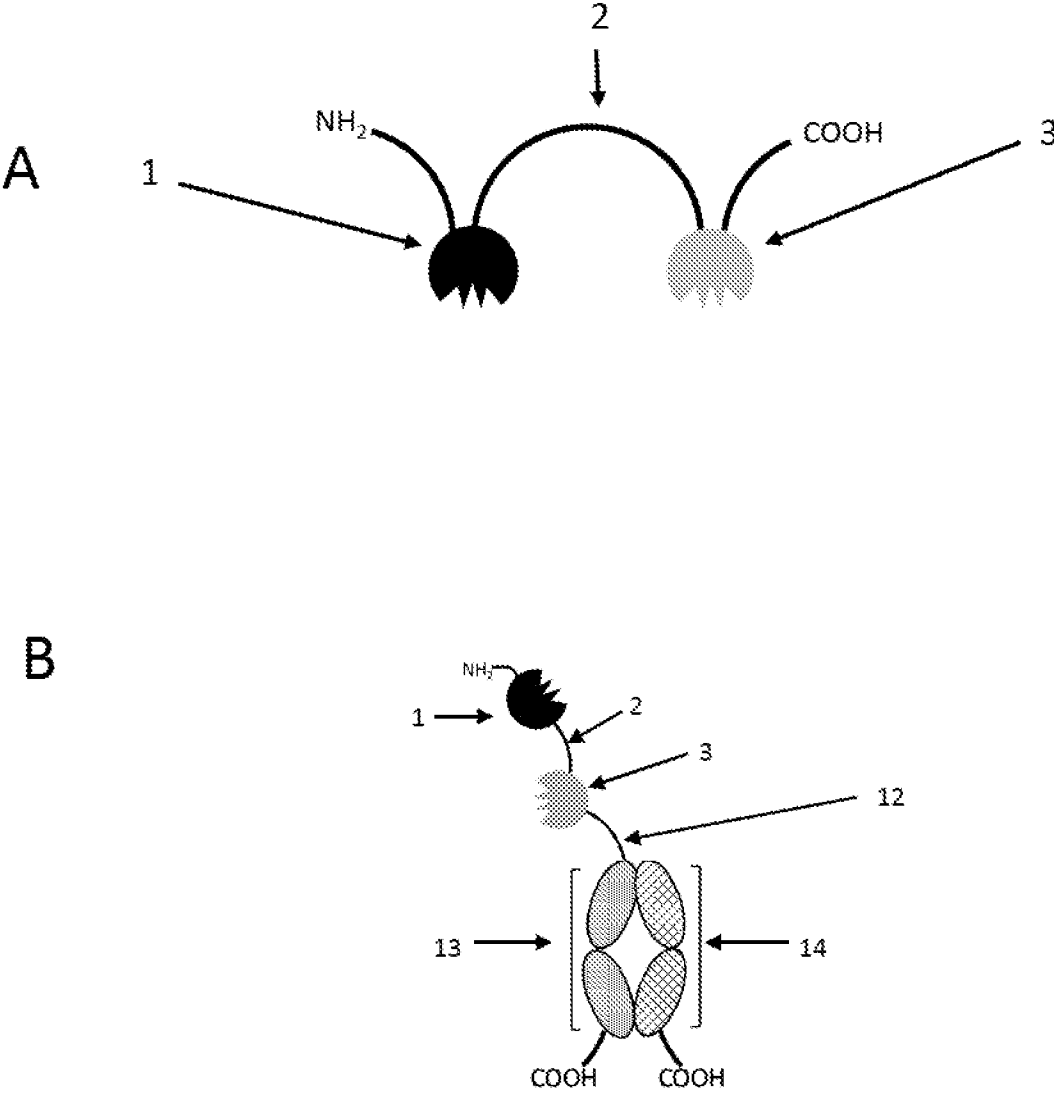


Figure 2

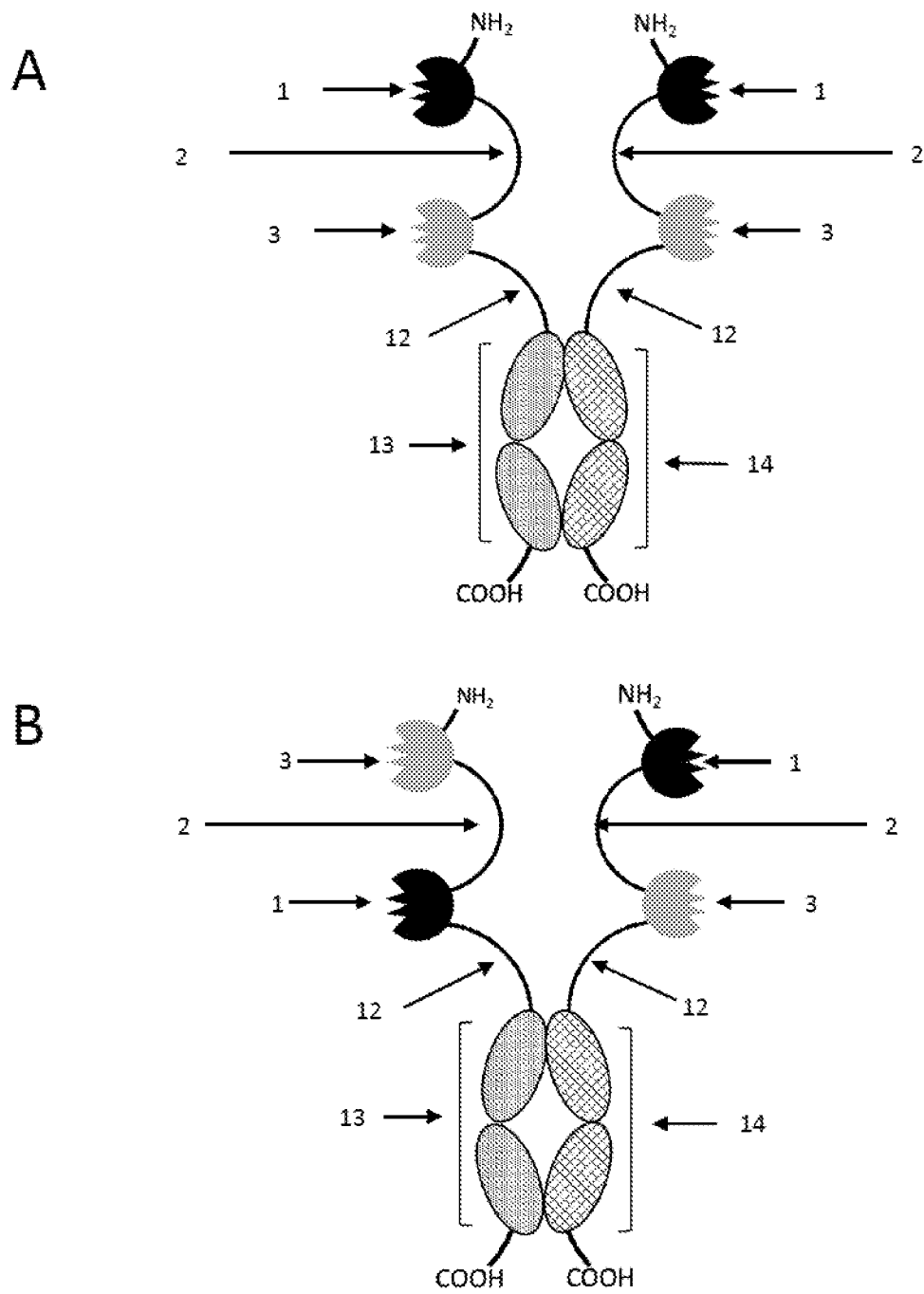


Figure 3

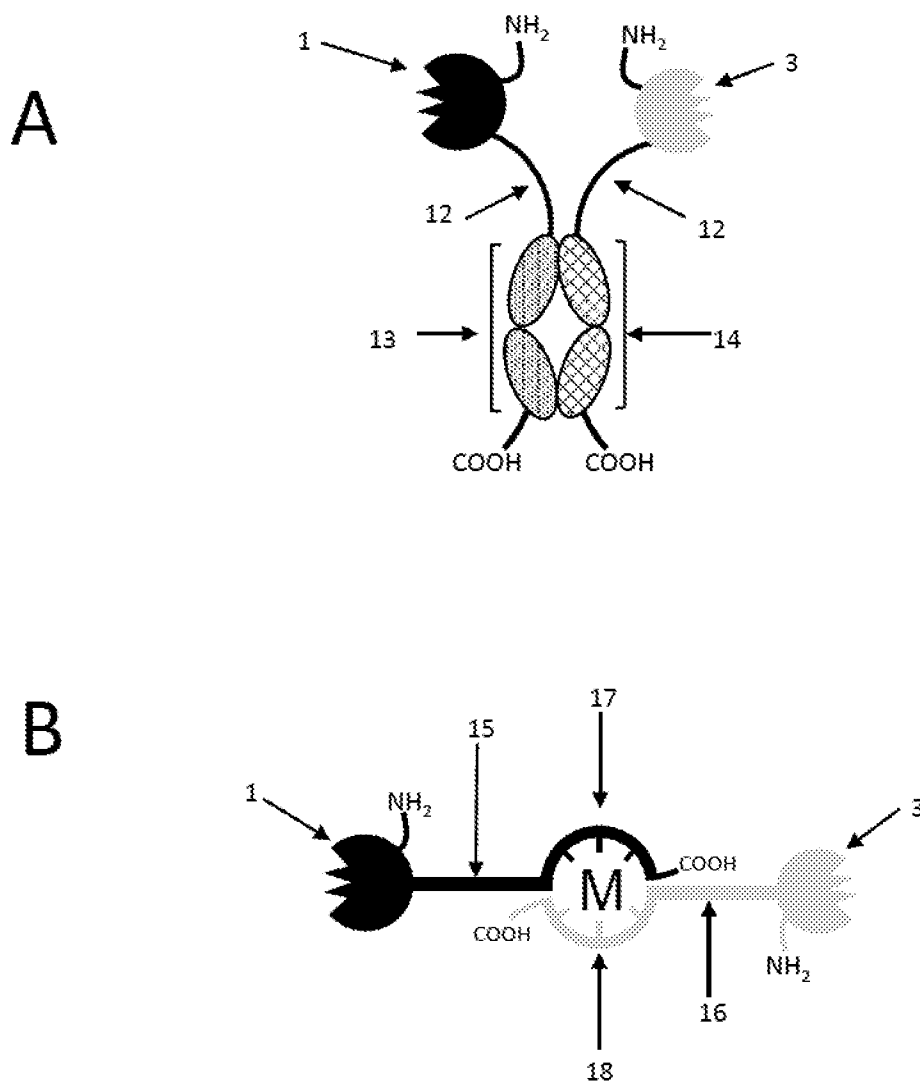


Figure 4

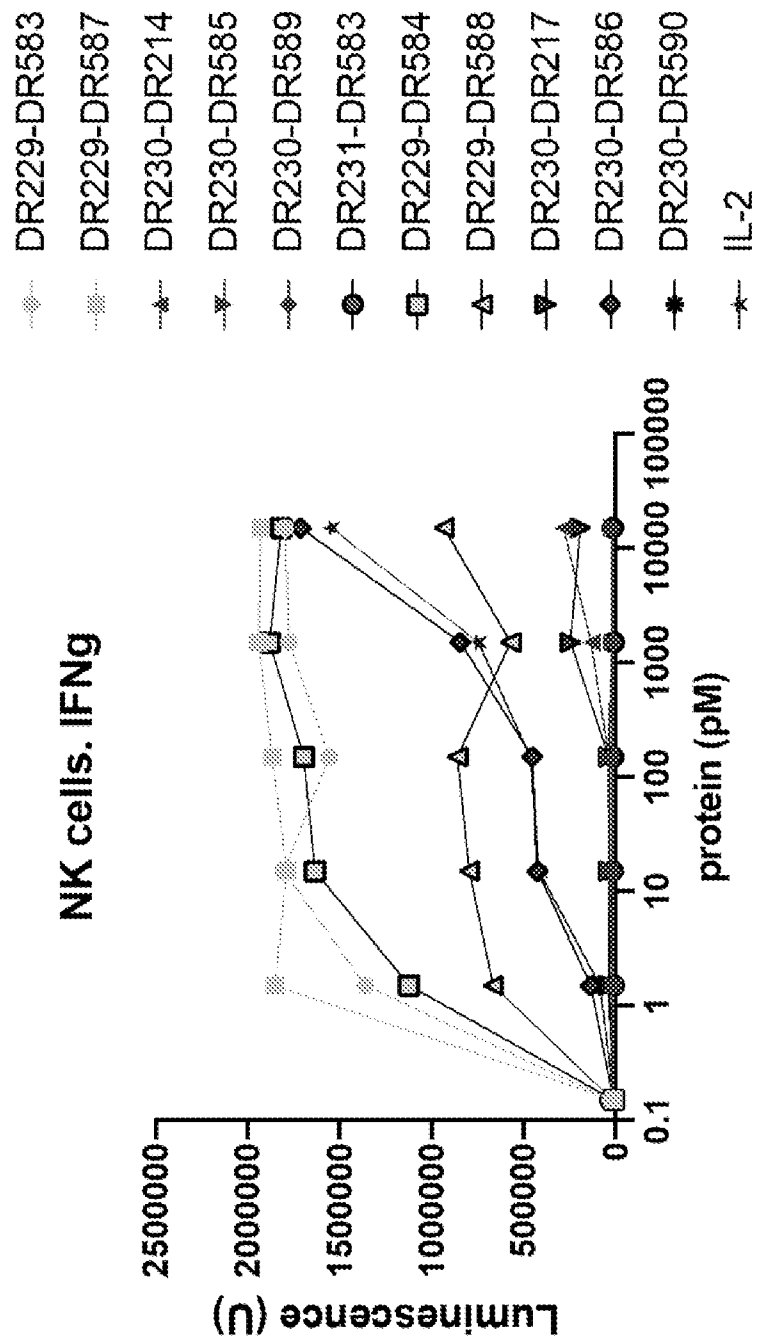


Figure 5

