(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 June 2016 (30.06.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/105450 A2

(51) **International Patent Classification**:
C07K 16/28 (2006.01)

(21) **International Application Number**:
PCT/US2015/000155

(22) **International Filing Date**:
22 December 2015 (22.12.2015)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:

62/095,625	22 December 2014 (22.12.2014)	US
62/095,635	22 December 2014 (22.12.2014)	US
62/095,610	22 December 2014 (22.12.2014)	US
62/095,647	22 December 2014 (22.12.2014)	US
62/159,160	8 May 2015 (08.05.2015)	US
62/174,371	11 June 2015 (11.06.2015)	US

(71) **Applicant**: **XENCOR, INC.** [US/US]; 111 West Lemon Avenue, Monrovia, CA 91016 (US).

(72) **Inventors**: **MOORE, Gregory**; c/o Xencor, Inc., 111 West Lemon Avenue, Monrovia, CA 91016 (US). **DESJARLAIS, John**; c/o Xencor, Inc., 111 West Lemon Avenue, Monrovia, CA 91016 (US). **CHU, Seung**; c/o Xencor, Inc., 111 West Lemon Avenue, Monrovia, CA 91016 (US). **LEE, Sung-Hyung**; c/o Xencor, Inc., 111 West Lemon Avenue, Monrovia, CA 91016 (US).

(74) **Agents**: **SILVA, Robin** et al.; Morgan, Lewis & Bockius, LLP, One Market, Spear Street Tower, San Francisco, CA 94105 (US).

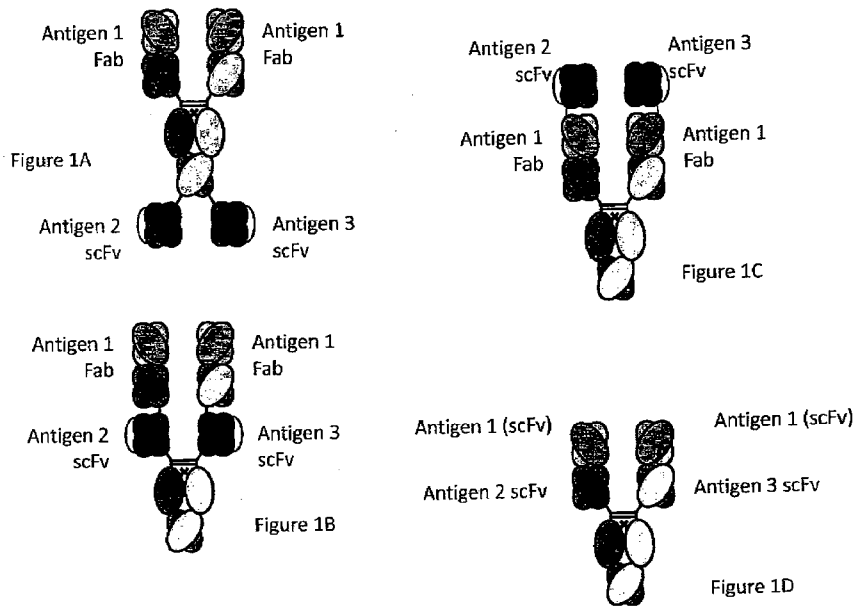
(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) **Title**: TRISPECIFIC ANTIBODIES

Figs. 1A-1D



(57) **Abstract**: The invention provides trispecific antibodies that co-engage CD3, CD8 and a tumor target antigen.

WO 2016/105450 A2

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

TRISPECIFIC ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Application Nos. 62/095,610, filed December 22, 2014, 62/095,625, filed December 22, 2014, 62/095,635, filed December 22, 2014, 62/095,647, filed December 22, 2014, 62/159,160, filed May 8, 2015 and 62/174,371, filed June 11, 2015, all of which are expressly incorporated herein by reference in their entirety, with particular reference to the figures, legends and claims therein.

BACKGROUND OF THE INVENTION

[0002] Antibody-based therapeutics have been used successfully to treat a variety of diseases, including cancer and autoimmune/inflammatory disorders. Yet improvements to this class of drugs are still needed, particularly with respect to enhancing their clinical efficacy. One avenue being explored is the engineering of additional and novel antigen binding sites into antibody-based drugs such that a single immunoglobulin molecule co-engages two different antigens. Such non-native or alternate antibody formats that engage two different antigens are often referred to as bispecifics. Because the considerable diversity of the antibody variable region (Fv) makes it possible to produce an Fv that recognizes virtually any molecule, the typical approach to bispecific generation is the introduction of new variable regions into the antibody.

[0003] A number of alternate antibody formats have been explored for bispecific targeting (Chames & Baty, 2009, mAbs 1[6]:1-9; Holliger & Hudson, 2005, Nature Biotechnology 23[9]:1126-1136; Kontermann, mAbs 4(2):182 (2012), all of which are expressly incorporated herein by reference). Initially, bispecific antibodies were made by fusing two cell lines that each produced a single monoclonal antibody (Milstein et al., 1983, Nature 305:537-540). Although the resulting hybrid hybridoma or quadroma did produce bispecific antibodies, they were only a minor population, and extensive purification was required to isolate the desired antibody. An engineering solution to this was the use of antibody fragments to make bispecifics. Because such fragments lack the complex quaternary structure of a full length

antibody, variable light and heavy chains can be linked in single genetic constructs. Antibody fragments of many different forms have been generated, including diabodies, single chain diabodies, tandem scFv's, and Fab_2 bispecifics (Chames & Baty, 2009, mAbs 1[6]:1-9; Holliger & Hudson, 2005, Nature Biotechnology 23[9]:1126-1136; expressly incorporated herein by reference). While these formats can be expressed at high levels in bacteria and may have favorable penetration benefits due to their small size, they clear rapidly *in vivo* and can present manufacturing obstacles related to their production and stability. A principal cause of these drawbacks is that antibody fragments typically lack the constant region of the antibody with its associated functional properties, including larger size, high stability, and binding to various Fc receptors and ligands that maintain long half-life in serum (i.e. the neonatal Fc receptor FcRn) or serve as binding sites for purification (i.e. protein A and protein G).

[0004] More recent work has attempted to address the shortcomings of fragment-based bispecifics by engineering dual binding into full length antibody -like formats (Wu et al., 2007, Nature Biotechnology 25[11]:1290-1297; USSN12/477,711; Michaelson et al., 2009, mAbs 1[2]:128-141; PCT/US2008/074693; Zuo et al., 2000, Protein Engineering 13[5]:361-367; USSN09/865,198; Shen et al., 2006, J Biol Chem 281[16]:10706-10714; Lu et al., 2005, J Biol Chem 280[20]:19665-19672; PCT/US2005/025472; expressly incorporated herein by reference). These formats overcome some of the obstacles of the antibody fragment bispecifics, principally because they contain an Fc region. One significant drawback of these formats is that, because they build new antigen binding sites on top of the homodimeric constant chains, binding to the new antigen is always bivalent.

[0005] For many antigens that are attractive as co-targets in a therapeutic bispecific format, the desired binding is monovalent rather than bivalent. For many immune receptors, cellular activation is accomplished by cross-linking of a monovalent binding interaction. The mechanism of cross-linking is typically mediated by antibody/antigen immune complexes, or via effector cell to target cell engagement. For example, the low affinity Fc gamma receptors (FcγRs) such as FcγRIIa, FcγRIIb, and FcγRIIIa bind monovalently to the antibody Fc region. Monovalent binding does not activate cells expressing these FcγRs; however, upon immune complexation or cell-to-cell contact, receptors are cross-linked and clustered

on the cell surface, leading to activation. For receptors responsible for mediating cellular killing, for example FcγRIIIa on natural killer (NK) cells, receptor cross-linking and cellular activation occurs when the effector cell engages the target cell in a highly avid format (Bowles & Weiner, 2005, J Immunol Methods 304:88-99, expressly incorporated by reference).. Similarly, on B cells the inhibitory receptor FcγRIIb downregulates B cell activation only when it engages into an immune complex with the cell surface B-cell receptor (BCR), a mechanism that is mediated by immune complexation of soluble IgG's with the same antigen that is recognized by the BCR (Heyman 2003, Immunol Lett 88[2]:157-161; Smith and Clatworthy, 2010, Nature Reviews Immunology 10:328-343; expressly incorporated by reference). As another example, CD3 activation of T-cells occurs only when its associated T-cell receptor (TCR) engages antigen-loaded MHC on antigen presenting cells in a highly avid cell-to-cell synapse (Kuhns et al., 2006, Immunity 24:133-139). Indeed nonspecific bivalent cross-linking of CD3 using an anti-CD3 antibody elicits a cytokine storm and toxicity (Perruche et al., 2009, J Immunol 183[2]:953-61; Chatenoud & Bluestone, 2007, Nature Reviews Immunology 7:622–632; expressly incorporated by reference). Thus for practical clinical use, the preferred mode of CD3 co-engagement for redirected killing of targets cells is monovalent binding that results in activation only upon engagement with the co-engaged target.

[0006] Thus while bispecifics generated from antibody fragments suffer biophysical and pharmacokinetic hurdles, a drawback of those built with full length antibody -like formats is that they engage co-target antigens multivalently in the absence of the primary target antigen, leading to nonspecific activation and potentially toxicity. The present invention solves this problem by introducing a novel set of trispecific formats that enable the multivalent co-engagement of distinct target antigens. In addition, the present invention provides novel heterodimerization variants that allow for better formation and purification of heterodimeric proteins, including antibodies.

SUMMARY OF THE INVENTIONS

[0007] Accordingly, the present invention provides trispecific antibodies. In one aspect, the invention provides tetravalent, trispecific antibodies comprising a) a first monomer comprising: i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain; ii) a first scFv domain; and b) a second monomer comprising: i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain; ii) a second scFv domain; and c) a light chain comprising a constant light domain and a variable light domain; wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen.

[0008] In an additional aspect, the tetravalent trispecific antibody above has a first and second variant Fc domains that comprise a pair of sets of amino acid substitutions selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q.

[0009] In some aspects, the tetravalent trispecific antibodies above have an antigen binding domain that binds CD8.

[0010] In some aspects, the tetravalent trispecific antibodies above have an antigen binding domain that binds CD123.

[0011] In some aspects, the tetravalent trispecific antibodies above have an antigen binding domain that binds CD38.

[0012] In some aspects, the tetravalent trispecific antibodies above have an antigen binding domain that binds CD20.

[0013] In some aspects, the tetravalent trispecific antibodies above have an antigen binding domain that binds CD19.

[0014] In a further aspect, the tetravalent trispecific antibodies above have one of said antigen binding domains that binds CD3 and another that binds CD8. In an additional aspect, the antibody binds a target tumor antigen.

[0015] In an additional aspect, the tetravalent trispecific antibodies above binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.30 and a variable light domain having the sequence L1.47.

[0016] In a further aspect, the tetravalent trispecific antibodies binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.32 and a variable light domain having the sequence L1.47.

[0017] In an additional aspect, the tetravalent trispecific antibodies bind CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.89 and a variable light domain having the sequence L1.47.

[0018] In a further aspect, the tetravalent trispecific antibodies bind CD3 and have a variable region comprising a variable heavy domain having the sequence of H1.90 and a variable light domain having the sequence L1.47.

[0019] In a further aspect, the tetravalent trispecific antibodies bind CD3 and have a variable region comprising a variable heavy domain having the sequence of H1.33 and a variable light domain having the sequence L1.47.

[0020] In a further aspect, the tetravalent trispecific antibodies bind CD3 and have a variable region comprising a variable heavy domain having the sequence of H1.31 and a variable light domain having the sequence L1.47.

[0021] In a further aspect, the tetravalent trispecific antibodies bind CD3 and have a variable region comprising a variable heavy domain having the sequence of H1.31 and a variable light domain having the sequence L1.47.

[0022] In a further aspect, the tetravalent trispecific antibodies bind CD38 and have a variable region comprising a variable heavy domain having the sequence of OTK10 H1.77 and a variable light domain having the sequence L1.24.

[0023] In a further aspect, the tetravalent trispecific antibodies bind CD38 and have a variable region comprising a variable heavy domain having the sequence of OTK10 H1 and a variable light domain having the sequence L1.24.

[0024] In a further aspect, the tetravalent trispecific antibodies bind CD38 and have a variable region comprising a variable heavy domain having the sequence of OTK10 H1 and a variable light domain having the sequence L1.

[0025] In a further aspect, the tetravalent trispecific antibodies bind CD20 and have a variable region comprising a variable heavy domain having the sequence of 2CB8 H1.202 and a variable light domain having the sequence L1.113.

[0026] In a further aspect, the tetravalent trispecific antibodies bind CD123 and have a variable region comprising a variable heavy domain having the sequence of 7G3 H1.109 and a variable light domain having the sequence L1.57.

[0027] In a further aspect, the tetravalent trispecific antibodies bind CD8 and have a variable region comprising a variable heavy domain having the sequence of OKT8 H1 and a variable light domain having the sequence L1.

[0028] In a further aspect, the tetravalent trispecific antibodies bind CD8 and have a variable region comprising a variable heavy domain having the sequence of OKT8 H2 and a variable light domain having the sequence L1.

[0029] In a further aspect, the tetravalent trispecific antibodies bind CD8 and have a variable region comprising a variable heavy domain having the sequence of 51.1 H1 and a variable light domain having the sequence L1.

[0030] In a further aspect, the tetravalent trispecific antibodies bind CD8 and have a variable region comprising a variable heavy domain having the sequence ofOKT8 H1 and a variable light domain having the sequence L2.

[0031] In an additional aspect, the tetravalent trispecific antibodies have first and second variant Fc domains comprise a pair of sets of amino acid substitutions selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q.

[0032] In further aspects, the tetravalent trispecific antibodies of the invention further comprises pI variants. These can include, but are not limited to, pI variants are selected from the sets of pI variants shown in Figure 31, as well as I199T/N203D/K274Q/R355Q/N384S/K392N/V397M/Q419E/DEL447 and N208D/Q295E N384D/Q418E/N421D.

[0033] In additional aspects, the tetravalent trispecific antibodies have the first and second scFv domains covalently attached to the C-terminus of said first and second heavy chain, respectively.

[0034] In further aspects, the tetravalent trispecific antibodies have the first and second scFv domains covalently attached to the N-terminus of said first and second heavy chain, respectively.

[0035] In additional aspects, the tetravalent trispecific antibodies have the first and second scFv domains are each attached between said Fc domain and the CH1 domain of said heavy chain.

[0036] In further aspects, the scFv domains are covalently attached using one or more domain linkers.

[0037] In an additional aspect, the scFv domains include a scFv linker. In some aspects, the scFv linker(s) are charged.

[0038] In an additional aspect, the invention provides nucleic acid compositions comprising: a) a first nucleic acid encoding said first monomer of any tetravalent, trispecific antibodies; b) a second nucleic acid encoding said second monomer of any any tetravalent, trispecific antibodies, respectively; and c) a third nucleic acid encoding said light chain of any tetravalent, trispecific antibodies, respectively.

[0039] In a further aspect, the invention provides expression vector compositions comprising: a) a first expression vector comprising said first nucleic acid; b) a second expression vector comprising said second nucleic acid; and c) a third expression vector comprising said third nucleic acid.

[0040] In an additional aspect, the invention provides host cells comprising either the nucleic acid compositions or the expression vector compositions.

[0041] In a further aspect, the invention provides methods of making a tetravalent trispecific antibody comprising culturing the host cells under conditions wherein said trispecific antibody is produced and recovering said antibody.

[0042] In an additional aspect the invention provides methods of treating a patient in need thereof comprising administering a tetravalent trispecific antibody.

[0043] In a further aspect, the invention provides trivalent trispecific antibodies comprising: a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain; b) a second monomer comprising: i) a second variant Fc domain; ii) a first scFv domain; and c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen.

[0044] In an additional aspect, the trispecific trispecific antibody above has a first and second variant Fc domains that comprise a pair of sets of amino acid substitutions selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q.

[0045] In some aspects, the trispecific trispecific antibodies above have an antigen binding domain that binds CD8.

[0046] In some aspects, the trispecific trispecific antibodies above have an antigen binding domain that binds CD123.

[0047] In some aspects, the trispecific trispecific antibodies above have an antigen binding domain that binds CD38.

[0048] In some aspects, the trispecific trispecific antibodies above have an antigen binding domain that binds CD20.

[0049] In some aspects, the trispecific trispecific antibodies above have an antigen binding domain that binds CD19.

[0050] In a further aspect, the trispecific trispecific antibodies above have one of said antigen binding domains that binds CD3 and another that binds CD8. In an additional aspect, the antibody binds a target tumor antigen.

[0051] In an additional aspect, the trispecific trispecific antibodies above binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.30 and a variable light domain having the sequence L1.47.

[0052] In a further aspect, the trispecific trispecific antibodies binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.32 and a variable light domain having the sequence L1.47.

[0053] In an additional aspect, the trispecific trispecific antibodies bind CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.89 and a variable light domain having the sequence L1.47.

[0054] In a further aspect, the trispecific trispecific antibodies bind CD3 and have a variable region comprising a variable heavy domain having the sequence of H1.90 and a variable light domain having the sequence L1.47.

[0055] In a further aspect, the trispecific trispecific antibodies bind CD3 and have a variable region comprising a variable heavy domain having the sequence of H1.33 and a variable light domain having the sequence L1.47.

[0056] In a further aspect, the trispecific trispecific antibodies bind CD3 and have a variable region comprising a variable heavy domain having the sequence of H1.31 and a variable light domain having the sequence L1.47.

[0057] In a further aspect, the trispecific trispecific antibodies bind CD3 and have a variable region comprising a variable heavy domain having the sequence of H1.31 and a variable light domain having the sequence L1.47.

[0058] In a further aspect, the trispecific trispecific antibodies bind CD38 and have a variable region comprising a variable heavy domain having the sequence of OTK10 H1.77 and a variable light domain having the sequence L1.24.

[0059] In a further aspect, the trispecific trispecific antibodies bind CD38 and have a variable region comprising a variable heavy domain having the sequence of OTK10 H1 and a variable light domain having the sequence L1.24.

[0060] In a further aspect, the trispecific trispecific antibodies bind CD38 and have a variable region comprising a variable heavy domain having the sequence of OTK10 H1 and a variable light domain having the sequence L1.

[0061] In a further aspect, the trispecific trispecific antibodies bind CD20 and have a variable region comprising a variable heavy domain having the sequence of 2CB8 H1.202 and a variable light domain having the sequence L1.113.

[0062] In a further aspect, the trispecific trispecific antibodies bind CD123 and have a variable region comprising a variable heavy domain having the sequence of 7G3 H1.109 and a variable light domain having the sequence L1.57.

[0063] In a further aspect, the trispecific trispecific antibodies bind CD8 and have a variable region comprising a variable heavy domain having the sequence of OKT8 H1 and a variable light domain having the sequence L1.

[0064] In a further aspect, the trispecific trispecific antibodies bind CD8 and have a variable region comprising a variable heavy domain having the sequence of OKT8 H2 and a variable light domain having the sequence L1.

[0065] In a further aspect, the trispecific trispecific antibodies bind CD8 and have a variable region comprising a variable heavy domain having the sequence of 51.1 H1 and a variable light domain having the sequence L1.

[0066] In a further aspect, the trispecific trispecific antibodies bind CD8 and have a variable region comprising a variable heavy domain having the sequence ofOKT8 H1 and a variable light domain having the sequence L2.

[0067] In an additional aspect, the trispecific trispecific antibodies have first and second variant Fc domains comprise a pair of sets of amino acid substitutions selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q.

[0068] In further aspects, the trispecific trispecific antibodies of the invention further comprises pI variants. These can include, but are not limited to, pI variants are selected from the sets of pI variants shown in Figure 31, as well as I199T/N203D/K274Q/R355Q/N384S/K392N/V397M/Q419E/DEL447 and N208D/Q295E N384D/Q418E/N421D.

[0069] In an additional aspect, the invention provides trivalent trispecific antibodies comprising: a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain; b) a second monomer comprising: i) a second variant Fc domain; ii) a first scFv domain; and c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first and said second variant Fc domains comprise a pair of sets of amino acid substitutions selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen.

[0070] In further aspect, the invention provides trivalent trispecific antibodies wherein one of said antigen binding domains binds CD3 and another binds CD8. Generally, the third antigen binding domain binds a tumor antigen.

[0071] In further aspect, the invention provides trivalent trispecific antibodies wherein said second scFv is covalently attached to said first monomer at the C-terminus.

[0072] In further aspect, the invention provides trivalent trispecific antibodies wherein said second scFv is covalently attached to said first monomer at the N-terminus.

[0073] In further aspect, the invention provides trivalent trispecific antibodies wherein said second scFv is covalently attached to said second monomer at the N-terminus.

[0074] In further aspect, the invention provides trivalent trispecific antibodies wherein said second scFv is covalently attached to said second monomer at the C-terminus.

[0075] In further aspect, the invention provides trivalent trispecific antibodies wherein said second scFv is covalently attached to said first monomer between the CH1 and Fc domain of the heavy chain.

[0076] In further aspect, the invention provides trivalent trispecific antibodies wherein said scFvs are attached using a domain linker.

[0077] In further aspect, the invention provides trivalent trispecific antibodies wherein at least one of said scFvs comprises a charged scFv linker.

[0078] In further aspects, the trivalent trispecific antibodies of the invention further comprises pI variants. These can include, but are not limited to, pI variants are selected from the sets of pI variants shown in Figure 31, as well as I199T/N203D/K274Q/R355Q/N384S/K392N/V397M/Q419E/DEL447 and N208D/Q295E N384D/Q418E/N421D.

[0079] In additional aspects, the invention provides tetravalent or trivalent trispecific antibodies as above wherein said first and/or second Fc domain further comprises amino acid substitution(s) selected from the group consisting of 434A, 434S, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I or V/434S, 436V/428L, 252Y, 252Y/254T/256E, 259I/308F/428L, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L, 236R, 328R, 236R/328R, 236N/267E, 243L, 298A and 299T.

[0080] In an additional aspect, the invention provides nucleic acid compositions comprising: a) a first nucleic acid encoding said first monomer of any trivalent, trispecific antibodies; b) a second nucleic acid encoding said second monomer of any

any trivalent, trispecific antibodies, respectively; and c) a third nucleic acid encoding said light chain of any trivalent, trispecific antibodies, respectively.

[0081] In a further aspect, the invention provides expression vector compositions comprising: a) a first expression vector comprising said first nucleic acid; b) a second expression vector comprising said second nucleic acid; and c) a third expression vector comprising said third nucleic acid.

[0082] In an additional aspect, the invention provides host cells comprising either the nucleic acid compositions or the expression vector compositions.

[0083] In a further aspect, the invention provides methods of making a trivalent trispecific antibody comprising culturing the host cells under conditions wherein said trispecific antibody is produced and recovering said antibody.

[0084] In an additional aspect the invention provides methods of treating a patient in need thereof comprising administering a trivalent trispecific antibody.

[0085] In an additional aspect, the invention provides trispecific antibody selected from the group consisting of XENP15242, XENP15243, XENP15244, XENP15264, XENP15265, XENP15266; XENP18951, XENP18952, XENP18953 and XENP18954.

BRIEF DESCRIPTION OF THE DRAWINGS

[0086] Figure 1A, 1B, 1C and 1D depict a number of different trispecific formats of the antibodies of the invention that are tetravalent (four antigens bound) but trispecific (three different antigens bound, e.g. one antigen has two antigen binding domains. Figure 1A depicts a "traditional" antibody with two Fab antigen binding domains to antigen 1 (sometimes referred to herein as "A1"), and a scFv covalently attached to the C-terminus of each heavy chain using a domain linker as outlined herein, each binding a different antigen, A2 and A3. The variable heavy and variable light domains of the scFv domains are covalently attached using a scFv linker as outlined herein. Thus, the antibody of Figure 1A comprises a first monomer comprising a first heavy chain and a C-terminally attached scFv, a second monomer comprising a second heavy chain and a C-terminally attached scFv, and

a light chain (with optional linkers as discussed herein). Figure 1B depicts a similar format except that rather than have the two scFv domains linked to the C-terminus of the heavy chains, they are linked internally within the heavy chain, generally using domain linkers, and generally between the Fc domain and the CH1 domain of the heavy chain. Thus the Figure 1B antibody comprising a first monomer comprising a heavy variable domain, a CH1 domain, a first scFv and an Fc domain, a second monomer comprising a heavy variable domain, a CH1 domain, a second scFv and an Fc domain, and a light chain (with optional linkers as discussed herein). Figure 1C is similar, example that the scFv domains are covalently attached to the N-terminus of the heavy chains. Thus Figure 1C comprises a first monomer comprising a first scFv and a first heavy chain, a second monomer comprising a second scFv and a second heavy chain, and a light chain (with optional linkers as discussed herein). Figure 1D depicts 4 scFv domains, two of which bind A1, comprising a first monomer comprising a first scFv, a second scFv and a first Fc domain, a second monomer comprising another first scFv, a third scFv and a second Fc domain, and a light chain (with optional linkers as discussed herein). Note additionally that in Figure 1D, the two scFvs that bind the same antigen could be on the same monomer rather than on different monomers, and only three scFvs rather than four can be also used.

[0087] Figure 2A, 2B and 2C depict a number of different trispecific formats that are trivalent and trispecific, e.g. three different antigens are each bound monovalently. Figure 2A has antigen 1A bound by a FAb, antigen 2 bound by a scFv attached to the N-terminus of an Fc domain of a first heavy chain using a domain linker, and a scFv attached to the C-terminus of the second heavy chain, again using a domain linker. Thus Figure 2A comprising a first monomer comprising a scFv and a first Fc domain, a second monomer comprising a heavy chain and a scFv, and a light chain (with optional linkers as discussed herein). Figure 2B is similar except that the third antigen binding domain is bound to the C-terminus of the first heavy chain; thus the antibody of Figure 2B comprises a first monomer comprising a scFv, a first Fc domain, and a scFv, the second monomer comprises a heavy chain, and a light chain. Figure 2C has a first monomer comprising a scFv covalently attached to a first Fc domain, a second monomer comprising a scFv covalently attached to a heavy chain, and a light chain (with optional linkers as discussed herein). Figure 2D takes

advantage of the fact that there is only a single light chain and thus attaches a scFv to the N-terminus of the light chain (although as will be appreciated by those in the art, this scFv can be attached to the C-terminus of the light chain as well). Thus the antibody of Figure 2D comprises a first monomer comprising a scFv covalently attached to a first Fc domain, a second monomer comprising a heavy chain, and a light chain with an attached scFv. Figure 2E is similar, except that the second scFv is attached internally to the heavy chain as discussed herein.

[0088] Figure 3A , 3B, 3C, 3D and 3E depict tetravalent, trispecific formats, where the tumor antigen binding is bivalent. All of the constructs depicted in Figure 3 include heterodimerization variants and optionally but preferably pI variants as described herein. Figure 3A depicts the use of anti-tumor Fabs (formed by a common light chain and the two heavy chains) and ant-CD8 and anti-CD3 scFvs attached at the C-terminus of the heavy chains. Figure 3B depicts the insertion of the scFvs between the Fab region of the tumor antigen binding domains and the Fc region of the heavy chains. As will be appreciated by those in the art for this figure as well as Figure 1 and Figure 2, this covalent linkage can be direct (e.g. no exogenous linker amino acid sequences used), or indirect, using one linker (e.g. a linker at the N- or C-terminus of the scFv sequences) or two linkers (e.g. at both N- and C-termini of the scFv). The linkers can be standard glycine-serine linkers as discussed herein or charged scFv linkers as depicted in Figure 33 or a combination (e.g. one uncharged, one charged). As will be appreciated by those in the art, the use of the scFv charged linkers is on the correct "strand" or monomer, preserving the "strandedness" of the pI changes for purification. Figure 3C depicts a format wherein the scFvs are at the N-terminus of the heavy chain. As for Figure 3B, this attachment may be direct or indirect using linkers, again optionally charged and preserving "strandedness". Figure 3D depicts the attachment of the scFv domains to the C-terminus of the light chains, again with optional linkers (either uncharged or using charged scFv linkers). Figure 3E depicts the attachment of the scFv domains to the N-terminus of the light chains, again with optional linkers (either uncharged or using charged scFv linkers). Figure 3F depicts the use of scFvs solely, again using optional linkers and optional charged scFv linkers.

[0089] Figure 4A, 4B, 4C, 4D, 4E and 4F depicts trivalent, trispecific formations of the invention. Figure 4A depicts a format with a first monomer with an anti-CD3 scFv linked to an Fc domain and a second monomer with a light chain and a heavy chain with an anti-CD8 scFv linked to its C-terminus. As for all the formats of Figures 1 and 2, these formats can optionally use direct linkages or linkers (either uncharged or charged scFvs, preserving "strandedness"). Figure 4B has a first monomer with an anti-CD3 scFv linked to an Fc domain and with an anti-CD8 scFv linked to its C-terminus, and a second monomer with a light chain and a heavy chain. Figure 4C depicts the anti-CD8 scFv linked to the N-terminus of the heavy chain of the second monomer. Figure 4D depicts the anti-CD8 scFv linked to the N-terminus of the light chain of the second monomer. Figure 4E depicts the anti-CD8 scFv linked to the C-terminus of the light chain of the second monomer. As will be appreciated by those in the art, while Figure 4A-4E depict one of the antigen binding domains as a Fab (depicted as the tumor antigen binding domain, although as discussed above this could also be either the anti-CD3 or anti-CD8 binding domains), this Fab could also be a scFv, that is, all three antigen binding domains could be scFv domains as depicted in Figure 4F. In addition, the antigen binding domains of Figure 4A-F can be switched as is depicted in Figure 3.

[0090] Figure 5A – 5E depict the possible combinations of the trivalent, trispecific antibodies of the invention when one of the antigen binding domains is a Fab and when one of the scFvs is attached to the C-terminus of the second monomer (although it can also be attached to the C-terminus of the first monomer). As for Figure 4, the Fab component could also be a third scFv.

[0091] Figure 6 is the schematic of the construct for XENP15242, XENP15243 and XENP15244.

[0092] Figure 7 is the schematic of the construct for XENP15264, XENP15265 and XENP15266.

[0093] Figure 8 is the schematic for XENP18951, XENP18952, XNEP18953 and XENP18954.

[0094] Figure 9 depicts the sequences for XENP18951, with the CDRs underlined, the standard scFV linker underlined (which, as for all scFv linkers herein can be charged or uncharged, as is depicted in Figure 33), a "/" between the variable and constant domains.

[0095] Figure 10 depicts the sequences of XENP18952, with the CDRs underlined, the charged scFV linker underlined (which, as for all scFv linkers herein can be charged or uncharged, as is depicted in Figure 33), a "/" between the variable and constant domains.

[0096] Figure 11 depicts the sequences of XENP18953, with the CDRs underlined, the charged scFV linker underlined (which, as for all scFv linkers herein can be charged or uncharged, as is depicted in Figure 33), a "/" between the variable and constant domains.

[0097] Figure 12 depicts the sequences of XENP18954, with the CDRs underlined, the charged scFV linker underlined (which, as for all scFv linkers herein can be charged or uncharged, as is depicted in Figure 33), a "/" between the variable and constant domains.

[0098] Figure 13 depicts the sequences of the "High CD3" anti-CD3_H1.30_L1.47 construct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined). As is true of all the sequences depicted in the Figures, this charged linker may be replaced by an uncharged linker or a different charged linker, as needed.

[0099] Figure 14 depicts the sequences of the "High-Int #1" Anti-CD3_H1.32_L1.47 construct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined). As is true of all the sequences depicted in the Figures, this charged linker may be replaced by an uncharged linker or a different charged linker, as needed.

[00100] Figure 15 depicts the sequences of the "High-Int #2" Anti-CD3_H1.89_L1.47 construct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined). As is true of all the sequences depicted in the Figures, this charged linker may be replaced by an uncharged linker or a different charged linker, as needed.

[00101] Figure 16 depicts the sequences of the "High-Int #3" Anti-CD3_H1.90_L1.47 construct, including the variable heavy and light domains (CDRs underlined), as well as the

individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined). As is true of all the sequences depicted in the Figures, this charged linker may be replaced by an uncharged linker or a different charged linker, as needed.

[00102] Figure 17 depicts the sequences of the "Int" Anti-CD3_H1.90_L1.47 construct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined). As is true of all the sequences depicted in the Figures, this charged linker may be replaced by an uncharged linker or a different charged linker, as needed.

[00103] Figure 18 depicts the sequences of the "Low" Anti-CD3_H1.31_L1.47 construct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined). As is true of all the sequences depicted in the Figures, this charged linker may be replaced by an uncharged linker or a different charged linker, as needed.

[00104] Figure 19 depicts the sequences of the High CD38: OKT10_H1.77_L1.24 construct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined).

[00105] Figure 20 depicts the sequences of the intermediate CD38: OKT10_H1L1.24 construct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined).

[00106] Figure 21 depicts the sequences of the Low CD38: OKT10_H1L1 construct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined).

[00107] Figure 22 depicts the sequences of the High CD20 C2B8_H1.202_L1.113 construct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined).

[00108] Figure 23 depicts the Low CD20 C2B8_H1L1 construct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined).

[00109] Figure 24 depicts the CD123 7G3_H1.109_L1.57 constuct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined).

[00110] Figure 25 depicts the CD8 OKT8_H1L1 constuct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined).

[00111] Figure 26 depicts the CD8 OKT8_H2L1 constuct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined).

[00112] Figure 27 depicts the CD8 51.1_H2L1 constuct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined).

[00113] Figure 28 depicts the CD8 51.1_H1L2 constuct, including the variable heavy and light domains (CDRs underlined), as well as the individual vl and vhCDRs, as well as an scFv construct with a charged linker (double underlined).

[00114] Figure 29 dpeicts the sequences of human CD20, human CD123, human CD3, and human CD38.

[00115] Figure 30A -30E depict useful pairs of heterodimerization variant sets (including skew and pI variants).

[00116] .Figure 31 depicts a list of isosteric variant antibody constant regions and their respective substituions. pI_(-) indicates lower pI variants, while pI_(+) indicates higher pI variants. These can be optionally and independently combined with other heterodimerization variants of the invention (and other variant types as well, as outlined herein). In addition, the variants are identified relative to IgG1, this is not limiting; that is, for example, while 208 is N in IgG1, G2, G3 and G4, and thus "N208D" covers all IgG1-4, at

position 384, for example, in IgG1, G2 and G4, the wild type residue is N but for IgG3 it is S. So "N384D" includes the situation where there may be a different wild type residue but the substitution at the position is a D.