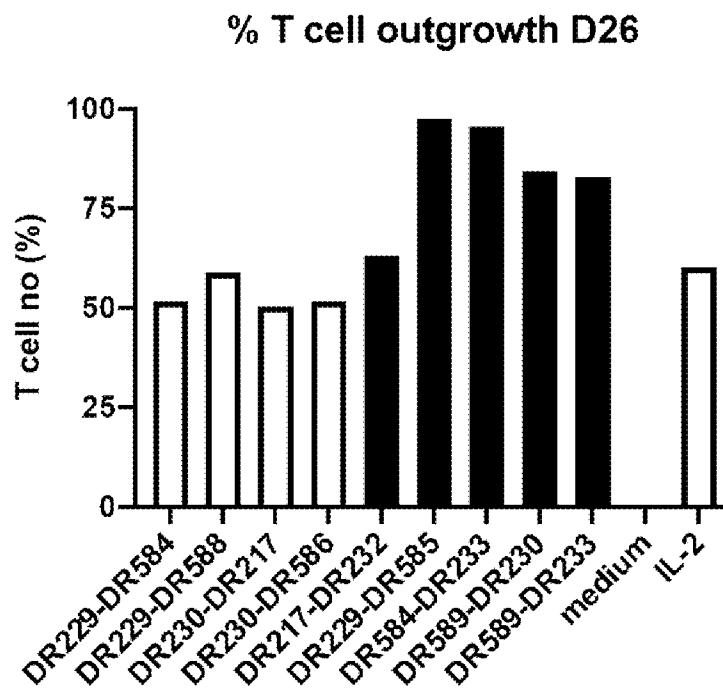
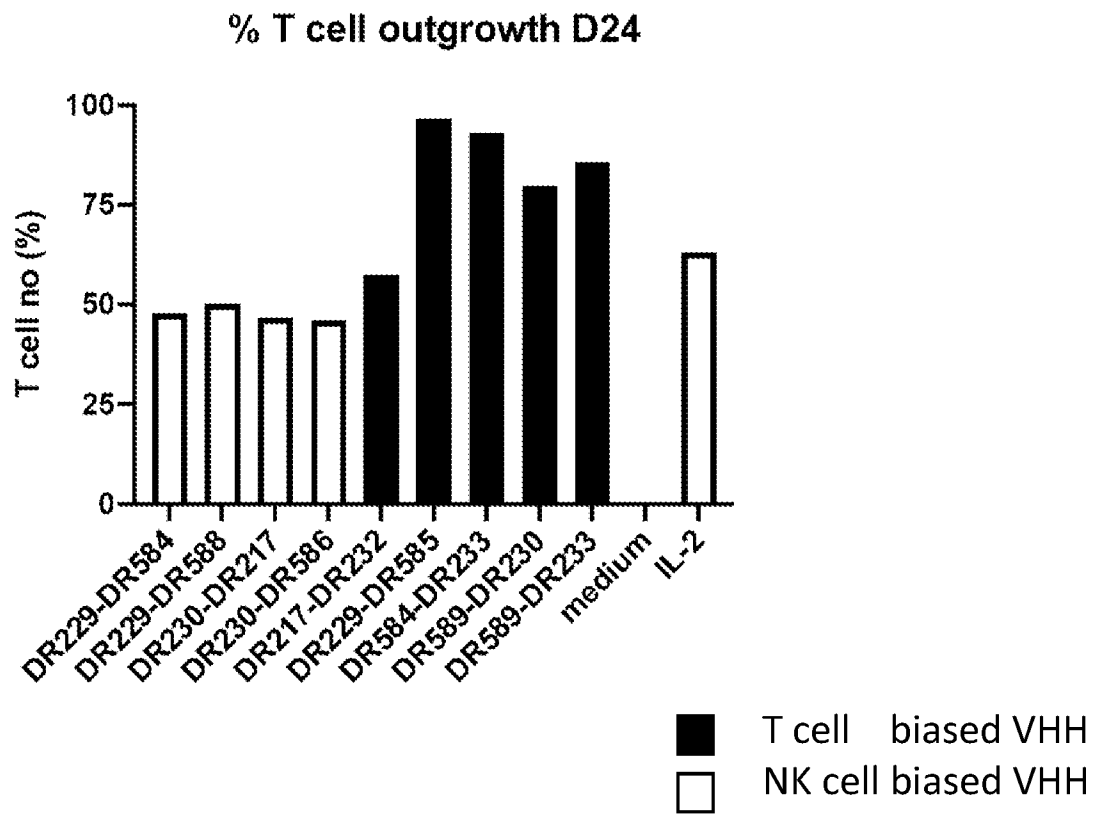


Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/044853

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/28; A61K 39/395 (2021.01)

CPC - C07K 16/2896; A61K 39/39541; A61K 39/395 (2021.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2018/0362655 A1 (AGENCY FOR SCIENCE TECHNOLOGY AND RESEARCH) 20 December 2018 (20.12.2018) entire document	1-5
A	WO 2016/097313 A1 (ABLYNX N.V.) 23 June 2016 (23.06.2016) entire document	1-5
A	US 8,258,268 B2 (WU et al) 04 September 2012 (04.09.2012) entire document	1-5

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

04 November 2021

Date of mailing of the international search report

DEC 17 2021

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/044853

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 6-29
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.