[00117] Figure 32 depict useful ablation variants that ablate FcγR binding (sometimes referred to as "knock outs" or "KO" variants). As is true for many if not all variants herein, these KO variants can be independently and optionally combined, both within the set described in Figure 32 and with any heterodimerization variants outlined herein, including steric and pI variants. For example, E233P/L234V/L235A/G236del can be combined with any other single or double variant from the list. In addition, while it is preferred in some embodiments that both monomers contain the same KO variants, it is possible to combine different KO variants on different monomers, as well as have only one monomer comprise the KO variant(s). Reference is also made to the Figures and Legends of USSN 61/913,870, all of which is expressly incorporated by reference in its entirety as it relates to "knock out" or "ablation" variants.

[00118] Figure 33 depicts a number of charged scFv linkers that find use in increasing or decreasing the pI of heterodimeric antibodies that utilize one or more scFv as a component. A single prior art scFv linker with a single charge is referenced as "Whitlow", from Whitlow et al., Protein Engineering 6(8):989-995 (1993). It should be noted that this linker was used for reducing aggregation and enhancing proteolytic stability in scFvs.

[00119] Figure 34 depicts a list of engineered heterodimer-skewing Fc variants with heterodimer yields (determined by HPLC-CIEX) and thermal stabilities (determined by DSC). Not determined thermal stability is denoted by "n.d.".

[00120] Figure 35 depicts the schematic for the XENP15242, XNEP15243 and XENP15244 constructs.

[00121] Figure 36 depicts the three sequences for XENP15242.

[00122] Figure 37 depicts the three sequences for XENP15243.

[00123] Figure 38 depicts the three sequences for XENP15244.

[00124] Figure 39 depicts the format for XENP15246, XENP15265 and XENP15266.

[00125] Figure 40 depicts the three sequences for XENP15246.

[00126] Figure 41 depicts the three sequences for XENP15265.

[00127] Figure 42 depicts the three sequences for XENP15266.

[00128] Figure 43 depict anti-CD8 variable heavy and variable light domains that find use in the present invention. These can be combined in any pairing, for example OKT8 H1 or H2 with L1, and 51.1 H0 or H1 with L0, or across original categories, such as OKT8 H1 with 51.1 L0, etc.

[00129] Figure 44 depicts the amino acid sequences of wild type constant regions from IgG1, IgG2, IgG3 and IgG4 and an IgG1/G2 hybrid that can be used with the variable heavy and light chains herein.

[00130] Figure 45 depicts a number of anti-CD123 (the alpha chain of IL-3) variable heavy and variable light chains, with the CDRs underlined, for use in the trispecific antibodies of the invention.

[00131] Figure 46 Table showing variants engineered to increase affinity and stability of 7G3_H1L1.

[00132] Figure 47 Table showing the properties of final affinity and stability optimized humanized variants of 7G3 (anti-CD123 antigen binding domain).

[00133] Figure 48 depicts Cell surface binding of XENP18953 to 50k purified human T cells. Detection was by a PE-labeled anti-human IgG Fc secondary antibody.

[00134] Figure 49 Top panel: Redirected T cell cytotoxicity (RTCC) assay, XENP18953, 24 h incubation, 20k JeKo cells, 400k purified human T cells. Detection was by flow cytometry. Bottom panel: Resulting T cell activation, measured by CD69 expression, after the RTCC assay described in the top panel.

[00135] Figure 50 shows a two dimensional matrix of possible specific combinations for the invention, starting with two antigen binding domains. The Top panel represents combinations with CD3 and a TTA. An "A" means that the CDRs of the referenced CD3 sequences can be combined with the CDRs of the TTA on the right hand side. That is, the vhCDRs from the variable heavy chain CD3 H1.30 sequence and the vlCDRs from the variable light chain of CD3 L1.57 sequence can be combined with the vhCDRs from the CD38 OKT10 H1.77 sequence and the vlCDRs from the OKT10L1.24 sequence. A "B" means that the CDRs from the CD3 constructs can be combined with the variable heavy and

light domains from the TTA. That is, the vhCDRs from the variable heavy chain CD3 H1.30 sequence and the vlCDRs from the variable light chain of CD3 L1.57 sequence can be combined with the variable heavy domain CD38 OKT10 H1.77 sequence and the OKT10L1.24 sequence. A "C" is reversed, such that the variable heavy domain and variable light domain from the CD3 sequences are used with the CDRs of the TTAs. A "D" is where both the variable heavy and variable light chains from each are combined. An "E" is where the scFv of the CD3 is used with the CDRs of the TTA, and an "F" is where the scFv of the CD3 is used with the variable heavy and variable light domains of the TTA antigen binding domain. In this top panel, the third antigen can either be a second antigen binding domain to the same target antigen (e.g. a different CD38 antigen binding domain), or to CD8. The bottom panel shows the possible combinations of specific CD3 and CD8 binding domains that can be combined, which then can have a TTA antigen binding domain from the top panel.

[00136] Figure 51 depicts a useful PMSA Fv region for use in the present invention, particular with CD3 and CD8 antigen binding domains (any combination of those shown in Figure 50, bottom panel, for example).

DETAILED DESCRIPTION OF THE INVENTION

[00137] The following applications are specifically incorporated by reference in their entirety, but in particular for the Figures, associated Legends, amino acid positions and amino acid sequences as well (particularly any heterodimerization variants): US Pub. 2014/0288275, WO2014/145806, 2013/0171095; 61/818,153; 61/778,148; 61/818,344; 14/207,489; 14/216,705; ; 61/913,870; 61/913,832; 61/972,172; 62/025,974; 62/084,741; 62/084,750; 62/085,003; 62/084,757; 62/085,117; 62/084,908; 62/085,027, 62/085,106 and 62/174,371. In particular, Figures and Figure Legends associated with binding constants and biochemical efficacy (RTCC assays, etc.) of components (such as antigen binding domains) are additionally specifically incorporated herein.

I. Definitions

[00138] In order that the application may be more completely understood, several definitions are set forth below. Such definitions are meant to encompass grammatical equivalents.

[00139] By "ablation" herein is meant a decrease or removal of activity. Thus for example, "ablating FcγR binding" means the Fc region amino acid variant has less than 50% starting binding as compared to an Fc region not containing the specific variant, with less than 70-80-90-95-98% loss of activity being preferred, and in general, with the activity being below the level of detectable binding in a Biacore assay. Of particular use in the ablation of FcγR binding are those shown in Figure 16.

[00140] By "ADCC" or "antibody dependent cell-mediated cytotoxicity" as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell. ADCC is correlated with binding to FcγRIIIa; increased binding to FcγRIIIa leads to an increase in ADCC activity.

[00141] By "ADCP" or antibody dependent cell-mediated phagocytosis as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell.

[00142] By "modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence or an alteration to a moiety chemically linked to a protein. For example, a modification may be an altered carbohydrate or PEG structure attached to a protein. By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. For clarity, unless otherwise noted, the amino acid modification is always to an amino acid coded for by DNA, e.g. the 20 amino acids that have codons in DNA and RNA.

[00143] By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with a different amino acid. In particular, in some embodiments, the substitution is to an amino acid that is not naturally occurring at the particular position, either not naturally occurring within the

organism or in any organism. For example, the substitution E272Y refers to a variant polypeptide, in this case an Fc variant, in which the glutamic acid at position 272 is replaced with tyrosine. In addition, the identification of the wild type position is not meant to be limiting. For most, if not all, amino acid subsitutions herein are relative to IgG1, although this applies equally to IgG2, G3 and G4. That is, there is an E at position 272 in all four isotypes. However, for other positions, there may be differences between the isotypes, such as at position 384, which is an N in IgG1, G2 and G4 and an S in IgG3. Accordingly, a listing of "E272Y" also includes "272Y", which indicates that whatever the wild type residue, the amino acid substitution is to a Y, and is not naturally occurring in the isotypes.

[00144] For clarity, a protein which has been engineered to change the nucleic acid coding sequence but not change the starting amino acid (for example exchanging CGG (encoding arginine) to CGA (still encoding arginine) to increase host organism expression levels) is not an "amino acid substitution"; that is, despite the creation of a new gene encoding the same protein, if the protein has the same amino acid at the particular position that it started with, it is not an amino acid substitution.

[00145] By "amino acid insertion" or "insertion" as used herein is meant the addition of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, -233E or 233E designates an insertion of glutamic acid after position 233 and before position 234. Additionally, -233ADE or A233ADE designates an insertion of AlaAspGlu after position 233 and before position 234.

[00146] By "amino acid deletion" or "deletion" as used herein is meant the removal of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, E233- or E233# or E233()designates a deletion of glutamic acid at position 233. Additionally, EDA233- or EDA233# designates a deletion of the sequence GluAspAla that begins at position 233.

[00147] By "variant protein" or "protein variant", or "variant" as used herein is meant a protein that differs from that of a parent protein by virtue of at least one amino acid modification. Protein variant may refer to the protein itself, a composition comprising the protein, or the amino sequence that encodes it. Preferably, the protein variant has at least

one amino acid modification compared to the parent protein, e.g. from about one to about seventy amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. As described below, in some embodiments the parent polypeptide, for example an Fc parent polypeptide, is a human wild type sequence, such as the Fc region from IgG1, IgG2, IgG3 or IgG4, although human sequences with variants can also serve as "parent polypeptides", for example the IgG1/2 hybrid of Figure 44. The protein variant sequence herein will preferably possess at least about 80% identity with a parent protein sequence, and most preferably at least about 90% identity, more preferably at least about 95-98-99% identity . Variant protein can refer to the variant protein itself, compositions comprising the protein variant, or the DNA sequence that encodes it. Accordingly, by "antibody variant" or "variant antibody" as used herein is meant an antibody that differs from a parent antibody by virtue of at least one amino acid modification, "IgG variant" or "variant IgG" as used herein is meant an antibody that differs from a parent IgG (again, in many cases, from a human IgG sequence) by virtue of at least one amino acid modification, and "immunoglobulin variant" or "variant immunoglobulin" as used herein is meant an immunoglobulin sequence that differs from that of a parent immunoglobulin sequence by virtue of at least one amino acid modification. "Fc variant" or "variant Fc" as used herein is meant a protein comprising an amino acid modification in an Fc domain. The Fc variants of the present invention are defined according to the amino acid modifications that compose them. Thus, for example, N434S or 434S is an Fc variant with the substitution serine at position 434 relative to the parent Fc polypeptide, wherein the numbering is according to the EU index. Likewise, M428L/N434S defines an Fc variant with the substitutions M428L and N434S relative to the parent Fc polypeptide. The identity of the WT amino acid may be unspecified, in which case the aforementioned variant is referred to as 428L/434S. It is noted that the order in which substitutions are provided is arbitrary, that is to say that, for example, 428L/434S is the same Fc variant as M428L/N434S, and so on. For all positions discussed in the present invention that relate to antibodies, unless otherwise noted, amino acid position numbering is according to the EU index. The EU index or EU index as in Kabat or EU numbering scheme refers to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85, hereby entirely incorporated by

reference.) The modification can be an addition, deletion, or substitution. Substitutions can include naturally occurring amino acids and, in some cases, synthetic amino acids. Examples include U.S. Pat. No. 6,586,207; WO 98/48032; WO 03/073238; US2004-0214988A1; WO 05/35727A2; WO 05/74524A2; J. W. Chin et al., (2002), Journal of the American Chemical Society 124:9026-9027; J. W. Chin, & P. G. Schultz, (2002), ChemBioChem 11:1135-1137; J. W. Chin, et al., (2002), PICAS United States of America 99:11020-11024; and, L. Wang, & P. G. Schultz, (2002), Chem. 1-10, all entirely incorporated by reference.

[00148] As used herein, "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The peptidyl group may comprise naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures, i.e. "analogs", such as peptoids (see Simon et al., PNAS USA 89(20):9367 (1992), entirely incorporated by reference). The amino acids may either be naturally occurring or synthetic (e.g. not an amino acid that is coded for by DNA); as will be appreciated by those in the art. For example, homo-phenylalanine, citrulline, ornithine and noreleucine are considered synthetic amino acids for the purposes of the invention, and both D- and L-(R or S) configured amino acids may be utilized. The variants of the present invention may comprise modifications that include the use of synthetic amino acids incorporated using, for example, the technologies developed by Schultz and colleagues, including but not limited to methods described by Cropp & Shultz, 2004, Trends Genet. 20(12):625-30, Anderson et al., 2004, Proc Natl Acad Sci USA 101 (2):7566-71, Zhang et al., 2003, 303(5656):371-3, and Chin et al., 2003, Science 301(5635):964-7, all entirely incorporated by reference. In addition, polypeptides may include synthetic derivatization of one or more side chains or termini, glycosylation, PEGylation, circular permutation, cyclization, linkers to other molecules, fusion to proteins or protein domains, and addition of peptide tags or labels.

[00149] By "residue" as used herein is meant a position in a protein and its associated amino acid identity. For example, Asparagine 297 (also referred to as Asn297 or N297) is a residue at position 297 in the human antibody IgG1.

[00150] By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region

in isolation, or this region in the context of a full length antibody, antibody fragment or Fab fusion protein. By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the VL and VH domains of a single antibody. As will be appreciated by those in the art, these generally are made up of two chains.

[00151] By "IgG subclass modification" or "isotype modification" as used herein is meant an amino acid modification that converts one amino acid of one IgG isotype to the corresponding amino acid in a different, aligned IgG isotype. For example, because IgG1 comprises a tyrosine and IgG2 a phenylalanine at EU position 296, a F296Y substitution in IgG2 is considered an IgG subclass modification.

[00152] By "non-naturally occurring modification" as used herein is meant an amino acid modification that is not isotypic. For example, because none of the IgGs comprise a serine at position 434, the substitution 434S in IgG1, IgG2, IgG3, or IgG4 (or hybrids thereof) is considered a non-naturally occurring modification.

[00153] By "amino acid" and "amino acid identity" as used herein is meant one of the 20 naturally occurring amino acids that are coded for by DNA and RNA.

[00154] By "effector function" as used herein is meant a biochemical event that results from the interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions include but are not limited to ADCC, ADCP, and CDC.

[00155] By "IgG Fc ligand" as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an IgG antibody to form an Fc/Fc ligand complex. Fc ligands include but are not limited to FcγRIs, FcγRIIs, FcγRIIIs, FcRn, C1q, C3, mannan binding lectin, mannose receptor, staphylococcal protein A, streptococcal protein G, and viral FcγR. Fc ligands also include Fc receptor homologs (FcRH), which are a family of Fc receptors that are homologous to the FcγRs (Davis et al., 2002, Immunological Reviews 190:123-136, entirely incorporated by reference). Fc ligands may include undiscovered molecules that bind Fc. Particular IgG Fc ligands are FcRn and Fc gamma receptors. By "Fc ligand" as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an antibody to form an Fc/Fc ligand complex.

[00156] By "Fc gamma receptor", "FcγR" or "FcqammaR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an FcγR gene. In humans this family includes but is not limited to FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIb-NA1 and FcγRIIb-NA2) (Jefferis et al., 2002, Immunol Lett 82:57-65, entirely incorporated by reference), as well as any undiscovered human FcγRs or FcγR isoforms or allotypes. An FcγR may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. Mouse FcγRs include but are not limited to FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16), and FcγRIII-2 (CD16-2), as well as any undiscovered mouse FcγRs or FcγR isoforms or allotypes.

[00157] By "FcRn" or "neonatal Fc Receptor" as used herein is meant a protein that binds the IgG antibody Fc region and is encoded at least in part by an FcRn gene. The FcRn may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. As is known in the art, the functional FcRn protein comprises two polypeptides, often referred to as the heavy chain and light chain. The light chain is beta-2-microglobulin and the heavy chain is encoded by the FcRn gene. Unless otherwise noted herein, FcRn or an FcRn protein refers to the complex of FcRn heavy chain with beta-2-microglobulin. A variety of FcRn variants used to increase binding to the FcRn receptor, and in some cases, to increase serum half-life, are shown in the Figure Legend of Figure 83.

[00158] By "parent polypeptide" as used herein is meant a starting polypeptide that is subsequently modified to generate a variant. The parent polypeptide may be a naturally occurring polypeptide, or a variant or engineered version of a naturally occurring polypeptide. Parent polypeptide may refer to the polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it. Accordingly, by "parent immunoglobulin" as used herein is meant an unmodified immunoglobulin polypeptide that is modified to generate a variant, and by "parent antibody" as used herein is meant an unmodified antibody that is modified to generate a variant antibody. It should

be noted that "parent antibody" includes known commercial, recombinantly produced antibodies as outlined below.

[00159] By "Fc" or "Fc region" or "Fc domain" as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain and in some cases, part of the hinge. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, the Fc domain comprises immunoglobulin domains Cγ2 and Cγ3 (Cγ2 and Cγ3) and the lower hinge region between Cγ1 (Cγ1) and Cγ2 (Cγ2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. In some embodiments, as is more fully described below, amino acid modifications are made to the Fc region, for example to alter binding to one or more FcγR receptors or to the FcRn receptor.

[00160] By "heavy constant region" herein is meant the CH1-hinge-CH2-CH3 portion of an antibody.

[00161] By "Fc fusion protein" or "immunoadhesin" herein is meant a protein comprising an Fc region, generally linked (optionally through a linker moiety, as described herein) to a different protein, such as a binding moiety to a target protein, as described herein. In some cases, one monomer of the heterodimeric antibody comprises an antibody heavy chain (either including an scFv or further including a light chain) and the other monomer is a Fc fusion, comprising a variant Fc domain and a ligand. In some embodiments, these "half antibody-half fusion proteins" are referred to as "Fusionbodies".

[00162] By "position" as used herein is meant a location in the sequence of a protein. Positions may be numbered sequentially, or according to an established format, for example the EU index for antibody numbering.

[00163] By "target antigen" as used herein is meant the molecule that is bound specifically by the variable region of a given antibody. A target antigen may be a protein,

carbohydrate, lipid, or other chemical compound. A wide number of suitable target antigens are described below.

[00164] By "strandedness" in the context of the monomers of the heterodimeric antibodies of the invention herein is meant that, similar to the two strands of DNA that "match", heterodimerization variants are incorporated into each monomer so as to preserve the ability to "match" to form heterodimers. For example, if some pI variants are engineered into monomer A (e.g. making the pI higher) then steric variants that are "charge pairs" that can be utilized as well do not interfere with the pI variants, e.g. the charge variants that make a pI higher are put on the same "strand" or "monomer" to preserve both functionalities. Similarly, for "skew" variants that come in pairs of a set as more fully outlined below, the skilled artisan will consider pI in deciding into which strand or monomer that incorporates one set of the pair will go, such that pI separation is maximized using the pI of the skews as well.

[00165] By "target cell" as used herein is meant a cell that expresses a target antigen.

[00166] By "variable region" as used herein is meant the region of an immunoglobulin that comprises one or more Ig domains substantially encoded by any of the V.kappa., V.lamda., and/or VH genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively.

[00167] By "wild type or WT" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

[00168] The antibodies of the present invention are generally isolated or recombinant. "Isolated," when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Ordinarily, an isolated polypeptide will be prepared by at least one purification step. An "isolated antibody," refers to an antibody which is substantially free of other antibodies having different antigenic specificities. "Recombinant" means the antibodies are generated using recombinant nucleic acid techniques in exogeneous host cells.

[00169] "Specific binding" or "specifically binds to" or is "specific for" a particular antigen or an epitope means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target.

[00170] Specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KD for an antigen or epitope of at least about 10-4 M, at least about 10-5 M, at least about 10-6 M, at least about 10-7 M, at least about 10-8 M, at least about 10-9 M, alternatively at least about 10-10 M, at least about 10-11 M, at least about 10-12 M, or greater, where KD refers to a dissociation rate of a particular antibody-antigen interaction. Typically, an antibody that specifically binds an antigen will have a KD that is 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for a control molecule relative to the antigen or epitope.

[00171] Also, specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KA or Ka for an antigen or epitope of at least 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for the epitope relative to a control, where KA or Ka refers to an association rate of a particular antibody-antigen interaction.

II. Overview of Multispecific Binding

[00172] Trispecific antibodies that co-engage CD3 and a tumor antigen target have been designed and used to redirect T cells to attack and lyse targeted tumor cells. Examples include the BiTE and DART formats, which monovalently engage CD3 and a tumor antigen. While the CD3-targeting approach has shown considerable promise, a common side effect of such therapies is the associated production of cytokines, often leading to toxic cytokine release syndrome. Because the anti-CD3 binding domain of the trispecific antibody engages all T cells, the high cytokine-producing CD4 T cell subset is recruited. Moreover, the CD4 T cell subset includes regulatory T cells, whose recruitment and expansion can potentially lead to immune suppression and have a negative impact on long-term tumor suppression. In addition, these formats do not contain Fc domains and show very short serum half-lives in patients.

[00173] While the CD3-targeting approach has shown considerable promise, a common side effect of such therapies is the associated production of cytokines, often leading to toxic cytokine release syndrome. Because the anti-CD3 binding domain of the trispecific antibody engages all T cells, the high cytokine-producing CD4 T cell subset is recruited. Moreover, the CD4 T cell subset includes regulatory T cells, whose recruitment and expansion can potentially lead to immune suppression and have a negative impact on long-term tumor suppression. One such possible way to reduce cytokine production and possibly reduce the activation of CD4 T cells is by reducing the affinity of the anti-CD3 domain for CD3. Thus, the invention includes the use of anti-CD3 binding domains that have "high", "intermediate" and "low" affinities such as are depicted in Figures.

[00174] In addition, the present invention is directed to an alternative solution of these issues, by including more selective T cell targets rather than the pan-T cell activator CD3. We demonstrate herein that trispecific antibodies designed to selectively recruit the CD8 T cell subset can target and kill tumor cells effectively. Selective CD8 recruitment leads to significantly reduced cytokine release, expanding the therapeutic window for T cell recruitment. Thus, antibodies of the invention can utilize anti-CD8 binding domains, whether in combination with anti-CD3 binding domains or in place of anti-CD3 binding domains.

[00175] Accordingly, as more fully described below, some aspects of the invention include antibody constructs that are generally trispecific, that is, they bind three different antigens. As shown in the Figures and described herein, the formation of the trispecific antibodies of the invention relies on the use of Fc domains (and, in some cases, includes heavy constant chain domains) that are heterodimeric; that is, each CH2-CH3 domain of the dimeric Fc domain has a different amino acid sequence as described herein (or, in the case where the format includes a full length heavy constant chain domain (CH1-hinge-CH2-CH3), where each heavy chain constant domain monomer has a different amino acid sequence. Thus, the trispecific antibodies of the invention are heterodimeric antibodies.

[00176] In addition, as is further discussed below and shown in the figures, the heterodimeric trispecific antibodies can take on a number of formats, which include combinations of single binding domains for antigens (e.g. the antibody binds an antigen

monovalently) or two binding domains for antigens (e.g. the antibody binds an antigen bivalently). In general, the trispecific formats of the invention utilize two scFv antigen binding domains and either one or two Fab antigen binding domains, although as shown in Figure 1 and Figure 2, three or four scFv domains can also be used.

[00177] Generally, the trispecific antibodies of the invention utilize different antigen binding domains to bind to three different antigens (sometimes referred to herein as "antigen 1" or "A1", "antigen-2" or "A2", and "antigen 3" or "A3"). The antigen binding domains bind to a combination of antigens including, but not limited to, target tumor antigens (TTAs), CD3 and/or CD8 as follows.

[00178] In some embodiments, the trispecific antibodies of the invention bind to CD3, and first and second tumor target antigens (TTA1 and TTA2). In this embodiment, generally the two TTAs are found on the same tumor type.

[00179] In some embodiments, the trispecific antibodies of the invention bind to CD8, and first and second tumor target antigens (TTA1 and TTA2). In this embodiment, generally the two TTAs are found on the same tumor type.

[00180] In some embodiments, the trispecific antibodies of the invention bind to CD3, CD8 and a tumor target antigen (TTA1). In this embodiment, generally the two TTAs are found on the same tumor type.

[00181] In some embodiments, the trispecific antibodies of the invention bind to three separate target tumor antigens. In this embodiment, generally the three TTAs are found on the same tumor type.

[00182] Importantly, when one of the antigens to be bound by the trispecific antibody is CD3, only one antigen binding domain will bind CD3; that is, CD3 is bound monovalently.

[00183] In addition, in the above embodiments, the antigen binding domain to CD3 can be "high" affinity, "intermediate" affinity, or "low" affinity; see the exemplary sequences in Figure 13 to Figure 18 . Similarly, the antigen bind domain to CD8 can be high or low (see exemplary sequences in Figure 25 to Figure 28) as is true for the TTA binding affinities as well (see exemplary sequences of Figure 19 to Figure 21 for CD38, Figure 22 and

Figure 23 for exemplary sequences for CD20, and Figure 24 for an exemplary sequence of CD123).

Antibodies

[00184] The present invention relates to the generation of trispecific antibodies, generally therapeutic antibodies. As is discussed below, the term "antibody" is used generally. Antibodies that find use in the present invention can take on a number of formats as described herein, including traditional antibodies as well as antibody derivatives, fragments and mimetics, described herein.

[00185] Traditional antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). Human light chains are classified as kappa and lambda light chains. The present invention is directed to the IgG class, which has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. Thus, "isotype" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. It should be understood that therapeutic antibodies can also comprise hybrids of isotypes and/or subclasses. For example, as shown in US Publication 2009/0163699, incorporated by reference, the present invention covers pI engineering of IgG1/G2 hybrids.

[00186] The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition, generally referred to in the art and herein as the "Fv domain" or "Fv region". In the variable region, three loops are gathered for each of the V domains of the heavy chain and light chain to form an antigen-binding site. Each of the loops is referred to as a complementarity-determining region (hereinafter referred to as a "CDR"), in which the variation in the amino acid sequence is most significant. "Variable" refers to the fact that certain segments of the variable region differ extensively in sequence

among antibodies. Variability within the variable region is not evenly distributed. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-15 amino acids long or longer.

[00187] Each VH and VL is composed of three hypervariable regions ("complementary determining regions," "CDRs") and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

[00188] The hypervariable region generally encompasses amino acid residues from about amino acid residues 24-34 (LCDR1; "L" denotes light chain), 50-56 (LCDR2) and 89-97 (LCDR3) in the light chain variable region and around about 31-35B (HCDR1; "H" denotes heavy chain), 50-65 (HCDR2), and 95-102 (HCDR3) in the heavy chain variable region; Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) and/or those residues forming a hypervariable loop (e.g. residues 26-32 (LCDR1), 50-52 (LCDR2) and 91-96 (LCDR3) in the light chain variable region and 26-32 (HCDR1), 53-55 (HCDR2) and 96-101 (HCDR3) in the heavy chain variable region; Chothia and Lesk (1987) J. Mol. Biol. 196:901-917. Specific CDRs of the invention are described below.

[00189] Throughout the present specification, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain variable region and residues 1-113 of the heavy chain variable region) and the EU numbering system for Fc regions (e.g, Kabat et al., supra (1991)). In some cases, internal "ACE" numbering is used for CDR numbering; see Figure 30.

[00190] The CDRs contribute to the formation of the antigen-binding, or more specifically, epitope binding site of antibodies. "Epitope" refers to a determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. Epitopes are groupings of molecules such as amino acids or sugar side chains and usually have specific structural characteristics, as well as specific charge characteristics. A single antigen may have more than one epitope.

[00191] The epitope may comprise amino acid residues directly involved in the binding (also called immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding, such as amino acid residues which are effectively blocked by the specifically antigen binding peptide; in other words, the amino acid residue is within the footprint of the specifically antigen binding peptide.

[00192] Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. Conformational and nonconformational epitopes may be distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[00193] An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Antibodies that recognize the same epitope can be verified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen, for example "binning."

[00194] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Kabat et al. collected numerous primary sequences of the variable regions of heavy chains and light chains. Based on the

degree of conservation of the sequences, they classified individual primary sequences into the CDR and the framework and made a list thereof (see SEQUENCES OF IMMUNOLOGICAL INTEREST, 5th edition, NIH publication, No. 91-3242, E.A. Kabat et al., entirely incorporated by reference).

[00195] In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the present invention are the heavy chain domains, including, the constant heavy (CH) domains and the hinge domains. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, "CH" domains in the context of IgG are as follows: "CH1" refers to positions 118-220 according to the EU index as in Kabat. "CH2" refers to positions 237-340 according to the EU index as in Kabat, and "CH3" refers to positions 341-447 according to the EU index as in Kabat. As shown herein and described below, the pI variants can be in one or more of the CH regions, as well as the hinge region, discussed below.

[00196] It should be noted that the sequences depicted herein start at the CH1 region, position 118; the variable regions are not included except as noted. For example, the first amino acid of SEQ ID NO: 2, while designated as position"1" in the sequence listing, corresponds to position 118 of the CH1 region, according to EU numbering.

[00197] Another type of Ig domain of the heavy chain is the hinge region. By "hinge" or "hinge region" or "antibody hinge region" or "immunoglobulin hinge region" herein is meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for IgG the antibody hinge is herein defined to include positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the numbering is according to the

EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the lower hinge is included, with the "lower hinge" generally referring to positions 226 or 230. As noted herein, pI variants can be made in the hinge region as well.

[00198] The light chain generally comprises two domains, the variable light domain (containing the light chain CDRs and together with the variable heavy domains forming the Fv region), and a constant light chain region (often referred to as CL or Cκ).

[00199] Another region of interest for additional substitutions, outlined below, is the Fc region.

[00200] Thus, the present invention provides different antibody domains. As described herein and known in the art, the heterodimeric antibodies of the invention comprise different domains within the heavy and light chains, which can be overlapping as well. These domains include, but are not limited to, the Fc domain, the CH1 domain, the CH2 domain, the CH3 domain, the hinge domain, the heavy constant domain (CH1-hinge-Fc domain or CH1-hinge-CH2-CH3), the variable heavy domain, the variable light domain, the light constant domain, FAb domains and scFv domains.

[00201] Thus, the "Fc domain" includes the -CH2-CH3 domain, and optionally a hinge domain. The heavy chain comprises a variable heavy domain and a constant domain, which includes a CH1-optional hinge-Fc domain comprising a CH2-CH3. The light chain comprises a variable light chain and the light constant domain.

[00202] Some embodiments of the invention comprise at least one scFv domain, which, while not naturally occurring, generally includes a variable heavy domain and a variable light domain, linked together by a scFv linker. As shown herein, there are a number of suitable scFv linkers that can be used, including traditional peptide bonds, generated by recombinant techniques.

[00203] The linker peptide may predominantly include the following amino acid residues: Gly, Ser, Ala, or Thr. The linker peptide should have a length that is adequate to

link two molecules in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. In one embodiment, the linker is from about 1 to 50 amino acids in length, preferably about 1 to 30 amino acids in length. In one embodiment, linkers of 1 to 20 amino acids in length may be used, with from about 5 to about 10 amino acids finding use in some embodiments. Useful linkers include glycine-serine polymers, including for example (GS)n, (GSGGS)n, (GGGGS)n, and (GGGS)n, where n is an integer of at least one (and generally from 3 to 4), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers. Alternatively, a variety of nonproteinaceous polymers, including but not limited to polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, may find use as linkers, that is may find use as linkers.

[00204] Other linker sequences may include any sequence of any length of CL/CH1 domain but not all residues of CL/CH1 domain; for example the first 5-12 amino acid residues of the CL/CH1 domains. Linkers can be derived from immunoglobulin light chain, for example Cκ or Cλ. Linkers can be derived from immunoglobulin heavy chains of any isotype, including for example Cγ1, Cγ2, Cγ3, Cγ4, Cα1, Cα2, Cδ, Cε, and Cμ. Linker sequences may also be derived from other proteins such as Ig-like proteins (e.g. TCR, FcR, KIR), hinge region-derived sequences, and other natural sequences from other proteins.

[00205] In some embodiments, the linker is a "domain linker", used to link any two domains as outlined herein together. While any suitable linker can be used, many embodiments utilize a glycine-serine polymer, including for example (GS)n, (GSGGS)n, (GGGGS)n, and (GGGS)n, where n is an integer of at least one (and generally from 3 to 4 to 5) as well as any peptide sequence that allows for recombinant attachment of the two domains with sufficient length and flexibility to allow each domain to retain its biological function. . In some cases, and with attention being paid to "strandedness", as outlined below, charged domain linkers, as used in some embodiments of scFv linkers can be used.

[00206] In some embodiments, the scFv linker is a charged scFv linker, a number of which are shown in Figure 33. Accordingly, the present invention further provides charged scFv linkers, to facilitate the separation in pI between a first and a second monomer. That is, by incorporating a charged scFv linker, either positive or negative (or both, in the case of

scaffolds that use scFvs on different monomers), this allows the monomer comprising the charged linker to alter the pI without making further changes in the Fc domains. These charged linkers can be substituted into any scFv containing standard linkers. Again, as will be appreciated by those in the art, charged scFv linkers are used on the correct "strand" or monomer, according to the desired changes in pI. For example, as discussed herein, to make triple F format heterodimeric antibody, the original pI of the Fv region for each of the desired antigen binding domains are calculated, and one is chosen to make an scFv, and depending on the pI, either positive or negative linkers are chosen.

[00207] Charged domain linkers can also be used to increase the pI separation of the monomers of the invention as well, and thus those included in Figure 33Figure 33 can be used in any embodiment herein where a linker is utilized.

[00208] Accordingly, in some embodiments the present invention provides heterodimeric antibodies that rely on the use of two different heavy chain variant Fc domains that will self-assemble to form heterodimeric antibodies.

[00209] In some embodiments, the antibodies are full length. By "full length antibody" herein is meant the structure that constitutes the natural biological form of an antibody, including variable and constant regions, including one or more modifications as outlined herein, particularly in the Fc domains to allow either heterodimerization formation or the purification of heterodimers away from homodimers. Full length antibodies generally include Fab and Fc domains, and can additionally contain extra antigen binding domains such as scFvs, as is generally depicted in the Figures.

[00210] In one embodiment, the antibody is an antibody fragment, as long as it contains at least one constant domain which can be engineered to produce heterodimers, such as pI engineering. Other antibody fragments that can be used include fragments that contain one or more of the CH1, CH2, CH3, hinge and CL domains of the invention that have been pI engineered. For example, Fc fusions are fusions of the Fc region (CH2 and CH3, optionally with the hinge region) fused to

another protein. A number of Fc fusions are known the art and can be improved by the addition of the heterodimerization variants of the invention. In the present case, antibody fusions can be made comprising CH1; CH1, CH2 and CH3; CH2; CH3; CH2 and CH3; CH1 and CH3, any or all of which can be made optionally with the hinge region, utilizing any combination of heterodimerization variants described herein.

Heterodimerization Antibodies

[00211] The present invention is directed to novel constructs to provide heterodimeric antibodies that allow binding to at least three antigens or ligands, e.g. to allow for trispecific binding. The heterodimeric antibody constructs are based on the self-assembling nature of the two Fc domains of the heavy chains of antibodies, e.g. two "monomers" that assemble into a "dimer". Heterodimeric antibodies are made by altering the amino acid sequence of each monomer as more fully discussed below. Thus, the present invention is generally directed to the creation of heterodimeric antibodies which can co-engage antigens in several ways, relying on amino acid variants in the constant regions that are different on each chain to promote heterodimeric formation and/or allow for ease of purification of heterodimers over the homodimers.

[00212] Thus, the present invention provides trispecific antibodies. These are made using two monomers that associate to form a heterodimeric protein.

[00213] In general, the trispecific antibodies fall into two categories: those that are tetravalent and trispecific, and those that are trivalent and trispecific. In this context, "specificity" refers to the number of different antigens that are bound by the antibody, and "valency" the number of antigens that can be bound by the antibody. Thus, "tetravalent trispecific" antibodies bind three different antigens (CD3, CD8 and a target turmor antigen, for example, or CD3 and two target tumor antigens, etc.) with one of them engaging the ligand bivalently, as shown in Figure 1, where it

is the tumor antigen that is bound bivalently. However, as will be appreciated by those in the art, the bivalent engagement can also be the CD8. For the reasons discussed above, bivalent engagement of CD3 is not preferred. "Trivalent trispecific" antibodies engage each of three different antigens monovalently. As outlined herein and in the figures, in general at least one Fab is used as one of the antigent binding domains, although this is not required (e.g. three scFv domains can be used). In the case of tetravalent trispecific antibodies, the bivalent antigen binding domain can be two Fabs, as it is preferred to only require one light chain in the constructs.

[00214] An ongoing problem in multispecific antibody technologies is how to make and isolate them. In general, these multispecific antibodies are made by including genes for each heavy and light chain into the host cells. This generally results in the formation of the desired heterodimer (A-B), as well as the two homodimers (A-A and B-B (not including the light chain heterodimeric issues)). However, a major obstacle in the formation of heterodimeric antibodies is the difficulty in purifying the heterodimeric antibodies away from the homodimeric antibodies and/or biasing the formation of the heterodimer over the formation of the homodimers.

[00215] There are a number of mechanisms that can be used to generate the heterodimers of the present invention. In addition, as will be appreciated by those in the art, these mechanisms can be combined to ensure high heterodimerization. Thus, amino acid variants that lead to the production of heterodimers are referred to as "heterodimerization variants". As discussed below, heterodimerization variants can include steric variants (e.g. the "knobs and holes" or "skew" variants described below and the "charge pairs" variants described below) as well as "pI variants", which allows purification of homodimers away from heterodimers. As is generally described in WO2014/145806, hereby incorporated by reference in its entirety and

specifically as below for the discussion of "heterodimerization variants", useful mechanisms for heterodimerization include "knobs and holes" ("KIH"; sometimes herein as "skew" variants (see discussion in WO2014/145806), "electrostatic steering" or "charge pairs" as described in WO2014/145806, pI variants as described in WO2014/145806, and general additional Fc variants as outlined in WO2014/145806 and below.

[00216] In the present invention, there are several basic mechanisms that can lead to ease of purifying heterodimeric antibodies; one relies on the use of pI variants, such that each monomer has a different pI, thus allowing the isoelectric purification of A-A, A-B and B-B dimeric proteins. Alternatively, some scaffold formats also allows separation on the basis of size. As is further outlined below, it is also possible to "skew" the formation of heterodimers over homodimers. Thus, a combination of steric heterodimerization variants and pI or charge pair variants find particular use in the invention. Additionally, as more fully outlined below, scaffolds that utilize scFv(s) can include charged scFv linkers (either positive or negative), that give a further pI boost for purification purposes. As will be appreciated by those in the art, some formats are useful with just charged scFv linkers and no additional pI adjustments, although the invention does provide the use of skew variants with charged scFv linkers as well (and combinations of Fc, FcRn and KO variants).

[00217] In the present invention that utilizes pI as a separation mechanism to allow the purification of heterodimeric proteins, amino acid variants can be introduced into one or both of the monomer polypeptides; that is, the pI of one of the monomers (referred to herein for simplicity as "monomer A") can be engineered away from monomer B, or both monomer A and B change be changed, with the pI of monomer A increasing and the pI of monomer B decreasing. As is outlined more fully below, the pI changes of either or both monomers can be done by removing or adding a charged residue (e.g. a neutral amino acid is replaced by a positively or

negatively charged amino acid residue, e.g. glycine to glutamic acid), changing a charged residue from positive or negative to the opposite charge (aspartic acid to lysine) or changing a charged residue to a neutral residue (e.g. loss of a charge; lysine to serine.). A number of these variants are shown in the Figures.

[00218] Accordingly, in this embodiment of the present invention provides for creating a sufficient change in pI in at least one of the monomers such that heterodimers can be separated from homodimers. As will be appreciated by those in the art, and as discussed further below, this can be done by using a "wild type" heavy chain constant region and a variant region that has been engineered to either increase or decrease it's pI (wt A-+B or wt A - -B), or by increasing one region and decreasing the other region (A+ -B- or A- B+).

[00219] Thus, in general, a component of some embodiments of the present invention are amino acid variants in the constant regions of antibodies that are directed to altering the isoelectric point (pI) of at least one, if not both, of the monomers of a dimeric protein to form "pI antibodies") by incorporating amino acid substitutions ("pI variants" or "pI substitutions") into one or both of the monomers. As shown herein, the separation of the heterodimers from the two homodimers can be accomplished if the pIs of the two monomers differ by as little as 0.1 pH unit, with 0.2, 0.3, 0.4 and 0.5 or greater all finding use in the present invention.

[00220] As will be appreciated by those in the art, the number of pI variants to be included on each or both monomer(s) to get good separation will depend in part on the starting pI of the components, for example the starting pI of the scFv and Fab of interest. That is, to determine which monomer to engineer or in which "direction" (e.g. more positive or more negative), the Fv sequences of the three target antigens are calculated and a decision is made from there. As is known in the art, different Fvs will have different starting pIs which are exploited in the present invention. In general, as outlined herein, the pIs are engineered to result in a total pI difference of

each monomer of at least about 0.1 logs, with 0.2 to 0.5 being preferred as outlined herein.

[00221] Furthermore, as will be appreciated by those in the art and outlined herein, in some embodiments, heterodimers can be separated from homodimers on the basis of size. As will be appreciated from the Figures, the heterodimers of many of these trispecific constructs can be separated from homodimers on the basis of size; see Figure 2 for example.

[00222] In the case where pI variants are used to achieve heterodimerization, by using the constant region(s) of the heavy chain(s), a more modular approach to designing and purifying trispecific proteins, including antibodies, is provided. Thus, in some embodiments, heterodimerization variants (including skew and purification heterodimerization variants) are not included in the variable regions, such that each individual antibody must be engineered. In addition, in some embodiments, the possibility of immunogenicity resulting from the pI variants is significantly reduced by importing pI variants from different IgG isotypes such that pI is changed without introducing significant immunogenicity. Thus, an additional problem to be solved is the elucidation of low pI constant domains with high human sequence content, e.g. the minimization or avoidance of non-human residues at any particular position.

[00223] A side benefit that can occur with this pI engineering is also the extension of serum half-life and increased FcRn binding. That is, as described in USSN 13/194,904 (incorporated by reference in its entirety), lowering the pI of antibody constant domains (including those found in antibodies and Fc fusions) can lead to longer serum retention in vivo. These pI variants for increased serum half life also facilitate pI changes for purification.

[00224] In addition, it should be noted that the pI variants of the heterodimerization variants give an additional benefit for the analytics and quality

control process of trispecific antibodies, as the ability to either eliminate, minimize and distinguish when homodimers are present is significant. Similarly, the ability to reliably test the reproducibility of the heterodimeric antibody production is important.

[00225] As will be appreciated by those in the art and discussed more fully below, the heterodimeric fusion proteins of the present invention can take on a wide variety of configurations, as are generally depicted in Figure 1 to Figure 5. Some figures depict "single ended" configurations, where there is one type of specificity on one "arm" of the molecule and a different specificity on the other "arm". Other figures depict "dual ended" configurations, where there is at least one type of specificity at the "top" of the molecule and one or more different specificities at the "bottom" of the molecule. Thus, the present invention is directed to novel immunoglobulin compositions that co-engage a first, second and third antigen, using three different antigent binding domains. First, second and third antigens of the invention are herein referred to as antigen-1, antigen-2 and antigen3, respectively.

Chimeric and Humanized Antibodies

[00226] In some embodiments, the antibody can be a mixture from different species, e.g. a chimeric antibody and/or a humanized antibody. In general, both "chimeric antibodies" and "humanized antibodies" refer to antibodies that combine regions from more than one species. For example, "chimeric antibodies" traditionally comprise variable region(s) from a mouse (or rat, in some cases) and the constant region(s) from a human. "Humanized antibodies" generally refer to non-human antibodies that have had the variable-domain framework regions swapped for sequences found in human antibodies. Generally, in a humanized antibody, the entire antibody, except the CDRs, is encoded by a polynucleotide of human origin or is identical to such an antibody except within its CDRs. The CDRs, some or all of

which are encoded by nucleic acids originating in a non-human organism, are grafted into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, e.g., WO 92/11018, Jones, 1986, Nature 321:522-525, Verhoeyen et al., 1988, Science 239:1534-1536, all entirely incorporated by reference. "Backmutation" of selected acceptor framework residues to the corresponding donor residues is often required to regain affinity that is lost in the initial grafted construct (US 5530101; US 5585089; US 5693761; US 5693762; US 6180370; US 5859205; US 5821337; US 6054297; US 6407213, all entirely incorporated by reference). The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin, and thus will typically comprise a human Fc region. Humanized antibodies can also be generated using mice with a genetically engineered immune system. Roque et al., 2004, Biotechnol. Prog. 20:639-654, entirely incorporated by reference. A variety of techniques and methods for humanizing and reshaping non-human antibodies are well known in the art (See Tsurushita & Vasquez, 2004, Humanization of Monoclonal Antibodies, Molecular Biology of B Cells, 533-545, Elsevier Science (USA), and references cited therein, all entirely incorporated by reference). Humanization methods include but are not limited to methods described in Jones et al., 1986, Nature 321:522-525; Riechmann et al.,1988; Nature 332:323-329; Verhoeyen et al., 1988, Science, 239:1534-1536; Queen et al., 1989, Proc Natl Acad Sci, USA 86:10029-33; He et al., 1998, J. Immunol. 160: 1029-1035; Carter et al., 1992, Proc Natl Acad Sci USA 89:4285-9, Presta et al., 1997, Cancer Res. 57(20):4593-9; Gorman et al., 1991, Proc. Natl. Acad. Sci. USA 88:4181-4185; O'Connor et al., 1998, Protein Eng 11:321-8, all entirely incorporated by reference. Humanization or other methods of reducing the immunogenicity of nonhuman antibody variable regions may include resurfacing methods, as described for example in Roguska et al., 1994, Proc. Natl. Acad. Sci. USA 91:969-973, entirely incorporated by reference. In one

embodiment, the parent antibody has been affinity matured, as is known in the art. Structure-based methods may be employed for humanization and affinity maturation, for example as described in USSN 11/004,590. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu et al., 1999, J. Mol. Biol. 294:151-162; Baca et al., 1997, J. Biol. Chem. 272(16):10678-10684; Rosok et al., 1996, J. Biol. Chem. 271(37): 22611-22618; Rader et al., 1998, Proc. Natl. Acad. Sci. USA 95: 8910-8915; Krauss et al., 2003, Protein Engineering 16(10):753-759, all entirely incorporated by reference. Other humanization methods may involve the grafting of only parts of the CDRs, including but not limited to methods described in USSN 09/810,510; Tan et al., 2002, J. Immunol. 169:1119-1125; De Pascalis et al., 2002, J. Immunol. 169:3076-3084, all entirely incorporated by reference.

[00227] As will be appreciated by those in the art, murine antibodies may be generated against a variety of different human target tumor antigens and then at least the variable heavy and variable light domaims are humanized, to be used either as Fabs or scFvs in the formats of the invention.

Additional Fc Variants

[00228] Furthermore, as outlined herein, for all Fc related formats, additional amino acid variants may be introduced into the antibodies of the invention, to add additional functionalities. For example, amino acid changes within the Fc region can be added (either to one monomer or both) to facilitiate increased ADCC or CDC (e.g. altered binding to Fcγ receptors); to allow or increase yield of the addition of toxins and drugs (e.g. for ADC), as well as to increase binding to FcRn and/or increase serum half-life of the resulting molecules.

FcγR Variants

[00229] Accordingly, there are a number of useful Fc substitutions that can be made to alter binding to one or more of the FcγR receptors. Substitutions that result

in increased binding as well as decreased binding can be useful. For example, it is known that increased binding to FcγRIIIa generally results in increased ADCC (antibody dependent cell-mediated cytotoxicity; the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell). Similarly, decreased binding to FcγRIIb (an inhibitory receptor) can be beneficial as well in some circumstances. Amino acid substitutions that find use in the present invention include those listed in USSNs 11/124,620 (particularly Figure 41), 11/174,287, 11/396,495, 11/538,406, all of which are expressly incorporated herein by reference in their entirety and specifically for the variants disclosed therein. Particular variants that find use include, but are not limited to, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L, 243A, 243L, 264A, 264V and 299T.

[00230] In addition, there are additional Fc substitutions that find use in increased binding to the FcRn receptor and increased serum half life, as specifically disclosed in USSN 12/341,769, hereby incorporated by reference in its entirety, including, but not limited to, 434S, 434A, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I or V/434S, 436V/428L and 259I/308F/428L.

[00231] Similarly, another category of functional variants are "Fcγ ablation variants" or "Fc knock out" (FcKO or KO) variants. In these embodiments, for some therapeutic applications, it is desirable to reduce or remove the normal binding of the Fc domain to one or more or all of the Fcγ receptors (e.g. FcγR1, FcγRIIa, FcγRIIb, FcγRIIIa, etc.) to avoid additional mechanisms of action. That is, for example, in many embodiments, particularly in the use of trispecific antibodies that bind CD3 monovalently and a tumor antigen on the other (e.g. CD19, her2/neu, etc.), it is generally desirable to ablate FcγRIIIa binding to eliminate or significantly reduce ADCC activity. These are shown in Figure 32.

[00232] In addition, while heterodimerization variants are generally added asymmetrically to the two monomers of the invention as discussed herein, FcγR variants and/or FcRn variants are generally, but not always, added to both monomers. That is, in the trispecific formats herein, FcγR variants, FcRn and ablation variants (and generally the same ablation variants) are added to both monomers.

[00233] As is further described herein and as will be appreciated by those in the art, any and all of the variants outlined herein can be optionally and independently combined with other variants.

[00234] The present invention is directed to the generation of trispecific antibodies that are mono- or divalent for each antigen binding domain. For heterodimeric trispecific antibodies, the present invention generally relies on the use of engineered or variant Fc domains that can self-assemble in production cells to produce heterodimeric proteins, and methods to generate and purify such heterodimeric proteins.

scFv Embodiments

[00235] In some embodiments of the present invention, the trispecific antibodies of the invention include at least one, and sometimes two (or even three), scFv regions, depending on the valency of antigen binding. In some embodiments, one (or both) monomer(s) comprises a heavy chain comprising a Fab linked to an Fc domain, e.g. a "typical" heavy chain, and a light chain. By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody, antibody fragment or Fab fusion protein. By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the VL and VH domains of a single antibody.

[00236] Several of the heterodimeric antibody embodiments described herein rely on the use of one or more scFv domains, comprising the variable heavy and variable light chains, covalently linked using a linker, forming an antigen binding domain. Some embodiments herein use "standard" linkers, usually linkers of glycine and serine, as is well known in the art and described above. Standard linkers are also used to add scFv domains either to an N- or C-terminus or internally within the construct.

[00237] The present invention further provides charged scFv linkers, to facilitate the separation in pI between a first and a second monomer. That is, by incorporating a charged scFv linker, either positive or negative (or both, in the case of scaffolds that use scFvs on different monomers), this allows the monomer comprising the charged linker to alter the pI without making further changes in the Fc domains. These charged linkers can be substituted into any scFv containing standard linkers. Again, as will be appreciated by those in the art, charged scFv linkers are used on the correct "strand" or monomer, according to the desired changes in pI. For example, as discussed herein, to make the trispecific heterodimeric antibodies, the original pI of the Fv region for each of the desired antigen binding domains are calculated, and those chosen for scFv formats, and depending on the pI, either positive or negative linkers are chosen, for example using linkers shown in Figure 33.

Heterodimeric Heavy Chain Constant Regions

[00238] Accordingly, the present invention provides heterodimeric antibodies based on the use of monomers containing variant heavy chain constant regions, and specifically the Fc domains, as a first domain. By "monomer" herein is meant one half of the heterodimeric protein. It should be noted that traditional antibodies are actually tetrameric (two heavy chains and two light chains). In the context of the present invention, one pair of heavy-light chains (if applicable, e.g. if the monomer

comprises an Fab) is considered a "monomer". Similarly, a heavy chain region comprising the scFv is considered a monomer. In the case where an Fv region is one fusion partner (e.g. heavy and light variable domains) and a non-antibody protein is another fusion partner, each "half" is considered a monomer. Essentially, each monomer comprises sufficient heavy chain constant region to allow heterodimerization engineering, whether that be all the constant region, e.g. Ch1-hinge-CH2-CH3, the Fc region (CH2-CH3), or just the CH3 domain.

[00239] The variant heavy chain constant regions can comprise all or part of the heavy chain constant region, including the full length construct, CH1-hinge-CH2-CH3, or portions thereof, including for example CH2-CH3 or CH3 alone. In addition, the heavy chain region of each monomer can be the same backbone (CH1-hinge-CH2-CH3 or CH2-CH3) or different. N- and C-terminal truncations and additions are also included within the definition; for example, some pI variants include the addition of charged amino acids to the C-terminus of the heavy chain domain.

[00240] Thus, in general, one monomer of the present "triple F" construct is a scFv region-hinge-Fc domain) and the other is (VH-CH1-hinge- CH2-CH3 plus associated light chain), with heterodimerization variants, including steric, isotypic, charge steering, and pI variants, Fc and FcRn variants, ablation variants, and additional antigen binding domains (with optional linkers) included in these regions.

[00241] In addition to the heterodimerization variants (e.g. steric and pI variants) outlined herein, the heavy chain regions may also contain additional amino acid substitutions, including changes for altering FcγR and FcRn binding as discussed below.

[00242] In addition, some monomers can utilize linkers between the variant heavy chain constant region and additional antigen binding sites. For the addition

of scFv domains, standard linkers as are known in the art can be used, or the charged scFv linkers described herein. The heterodimerization variants include a number of different types of variants, including, but not limited to, steric variants (including charge variants) and pI variants, that can be optionally and independently combined with any other variants. In these embodiments, it is important to match "monomer A" with "monomer B"; that is, if a heterodimeric antibody relies on both steric variants and pI variants, these need to be correctly matched to each monomer: e.g. the set of steric variants that work (1 set on monomer A, 1 set on monomer B) is combined with pI variant sets (1 set on monomer A, 1 set on monomer B), such that the variants on each monomer are designed to achieve the desired function, keeping in mind the pI "strandedness" such that steric variants that may alter pI are put on the appropriate monomer.

[00243] It is important to note that the heterodimerization variants outlined herein, can be optionally and independently combined with any other variants, and on any other monomer. That is, what is important for the heterodimerization is that there are "sets" of variants, one set for one monomer and one set for the other. Whether these are combined from the Figures 1 to 1 (e.g. monomer 1 listings can go together) or switched (monomer 1 pI variants with monomer 2 steric variants) is irrelevant. However, as noted herein, "strandedness" should be preserved when combinations are made as outlined above. Furthermore, for the additional Fc variants (such as for FcγR binding, FcRn binding, etc.), either monomer, or both monomers, can include any of the listed variants, independently and optionally. In some cases, both monomers have the additional variants and in some only one monomer has the additional variants, or they can be combined.

Heterodimerization Variants

[00244] The present invention provides heterodimeric proteins, including heterodimeric antibodies in a variety of formats, which utilize heterodimeric

variants to allow for heterodimeric formation and/or purification away from homodimers.

Steric Variants

[00245] In some embodiments, the formation of heterodimers can be facilitated by the addition of steric variants. That is, by changing amino acids in each heavy chain, different heavy chains are more likely to associate to form the heterodimeric structure than to form homodimers with the same Fc amino acid sequences. Suitable steric variants are included in Figure 34, and in Figure 30.

[00246] One mechanism is generally referred to in the art as "knobs and holes", referring to amino acid engineering that creates steric influences to favor heterodimeric formation and disfavor homodimeric formation can also optionally be used; this is sometimes referred to as "knobs and holes", as described in USSN 61/596,846, Ridgway et al., Protein Engineering 9(7):617 (1996); Atwell et al., J. Mol. Biol. 1997 270:26; US Patent No. 8,216,805, all of which are hereby incorporated by reference in their entirety. The Figures identify a number of "monomer A – monomer B" pairs that rely on "knobs and holes". In addition, as described in Merchant et al., Nature Biotech. 16:677 (1998), these "knobs and hole" mutations can be combined with disulfide bonds to skew formation to heterodimerization.

[00247] An additional mechanism that finds use in the generation of heterodimers is sometimes referred to as "electrostatic steering" as described in Gunasekaran et al., J. Biol. Chem. 285(25):19637 (2010), hereby incorporated by reference in its entirety. This is sometimes referred to herein as "charge pairs". In this embodiment, electrostatics are used to skew the formation towards heterodimerization. As those in the art will appreciate, these may also have have an effect on pI, and thus on purification, and thus could in some cases also be considered pI variants. However, as these were generated to force heterodimerization and were not used as purification tools, they are classified as

"steric variants". These include, but are not limited to, D221E/P228E/L368E paired with D221R/P228R/K409R (e.g. these are "monomer corresponding sets) and C220E/P228E/368E paired with C220R/E224R/P228R/K409R.

[00248] Additional monomer A and monomer B variants that can be combined with other variants, optionally and independently in any amount, such as pI variants outlined herein or other steric variants that are shown in Figure 37 of US 2012/0149876, the figure and legend and SEQ ID NOs of which are incorporated expressly by reference herein.

[00249] In some embodiments, the steric variants outlined herein can be optionally and independently incorporated with any pI variant (or other variants such as Fc variants, FcRn variants, etc.) into one or both monomers, and can be independently and optionally included or excluded from the proteins of the invention.

pI (Isoelectric point) Variants for Heterodimers

[00250] In general, as will be appreciated by those in the art, there are two general categories of pI variants: those that increase the pI of the protein (basic changes) and those that decrease the pI of the protein (acidic changes). Increasing pI can be done by substituting basic amino acids (such as R and K) for neutral residues, substituting a neutral amino acid for an acidic one, or substituting an acidic amino acid with a basic one. Decreasing pI can be done similarly, by substituting acidic amino acids (such as D and E) for neutral residues, substituting a neutral amino acid for a basic one, or substituting a basic amino acid with an acidic one. As described herein, all combinations of these variants can be done: one monomer may be wild type, or a variant that does not display a significantly different pI from wild-type, and the other can be either more basic or more acidic. Alternatively, each monomer is changed, one to more basic and one to more acidic.

[00251] Preferred examples of isotypic pI variants are shown in .Figure 31, as well as in Figures 29, 30 and 31 from US Pub. 2014/0288275, incorporated herein by reference in their entirety (and the corresponding Figure legends).

Heavy Chain pI Changes

[00252] A number of pI variants are shown in Figures. As outlined herein and shown in the figures, these changes are shown relative to IgG1, but all isotypes can be altered this way, as well as isotype hybrids. In the case where the heavy chain constant domain is from IgG2-4, R133E and R133Q can also be used.

Antibody Heterodimers Light chain variants

[00253] In the case of antibody based heterodimers, e.g. where at least one of the monomers comprises a light chain in addition to the heavy chain domain, pI variants can also be made in the light chain. Amino acid substitutions for lowering the pI of the light chain include, but are not limited to, K126E, K126Q, K145E, K145Q, N152D, S156E, K169E, S202E, K207E and adding peptide DEDE at the c-terminus of the light chain. Changes in this category based on the constant lambda light chain include one or more substitutions at R108Q, Q124E, K126Q, N138D, K145T and Q199E. In addition, increasing the pI of the light chains can also be done.

Isotypic Variants

[00254] In addition, many embodiments of the invention rely on the "importation" of pI amino acids at particular positions from one IgG isotype into another, thus reducing or eliminating the possibility of unwanted immunogenicity being introduced into the variants. A number of these are shown in Figures 9 and 10. That is, IgG1 is a common isotype for therapeutic antibodies for a variety of reasons, including high effector function. However, the heavy constant region of IgG1 has a higher pI than that of IgG2 (8.10 versus 7.31). By introducing IgG2 residues at particular positions into the IgG1 backbone, the pI of the resulting monomer is lowered (or increased) and additionally exhibits longer serum half-life.

For example, IgG1 has a glycine (pI 5.97) at position 137, and IgG2 has a glutamic acid (pI 3.22); importing the glutamic acid will affect the pI of the resulting protein. As is described below, a number of amino acid substitutions are generally required to significant affect the pI of the variant antibody (from 1, 2, 3, 4, 5, 6, 7,8 , 9 and 10 amino acid changes all finding use herein). However, it should be noted as discussed below that even changes in IgG2 molecules allow for increased serum half-life. Preferred combinations of isotypic pI variants are shown in .Figure 31, as well as in Figures 29, 30 and 31 from US Pub. 2014/0288275, incorporated herein by reference in their entirety (and the corresponding Figure legends).

[00255] In other embodiments, non-isotypic amino acid changes are made, either to reduce the overall charge state of the resulting protein (e.g. by changing a higher pI amino acid to a lower pI amino acid), or to allow accommodations in structure for stability, etc. as is more further described below.

[00256] In addition, by pI engineering both the heavy and light constant domains, significant changes in each monomer of the heterodimer can be seen. As discussed herein, having the pIs of the two monomers differ by at least 0.5 can allow separation by ion exchange chromatography or isoelectric focusing, or other methods sensitive to isoelectric point.

Calculating pI

[0100] The pI of each monomer can depend on the pI of the variant heavy chain constant domain and the pI of the total monomer, including the variant heavy chain constant domain and the fusion partner. Thus, in some embodiments, the change in pI is calculated on the basis of the variant heavy chain constant domain, using the chart in Figure 24 of US Pub. 2014/0288275, incorporated herein by reference in its entirety (and the corresponding Figure legend). As discussed herein, which monomer to engineer is generally decided by the inherent pI of the Fv and scaffold regions. Alternatively, the pI of each monomer can be compared.

pI Variants that also confer better FcRn in vivo binding

[00257] In the case where the pI variant decreases the pI of the monomer, they can have the added benefit of improving serum retention in vivo.

[00258] Although still under examination, Fc regions are believed to have longer half-lives *in vivo*, because binding to FcRn at pH 6 in an endosome sequesters the Fc (Ghetie and Ward, 1997 Immunol Today. 18(12): 592-598, entirely incorporated by reference). The endosomal compartment then recycles the Fc to the cell surface. Once the compartment opens to the extracellular space, the higher pH, ~7.4, induces the release of Fc back into the blood. In mice, Dall' Acqua et al. showed that Fc mutants with increased FcRn binding at pH 6 and pH 7.4 actually had reduced serum concentrations and the same half life as wild-type Fc (Dall' Acqua et al. 2002, J. Immunol. 169:5171-5180, entirely incorporated by reference). The increased affinity of Fc for FcRn at pH 7.4 is thought to forbid the release of the Fc back into the blood. Therefore, the Fc mutations that will increase Fc's half-life *in vivo* will ideally increase FcRn binding at the lower pH while still allowing release of Fc at higher pH. The amino acid histidine changes its charge state in the pH range of 6.0 to 7.4. Therefore, it is not surprising to find His residues at important positions in the Fc/FcRn complex.

[00259] Recently it has been suggested that antibodies with variable regions that have lower isoelectric points may also have longer serum half-lives (Igawa et al., 2010 PEDS. 23(5): 385-392, entirely incorporated by reference). However, the mechanism of this is still poorly understood. Moreover, variable regions differ from antibody to antibody. Constant region variants with reduced pI and extended half-life would provide a more modular approach to improving the pharmacokinetic properties of antibodies, as described herein.

[00260] pI variants that find use in this embodiment, as well as their use for purification optimization, are disclosed in Figure 20.

Combination of Heterodimeric Variants

[00261] As will be appreciated by those in the art, all of the recited heterodimerization variants can be optionally and independently combined in any way, as long as they retain their "strandedness" or "monomer partition". In addition, all of these variants can be combined into any of the heterodimerization formats of Figure 1 to Figure 5.

[00262] In the case of pI variants, while embodiments finding particular use are shown in the Figures, other combinations can be generated, following the basic rule of altering the pI difference between two monomers to facilitate purification.

[00263] Additionally, any and all heterodimerization variants (including skew and pI variants) can also be independently and optionally combined with FcγR (ablation, high ADCC, etc.) and FcRn variants.

[00264] The trispecific heterodimeric antibodies of the invention include antigen binding domains (generally Fab or scFv domains) that bind to combination of target antigens.

Trispecific Heterodimeric formats

[00265] As are shown in the Figures, there are a number of suitable trispecific heterodimeric antibody formats, generally either tetravalent and trispecific, or trivalent and trispecific.

Target Antigens

[00266] The trispecific antibodies of the invention bind to three different target antigens.

[00267] CD3 Antigen Binding Domains

[00268] In many embodiments, one of the antigen binding domains of the invention binds to human CD3ε, an effector cell antigen, as is known in the art to be useful in bispecific formats. In this embodiment, the trispecific antibody has a single

antigen binding domain to CD3. In many embodiments, the anti-CD3 binding domain is a scFv, usually, but not always, with a charged scFv linker as is described herein.

[00269] There are a wide variety of known and useful anti-CD3 binding domains, including those of varying binding affinity as are shown in Figure 13 to Figure 18. Additional useful anti-CD3 pairs of sequences (variable heavy and variable light sequences) include, but are not limited to, H1.31_L1.47, H1.32_L1.47, H1.33_L1.47, H1.89_L1.47, H1.90_L1.47, H1_L1.4, H1.3_L1.4, H1.4_L1.4, H1.5_L1.4, H1.6_L1.4, H1.7_L1.4, H1.8_L1.4, H1.9_L1.4, H1.10_L1.4, H1.11_L1.4, H1.12_L1.4, H1.13_L1.4, H1.14_L1.4, H1.15_L1.4, H1.16_L1.4, H1.17_L1.4, H1.18_L1.4, H1.19_L1.4, H1.20_L1.4, H1.21_L1.4, H1.22_L1.4, H1.23_L1.4, H1.24_L1.4, H1.25_L1.4, H1.26_L1.4, H1.27_L1.4, H1.28_L1.4, H1.29_L1.30, H1_L1.10, H1_L1.11, H1_L1.12, H1_L1.13, H1_L1.14, H1_L1.15, H1_L1.16, H1_L1.17, H1_L1.18, H1_L1.19, H1_L1.20, H1_L1.21, H1_L1.22, H1_L1.23, H1_L1.24, H1_L1.25, H1_L1.26, H1_L1.27, H1_L1.28, H1_L1.29, H1_L3.1, H1_L5.1, H1_L1.31, H1_L1.32, H1.38_L1.4, H1_L1.33, H1_L1.34, H1_L1.35, H1_L1.36, H1_L1.37, H1_L1.38, H1_L1.39, H1.30_L1.4, H1.31_L1.4, H1.32_L1.4, H1.33_L1.4, H1.34_L1.4, H1.35_L1.4, H1.36_L1.4, H1.37_L1.4, H1.37_L1.32, H1_L1.40, H1.30_L1.40, H1.8_L1.40, H1.39_L1.40, H1.40_L1.40, H1.30_L1.41, H1.8_L1.41, H1.39_L1.41, H1.40_L1.41, H1.30_L1.42, H1.8_L1.42, H1.39_L1.42, H1.40_L1.42, H1.30_L1.43, H1.8_L1.43, H1.39_L1.43, H1.40_L1.43, H1.30_L1.44, H1.8_L1.44, H1.39_L1.44, H1.40_L1.44, H1.30_L1.45, H1.8_L1.45, H1.39_L1.45, H1.40_L1.45, H1.30_L1.46, H1.8_L1.46, H1.39_L1.46, H1.40_L1.46, H1.30_L1.47, H1.8_L1.47, H1.39_L1.47, H1.40_L1.47, as are shown in Figures 2 and 6 of US Pub. 2014/0288275, all of which are expressly incorporated by reference in their entirety herein.

[00270] CD8 Antigen Binding Domains

[00271] In some embodiments, one or two of the antigen binding domains of the invention binds to human CD8, an effector cell antigen. In many embodiments, the anti-CD8 binding domain is a scFv, usually, but not always, with a charged scFv linker as is described herein.

[00272] There are a wide variety of known and useful anti-CD8 binding domains, including those of varying binding affinity as are shown in Figure 25 to Figure 28. Figure 13 Additional useful anti-CD8 pairs of sequences (variable heavy and variable light sequences) include, but are not limited to, those depicted in Figure 43, or other known anti-CD8 sequences.

CD20 Antigen Binding Domains

[00273] In some embodiments, one or two of the antigen binding domains of the invention binds to human CD20, a tumor antigen. As outlined herein, the anti-CD38 antigen binding domain(s) can be either Fabs or scFvs, or both. In addition, when a single CD20 antigen binding domain is used, some embodiments utilize a CD3 and a CD8 binding domain. When two CD20 antigen binding domains are used, the third antigen binding domain can bind either CD3 or CD8.

[00274] There are a wide variety of known and useful anti-CD20 binding domains, including those of varying binding affinity as are shown in Figure 22 and Figure 23.

[00275] CD38 Antigen Binding Domains

[00276] In some embodiments, one or two of the antigen binding domains of the invention binds to human CD38, a target tumor cell antigen. As outlined herein, the anti-CD38 antigen binding domain(s) can be either Fabs or scFvs, or both. In addition, when a single CD38 antigen binding domain is used, some embodiments utilize a CD3 and a CD8 binding domain. When two CD38 antigen binding domains are used, the third antigen binding domain can bind either CD3 or CD8.

[00277] There are a wide variety of known and useful anti-CD38 binding domains, including those of varying binding affinity as are shown in Figure 19 to Figure 21Figure 25.

[00278] CD123 Antigen Binding Domains

[00279] In some embodiments, one or two of the antigen binding domains of the invention binds to human CD123, a target tumor cell antigen. As outlined herein, the anti-CD123 antigen binding domain(s) can be either Fabs or scFvs, or both. In addition, when a single CD123 antigen binding domain is used, some embodiments utilize a CD3 and a CD8 binding domain. When two CD123 antigen binding domains are used, the third antigen binding domain can bind either CD3 or CD8.

[00280] There are a wide variety of known and useful anti-CD123 binding domains, including those of varying binding affinity as are shown in Figure 24 and Figure 45 and Figure 46.

[00281] PMSA Antigen Binding Domains

[00282] In some embodiments, one or two of the antigen binding domains of the invention binds to human prostate membrane specific antigen (PMSA) a target tumor cell antigen. As outlined herein, the anti-PSMA antigen binding domain(s) can be either Fabs or scFvs, or both. In addition, when a single PSMA antigen binding domain is used, some embodiments utilize a CD3 and a CD8 binding domain. When two PSMA antigen binding domains are used, the third antigen binding domain can bind either CD3 or CD8.

[00283] There are a wide variety of known and useful anti-CD38 binding domains, including those of varying binding affinity as are shown in Figure 51.

[00284] Target Tumor Antigens

[00285] The trispecific antibodies of the invention have three different antigen binding domains that bind three different antigens.

[00286] Suitable target tumor antigens include, but are not limited to,CD20, CD38, CD123; ROR1, ROR2, BCMA; PSMA; SSTR2; SSTR5, CD19, FLT3, CD33, PSCA, ADAM 17, CEA, Her2, EGFR, EGFR-vIII, CD30, FOLR1, GD-2, CA-IX, Trop-2,

CD70, CD38, mesothelin, EphA2, CD22, CD79b, GPNMB, CD56, CD138, CD52, CD74, CD30, CD123, RON, ERBB2, and EGFR.

[00287] Particular suitable applications of the heterodimeric antibodies herein are co-target pairs for which it is beneficial or critical to engage each target antigen monovalently. Such antigens may be, for example, immune receptors that are activated upon immune complexation. Cellular activation of many immune receptors occurs only by cross-linking, acheived typically by antibody/antigen immune complexes, or via effector cell to target cell engagement. For some immune receptors, for example the CD3 signaling receptor on T cells, activation only upon engagement with co-engaged target is critical, as nonspecifiic cross-linking in a clinical setting can elicit a cytokine storm and toxicity. Therapeutically, by engaging such antigens monovalently rather than multivalently, using the immunoglobulins herein, such activation occurs only in response to cross-linking only in the microenvironment of the primary target antigen. The ability to target three different antigens with different valencies is a novel and useful aspect of the present invention. Examples of target antigens for which it may be therapeutically beneficial or necessary to co-engage monovalently include but are not limited to immune activating receptors such as CD3, FcγRs, toll-like receptors (TLRs) such as TLR4 and TLR9, cytokine, chemokine, cytokine receptors, and chemokine receptors. In many embodiments, one of the antigen binding sites binds to CD3, and in some embodiments it is the scFv-containing monomer.

[00288] Virtually any antigen may be targeted by the immunoglobulins herein, including but not limited to proteins, subunits, domains, motifs, and/or epitopes belonging to the following list of target antigens, which includes both soluble factors such as cytokines and membrane-bound factors, including transmembrane receptors: 17-IA, 4-1BB, 4Dc, 6-keto-PGF1a, 8-iso-PGF2a, 8-oxo-dG, A1 Adenosine Receptor, A33, ACE, ACE-2, Activin, Activin A, Activin AB, Activin B, Activin C,

Activin RIA, Activin RIA ALK-2, Activin RIB ALK-4, Activin RIIA, Activin RIIB, ADAM, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADAM8, ADAM9, ADAMTS, ADAMTS4, ADAMTS5, Addressins, aFGF, ALCAM, ALK, ALK-1, ALK-7, alpha-1-antitrypsin, alpha-V/beta-1 antagonist, ANG, Ang, APAF-1, APE, APJ, APP, APRIL, AR, ARC, ART, Artemin, anti-Id, ASPARTIC, Atrial natriuretic factor, av/b3 integrin, Axl, b2M, B7-1, B7-2, B7-H, B-lymphocyte Stimulator (BlyS), BACE, BACE-1, Bad, BAFF, BAFF-R, Bag-1, BAK, Bax, BCA-1, BCAM, Bcl, BCMA, BDNF, b-ECGF, bFGF, BID, Bik, BIM, BLC, BL-CAM, BLK, BMP, BMP-2 BMP-2a, BMP-3 Osteogenin, BMP-4 BMP-2b, BMP-5, BMP-6 Vgr-1, BMP-7 (OP-1), BMP-8 (BMP-8a, OP-2), BMPR, BMPR-IA (ALK-3), BMPR-IB (ALK-6), BRK-2, RPK-1, BMPR-II (BRK-3), BMPs, b-NGF, BOK, Bombesin, Bone-derived neurotrophic factor, BPDE, BPDE-DNA, BTC, complement factor 3 (C3), C3a, C4, C5, C5a, C10, CA125, CAD-8, Calcitonin, cAMP, carcinoembryonic antigen (CEA), carcinoma-associated antigen, Cathepsin A, Cathepsin B, Cathepsin C/DPPI, Cathepsin D, Cathepsin E, Cathepsin H, Cathepsin L, Cathepsin O, Cathepsin S, Cathepsin V, Cathepsin X/Z/P, CBL, CCI, CCK2, CCL, CCL1, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/10, CCR, CCR1, CCR10, CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CD1, CD2, CD3, CD3E, CD4, CD5, CD6, CD7, CD8, CD10, CD11a, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD25, CD27L, CD28, CD29, CD30, CD30L, CD32, CD33 (p67 proteins), CD34, CD38, CD40, CD40L, CD44, CD45, CD46, CD49a, CD52, CD54, CD55, CD56, CD61, CD64, CD66e, CD74, CD80 (B7-1), CD89, CD95, CD123, CD137, CD138, CD140a, CD146, CD147, CD148, CD152, CD164, CEACAM5, CFTR, cGMP, CINC, Clostridium botulinum toxin, Clostridium perfringens toxin, CKb8-1, CLC, CMV, CMV UL, CNTF, CNTN-1, COX, C-Ret, CRG-2, CT-1, CTACK, CTGF, CTLA-4, CX3CL1, CX3CR1, CXCL, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12,

CXCL13, CXCL14, CXCL15, CXCL16, CXCR, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, cytokeratin tumor-associated antigen, DAN, DCC, DcR3, DC-SIGN, Decay accelerating factor, des(1-3)-IGF-I (brain IGF-1), Dhh, digoxin, DNAM-1, Dnase, Dpp, DPPIV/CD26, Dtk, ECAD, EDA, EDA-A1, EDA-A2, EDAR, EGF, EGFR (ErbB-1), EMA, EMMPRIN, ENA, endothelin receptor, Enkephalinase, eNOS, Eot, eotaxin1, EpCAM, Ephrin B2/ EphB4, EPO, ERCC, E-selectin, ET-1, Factor IIa, Factor VII, Factor VIIIc, Factor IX, fibroblast activation protein (FAP), Fas, FcR1, FEN-1, Ferritin, FGF, FGF-19, FGF-2, FGF3, FGF-8, FGFR, FGFR-3, Fibrin, FL, FLIP, Flt-3, Flt-4, Follicle stimulating hormone, Fractalkine, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, FZD10, G250, Gas 6, GCP-2, GCSF, GD2, GD3, GDF, GDF-1, GDF-3 (Vgr-2), GDF-5 (BMP-14, CDMP-1), GDF-6 (BMP-13, CDMP-2), GDF-7 (BMP-12, CDMP-3), GDF-8 (Myostatin), GDF-9, GDF-15 (MIC-1), GDNF, GDNF, GFAP, GFRa-1, GFR-alpha1, GFR-alpha2, GFR-alpha3, GITR, Glucagon, Glut 4, glycoprotein IIb/IIIa (GP IIb/IIIa), GM-CSF, gp130, gp72, GRO, Growth hormone releasing factor, Hapten (NP-cap or NIP-cap), HB-EGF, HCC, HCMV gB envelope glycoprotein, HCMV) gH envelope glycoprotein, HCMV UL, Hemopoietic growth factor (HGF), Hep B gp120, heparanase, Her2, Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), herpes simplex virus (HSV) gB glycoprotein, HSV gD glycoprotein, HGFA, High molecular weight melanoma-associated antigen (HMW-MAA), HIV gp120, HIV IIIB gp 120 V3 loop, HLA, HLA-DR, HM1.24, HMFG PEM, HRG, Hrk, human cardiac myosin, human cytomegalovirus (HCMV), human growth hormone (HGH), HVEM, I-309, IAP, ICAM, ICAM-1, ICAM-3, ICE, ICOS, IFNg, Ig, IgA receptor, IgE, IGF, IGF binding proteins, IGF-1R, IGFBP, IGF-I, IGF-II, IL, IL-1, IL-1R, IL-2, IL-2R, IL-4, IL-4R, IL-5, IL-5R, IL-6, IL-6R, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-18, IL-18R, IL-23, interferon (INF)-alpha, INF-beta, INF-gamma, Inhibin, iNOS, Insulin A-chain, Insulin B-chain, Insulin-like growth factor 1, integrin alpha2, integrin alpha3, integrin alpha4, integrin alpha4/beta1, integrin alpha4/beta7, integrin alpha5 (alphaV), integrin alpha5/beta1, integrin alpha5/beta3, integrin

alpha6, integrin beta1, integrin beta2, interferon gamma, IP-10, I-TAC, JE, Kallikrein 2, Kallikrein 5, Kallikrein 6, , Kallikrein 11, Kallikrein 12, Kallikrein 14, Kallikrein 15, Kallikrein L1, Kallikrein L2, Kallikrein L3, Kallikrein L4, KC, KDR, Keratinocyte Growth Factor (KGF), laminin 5, LAMP, LAP, LAP (TGF- 1), Latent TGF-1, Latent TGF-1 bp1, LBP, LDGF, LECT2, Lefty, Lewis-Y antigen, Lewis-Y related antigen, LFA-1, LFA-3, Lfo, LIF, LIGHT, lipoproteins, LIX, LKN, Lptn, L-Selectin, LT-a, LT-b, LTB4, LTBP-1, Lung surfactant, Luteinizing hormone, Lymphotoxin Beta Receptor, Mac-1, MAdCAM, MAG, MAP2, MARC, MCAM, MCAM, MCK-2, MCP, M-CSF, MDC, Mer, METALLOPROTEASES, MGDF receptor, MGMT, MHC (HLA-DR), MIF, MIG, MIP, MIP-1-alpha, MK, MMAC1, MMP, MMP-1, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-2, MMP-24, MMP-3, MMP-7, MMP-8, MMP-9, MPIF, Mpo, MSK, MSP, mucin (Muc1), MUC18, Muellerian-inhibitin substance, Mug, MuSK, NAIP, NAP, NCAD, N-Cadherin, NCA 90, NCAM, NCAM, Neprilysin, Neurotrophin-3,-4, or -6, Neurturin, Neuronal growth factor (NGF), NGFR, NGF-beta, nNOS, NO, NOS, Npn, NRG-3, NT, NTN, OB, OGG1, OPG, OPN, OSM, OX40L, OX40R, p150, p95, PADPr, Parathyroid hormone, PARC, PARP, PBR, PBSF, PCAD, P-Cadherin, PCNA, PDGF, PDGF, PDK-1, PECAM, PEM, PF4, PGE, PGF, PGI2, PGJ2, PIN, PLA2, placental alkaline phosphatase (PLAP), PlGF, PLP, PP14, Proinsulin, Prorelaxin, Protein C, PS, PSA, PSCA, prostate specific membrane antigen (PSMA), PTEN, PTHrp, Ptk, PTN, R51, RANK, RANKL, RANTES, RANTES, Relaxin A-chain, Relaxin B-chain, renin, respiratory syncytial virus (RSV) F, RSV Fgp, Ret, Rheumatoid factors, RLIP76, RPA2, RSK, S100, SCF/KL, SDF-1, SERINE, Serum albumin, sFRP-3, Shh, SIGIRR, SK-1, SLAM, SLPI, SMAC, SMDF, SMOH, SOD, SPARC, Stat, STEAP, STEAP-II, TACE, TACI, TAG-72 (tumor-associated glycoprotein-72), TARC, TCA-3, T-cell receptors (e.g., T-cell receptor alpha/beta), TdT, TECK, TEM1, TEM5, TEM7, TEM8, TERT, testicular PLAP-like alkaline phosphatase, TfR, TGF, TGF-alpha, TGF-beta, TGF-beta Pan Specific, TGF-beta RI (ALK-5), TGF-beta RII, TGF-beta RIIb, TGF-beta RIII, TGF-beta1, TGF-beta2, TGF-

beta3, TGF-beta4, TGF-beta5, Thrombin, Thymus Ck-1, Thyroid stimulating hormone, Tie, TIMP, TIQ, Tissue Factor, TMEFF2, Tmpo, TMPRSS2, TNF, TNF-alpha, TNF-alpha beta, TNF-beta2, TNFc, TNF-RI, TNF-RII, TNFRSF10A (TRAIL R1 Apo-2, DR4), TNFRSF10B (TRAIL R2 DR5, KILLER, TRICK-2A, TRICK-B), TNFRSF10C (TRAIL R3 DcR1, LIT, TRID), TNFRSF10D (TRAIL R4 DcR2, TRUNDD), TNFRSF11A (RANK ODF R, TRANCE R), TNFRSF11B (OPG OCIF, TR1), TNFRSF12 (TWEAK R FN14), TNFRSF13B (TACI), TNFRSF13C (BAFF R), TNFRSF14 (HVEM ATAR, HveA, LIGHT R, TR2), TNFRSF16 (NGFR p75NTR), TNFRSF17 (BCMA), TNFRSF18 (GITR AITR), TNFRSF19 (TROY TAJ, TRADE), TNFRSF19L (RELT), TNFRSF1A (TNF RI CD120a, p55-60), TNFRSF1B (TNF RII CD120b, p75-80), TNFRSF26 (TNFRH3), TNFRSF3 (LTbR TNF RIII, TNFC R), TNFRSF4 (OX40 ACT35, TXGP1 R), TNFRSF5 (CD40 p50), TNFRSF6 (Fas Apo-1, APT1, CD95), TNFRSF6B (DcR3 M68, TR6), TNFRSF7 (CD27), TNFRSF8 (CD30), TNFRSF9 (4-1BB CD137, ILA), TNFRSF21 (DR6), TNFRSF22 (DcTRAIL R2 TNFRH2), TNFRST23 (DcTRAIL R1 TNFRH1), TNFRSF25 (DR3 Apo-3, LARD, TR-3, TRAMP, WSL-1), TNFSF10 (TRAIL Apo-2 Ligand, TL2), TNFSF11 (TRANCE/RANK Ligand ODF, OPG Ligand), TNFSF12 (TWEAK Apo-3 Ligand, DR3 Ligand), TNFSF13 (APRIL TALL2), TNFSF13B (BAFF BLYS, TALL1, THANK, TNFSF20), TNFSF14 (LIGHT HVEM Ligand, LTg), TNFSF15 (TL1A/VEGI), TNFSF18 (GITR Ligand AITR Ligand, TL6), TNFSF1A (TNF-a Conectin, DIF, TNFSF2), TNFSF1B (TNF-b LTa, TNFSF1), TNFSF3 (LTb TNFC, p33), TNFSF4 (OX40 Ligand gp34, TXGP1), TNFSF5 (CD40 Ligand CD154, gp39, HIGM1, IMD3, TRAP), TNFSF6 (Fas Ligand Apo-1 Ligand, APT1 Ligand), TNFSF7 (CD27 Ligand CD70), TNFSF8 (CD30 Ligand CD153), TNFSF9 (4-1BB Ligand CD137 Ligand), TP-1, t-PA, Tpo, TRAIL, TRAIL R, TRAIL-R1, TRAIL-R2, TRANCE, transferring receptor, TRF, Trk, TROP-2, TSG, TSLP, tumor-associated antigen CA 125, tumor-associated antigen expressing Lewis Y related carbohydrate, TWEAK, TXB2, Ung, uPAR, uPAR-1, Urokinase, VCAM, VCAM-1, VECAD, VE-Cadherin, VE-cadherin-2, VEFGR-1 (flt-1), VEGF, VEGFR, VEGFR-3 (flt-4), VEGI,

VIM, Viral antigens, VLA, VLA-1, VLA-4, VNR integrin, von Willebrands factor, WIF-1, WNT1, WNT2, WNT2B/13, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9A, WNT9B, WNT10A, WNT10B, WNT11, WNT16, XCL1, XCL2, XCR1, XCR1, XEDAR, XIAP, XPD, and receptors for hormones and growth factors.

[00289] Exemplary antigens that may be targeted specifically by the immunoglobulins of the invention include but are not limited to: CD20, CD19, Her2, EGFR, EpCAM, CD3, FcγRIIIa (CD16), FcγRIIa (CD32a), FcγRIIb (CD32b), FcγRI (CD64), Toll-like receptors (TLRs) such as TLR4 and TLR9, cytokines such as IL-2, IL-5, IL-13, IL-12, IL-23, and TNFα, cytokine receptors such as IL-2R, chemokines, chemokine receptors, growth factors such as VEGF and HGF, and the like. . To form the trispecific antibodies of the invention, antibodies to any combination of these antigens can be made; that is, each of these antigens can be optionally and independently included or excluded from a trispecific antibody according to the present invention.

[00290] Particularly preferred combinations for trispecific antibodies are an antigen-binding domain to CD3 and an antigen binding domain selected from CD19, CD20, CD38 and CD123.

Nucleic acids of the Invention

[00291] The invention further provides nucleic acid compositions encoding the trispecific antibodies of the invention. As will be appreciated by those in the art, the nucleic acid compositions will depend on the format and scaffold of the heterodimeric protein. For example, most of the formats outlined in Figures 1 to 5 use three amino acid sequences. For example, Figure 1A uses a first nucleic acid encoding a first monomer comprising a heavy chain and a scFv, a second nucleic acid encoding a second amino acid monomer comprising a heavy chain and an scFv, and a third nucleic acid encoding a light chain. These three nucleic acid sequences

can be incorporated into one or more expression vectors for expression. Similarly, some formats (e.g. Figures 2E) only two nucleic acids are needed; again, they can be put into one or two expression vectors.

[00292] As is known in the art, the nucleic acids encoding the components of the invention can be incorporated into expression vectors as is known in the art, and depending on the host cells used to produce the heterodimeric antibodies of the invention. Generally the nucleic acids are operably linked to any number of regulatory elements (promoters, origin of replication, selectable markers, ribosomal binding sites, inducers, etc.). The expression vectors can be extra-chromosomal or integrating vectors.

[00293] The nucleic acids and/or expression vectors of the invention are then transformed into any number of different types of host cells as is well known in the art, including mammalian, bacterial, yeast, insect and/or fungal cells, with mammalian cells (e.g. CHO cells), finding use in many embodiments.

[00294] In some embodiments, nucleic acids encoding each monomer and the optional nucleic acid encoding a light chain, as applicable depending on the format, are each contained within a single expression vector, generally under different or the same promoter controls. In embodiments of particular use in the present invention, each of these two or three nucleic acids are contained on a different expression vector. As shown herein and in PCT/US2015/23411, hereby incorporated by reference, different vector ratios can be used to drive heterodimer formation. That is, surprisingly, while the proteins comprise first monomer:second monomer:light chains (in the case of many of the embodiments herein that have three polypeptides comprising the heterodimeric antibody) in a 1:1:2 ratio, these are not the ratios that give the best results.

[00295] The heterodimeric antibodies of the invention are made by culturing host cells comprising the expression vector(s) as is well known in the art. Once produced, traditional antibody purification steps are done, including an ion exchange chromotography step. As discussed herein, having the pIs of the two monomers differ by at least 0.5 can allow separation by ion exchange chromatography or isoelectric focusing, or other methods sensitive to isoelectric point. That is, the inclusion of pI substitutions that alter the isoelectric

point (pI) of each monomer so that such that each monomer has a different pI and the heterodimer also has a distinct pI, thus facilitating isoelectric purification of the heterodimer (e.g., anionic exchange columns, cationic exchange columns). These substitutions also aid in the determination and monitoring of any contaminating homodimeric monomers post-purification (e.g., IEF gels, cIEF, and analytical IEX columns).

Treatments

[00296] Once made, the compositions of the invention find use in a number of applications. CD20, CD38 and CD123 are all unregulated in many hematopoeitic malignancies and in cell lines derived from various hematopoietic malignancies, accordingly, the heterodimeric antibodies of the invention find use in treating cancer, including but not limited to, all B cell lymphomas and leukemias, including but not limited to non-Hodgkin's lymphoma (NHL), Burkitt's lymphoma (BL), multiple myeloma (MM), B chronic lymphocytic leukemia (B-CLL), B and T acute lymphocytic leukemia (ALL), T cell lymphoma (TCL), acute myeloid leukemia (AML), hairy cell leukemia (HCL), Hodgkin's Lymphoma (HL), chronic lymphocytic leukemia (CLL), non-Hodgkin's lymphoma, and chronic myeloid leukemia (CML).

[00297] Accordingly, the heterodimeric compositions of the invention find use in the treatment of these cancers.

Antibody Compositions for In Vivo Administration

[00298] Formulations of the antibodies used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride;

benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[00299] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to provide antibodies with other specifcities. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine, growth inhibitory agent and/or small molecule antagonist. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[00300] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[00301] The formulations to be used for in vivo administration should be sterile, or nearly so. This is readily accomplished by filtration through sterile filtration membranes.

[00302] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and .gamma. ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

[00303] When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37oC, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S--S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Administrative modalities

[00304] The antibodies and chemotherapeutic agents of the invention are administered to a subject, in accord with known methods, such as intravenous

administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous or subcutaneous administration of the antibody is preferred.

Treatment modalities

[00305] In the methods of the invention, therapy is used to provide a positive therapeutic response with respect to a disease or condition. By "positive therapeutic response" is intended an improvement in the disease or condition, and/or an improvement in the symptoms associated with the disease or condition. For example, a positive therapeutic response would refer to one or more of the following improvements in the disease: (1) a reduction in the number of neoplastic cells; (2) an increase in neoplastic cell death; (3) inhibition of neoplastic cell survival; (5) inhibition (i.e., slowing to some extent, preferably halting) of tumor growth; (6) an increased patient survival rate; and (7) some relief from one or more symptoms associated with the disease or condition.

[00306] Positive therapeutic responses in any given disease or condition can be determined by standardized response criteria specific to that disease or condition. Tumor response can be assessed for changes in tumor morphology (i.e., overall tumor burden, tumor size, and the like) using screening techniques such as magnetic resonance imaging (MRI) scan, x-radiographic imaging, computed tomographic (CT) scan, bone scan imaging, endoscopy, and tumor biopsy sampling including bone marrow aspiration (BMA) and counting of tumor cells in the circulation.

[00307] In addition to these positive therapeutic responses, the subject undergoing therapy may experience the beneficial effect of an improvement in the symptoms associated with the disease.

[00308] Thus for B cell tumors, the subject may experience a decrease in the so-called B symptoms, i.e., night sweats, fever, weight loss, and/or urticaria. For pre-

malignant conditions, therapy with an trispecific therapeutic agent may block and/or prolong the time before development of a related malignant condition, for example, development of multiple myeloma in subjects suffering from monoclonal gammopathy of undetermined significance (MGUS).

[00309] An improvement in the disease may be characterized as a complete response. By "complete response" is intended an absence of clinically detectable disease with normalization of any previously abnormal radiographic studies, bone marrow, and cerebrospinal fluid (CSF) or abnormal monoclonal protein in the case of myeloma.

[00310] Such a response may persist for at least 4 to 8 weeks, or sometimes 6 to 8 weeks, following treatment according to the methods of the invention. Alternatively, an improvement in the disease may be categorized as being a partial response. By "partial response" is intended at least about a 50% decrease in all measurable tumor burden (i.e., the number of malignant cells present in the subject, or the measured bulk of tumor masses or the quantity of abnormal monoclonal protein) in the absence of new lesions, which may persist for 4 to 8 weeks, or 6 to 8 weeks.

[00311] Treatment according to the present invention includes a "therapeutically effective amount" of the medicaments used. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result.

[00312] A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the medicaments to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects.

[00313] A "therapeutically effective amount" for tumor therapy may also be measured by its ability to stabilize the progression of disease. The ability of a compound to inhibit cancer may be evaluated in an animal model system predictive of efficacy in human tumors.

[00314] Alternatively, this property of a composition may be evaluated by examining the ability of the compound to inhibit cell growth or to induce apoptosis by in vitro assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound may decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

[00315] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[00316] The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[00317] The efficient dosages and the dosage regimens for the trispecific antibodies used in the present invention depend on the disease or condition to be treated and may be determined by the persons skilled in the art.

[00318] An exemplary, non-limiting range for a therapeutically effective amount of an trispecific antibody used in the present invention is about 0.1-100 mg/kg, such as about 0.1-50 mg/kg, for example about 0.1-20 mg/kg, such as about 0.1-10 mg/kg, for instance about 0.5, about such as 0.3, about 1, or about 3 mg/kg. In another embodiment, he antibody is administered in a dose of 1 mg/kg or more, such as a dose of from 1 to 20 mg/kg, e.g. a dose of from 5 to 20 mg/kg, e.g. a dose of 8 mg/kg.

[00319] A medical professional having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, a physician or a veterinarian could start doses of the medicament employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[00320] In one embodiment, the trispecific antibody is administered by infusion in a weekly dosage of from 10 to 500 mg/kg such as of from 200 to 400 mg/kg Such administration may be repeated, e.g., 1 to 8 times, such as 3 to 5 times. The administration may be performed by continuous infusion over a period of from 2 to 24 hours, such as of from 2 to 12 hours.

[00321] In one embodiment, the trispecific antibody is administered by slow continuous infusion over a long period, such as more than 24 hours, if required to reduce side effects including toxicity.

[00322] In one embodiment the trispecific antibody is administered in a weekly dosage of from 250 mg to 2000 mg, such as for example 300 mg, 500 mg, 700 mg, 1000 mg, 1500 mg or 2000 mg, for up to 8 times, such as from 4 to 6 times. The

administration may be performed by continuous infusion over a period of from 2 to 24 hours, such as of from 2 to 12 hours. Such regimen may be repeated one or more times as necessary, for example, after 6 months or 12 months. The dosage may be determined or adjusted by measuring the amount of compound of the present invention in the blood upon administration by for instance taking out a biological sample and using anti-idiotypic antibodies which target the antigen binding region of the trispecific antibody.

[00323] In a further embodiment, the trispecific antibody is administered once weekly for 2 to 12 weeks, such as for 3 to 10 weeks, such as for 4 to 8 weeks.

[00324] In one embodiment, the trispecific antibody is administered by maintenance therapy, such as, e.g., once a week for a period of 6 months or more.

[00325] In one embodiment, the trispecific antibody is administered by a regimen including one infusion of an trispecific antibody followed by an infusion of an trispecific antibody conjugated to a radioisotope. The regimen may be repeated, e.g., 7 to 9 days later.

[00326] As non-limiting examples, treatment according to the present invention may be provided as a daily dosage of an antibody in an amount of about 0.1-100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 after initiation of treatment, or any combination thereof, using single or divided doses of every 24, 12, 8, 6, 4, or 2 hours, or any combination thereof.

[00327] In some embodiments the trispecific antibody molecule thereof is used in combination with one or more additional therapeutic agents, e.g. a chemotherapeutic agent. Non-limiting examples of DNA damaging

chemotherapeutic agents include topoisomerase I inhibitors (e.g., irinotecan, topotecan, camptothecin and analogs or metabolites thereof, and doxorubicin); topoisomerase II inhibitors (e.g., etoposide, teniposide, and daunorubicin); alkylating agents (e.g., melphalan, chlorambucil, busulfan, thiotepa, ifosfamide, carmustine, lomustine, semustine, streptozocin, decarbazine, methotrexate, mitomycin C, and cyclophosphamide); DNA intercalators (e.g., cisplatin, oxaliplatin, and carboplatin); DNA intercalators and free radical generators such as bleomycin; and nucleoside mimetics (e.g., 5-fluorouracil, capecitibine, gemcitabine, fludarabine, cytarabine, mercaptopurine, thioguanine, pentostatin, and hydroxyurea).

[00328] Chemotherapeutic agents that disrupt cell replication include: paclitaxel, docetaxel, and related analogs; vincristine, vinblastin, and related analogs; thalidomide, lenalidomide, and related analogs (e.g., CC-5013 and CC-4047); protein tyrosine kinase inhibitors (e.g., imatinib mesylate and gefitinib); proteasome inhibitors (e.g., bortezomib); NF-κB inhibitors, including inhibitors of IκB kinase; antibodies which bind to proteins overexpressed in cancers and thereby downregulate cell replication (e.g., trastuzumab, rituximab, cetuximab, and bevacizumab); and other inhibitors of proteins or enzymes known to be upregulated, over-expressed or activated in cancers, the inhibition of which downregulates cell replication.

[00329] In some embodiments, the antibodies of the invention can be used prior to, concurrent with, or after treatment with Velcade® (bortezomib).

[00330] All cited references are herein expressly incorporated by reference in their entirety.

[00331] Whereas particular embodiments of the invention have been described above for purposes of illustration, it will be appreciated by those skilled in the art that numerous variations of the details may be made without departing from the invention as described in the appended claims.

EXAMPLES

[00332] Examples are provided below to illustrate the present invention. These examples are not meant to constrain the present invention to any particular application or theory of operation. For all constant region positions discussed in the present invention, numbering is according to the EU index as in Kabat (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda, entirely incorporated by reference). Those skilled in the art of antibodies will appreciate that this convention consists of nonsequential numbering in specific regions of an immunoglobulin sequence, enabling a normalized reference to conserved positions in immunoglobulin families. Accordingly, the positions of any given immunoglobulin as defined by the EU index will not necessarily correspond to its sequential sequence.

[00333] As for all the heterodimeric antibodies herein, genes encoding the heavy and light chains of the antibodies can be constructed in the mammalian expression vector pTT5. The human IgG1 constant chain gene can be obtained from IMAGE clones and subcloned into the pTT5 vector. VH and VL genes encoding the anti-VEGF antibodies can be synthesized commercially (Blue Heron Biotechnologies, Bothell WA), and subcloned into the vectors encoding the appropriate CL and IgG1 constant chains. Amino acid modifications can be constructed using site-directed mutagenesis using the QuikChange® site-directed mutagenesis methods (Stratagene, La Jolla CA). All DNA will be sequenced to confirm the fidelity of the sequences.

[00334] Plasmids containing heavy chain gene (VH-Cγ1-Cγ2-Cγ3) can be co-transfected with plasmid containing light chain gene (VL-Cκ) into 293E cells using llipofectamine (Invitrogen, Carlsbad CA) and grown in FreeStyle 293 media (Invitrogen, Carlsbad CA). After 5 days of growth, the antibodies can be purified

from the culture supernatant by protein A affinity using the MabSelect resin (GE Healthcare). Antibody concentrations can be determined by bicinchoninic acid (BCA) assay (Pierce).

[00335] The pI engineered mAbs can be generally characterized by SDS PAGE on an Agilent Bioanalyzer, by size exclusion chromatography (SEC), isoelectric focusing (IEF) gel electrophoresis, binding to antigen by Biacore, and differential scanning calorimetry (DSC). All mAbs can show high purity on SDS-PAGE and SEC. IEF gels to indicate that each variant has the designed isoelectric point. Generally the binding analysis on Biacore will show that pI engineered variants bind to antigen with similar affinity as the parent antibodies, to indicate that the designed substitutions do not perturb the function of the mAb. DSC can be run to show which variants generally have high thermostability.

[00336] Pharmacokinetic experiments for serum half life as appropriate can be performed in B6 mice that are homozygous knock-outs for murine FcRn and heterozygous knock-ins of human FcRn (mFcRn-/-, hFcRn+) (Petkova et al., 2006, Int Immunol 18(12):1759-69, entirely incorporated by reference), herein referred to as hFcRn or hFcRn+ mice.

[00337] A single, intravenous tail vein injection of antibody (2 mg/kg) can be given to groups of 4-7 female mice randomized by body weight (20-30g range). Blood (~50ul) is drawn from the orbital plexus at each time point, processed to serum, and stored at -80°C until analysis. Antibody concentrations are determined using an ELISA assay. Serum concentration of antibody is measured using recombinant antigen as capture reagent, and detection is carried out with biotinylated anti-human kappa antibody and europium-labeled streptavidin. The time resolved fluorescence signal is collected. PK parameters are determined for individual mice with a non-compartmental model using WinNonLin (Pharsight Inc,

Mountain View CA). Nominal times and dose are used with uniform weighing of points.

[00338] Purifying mixtures of antibody variants with modified isolectric points.

[00339] Variants are first purified by Protein A, and then loaded onto a GE Healthcare HiTrap SP HP cation exchange column in 50 mM MES (pH 6.0) and eluted with an NaCl gradient. Following elution, fractions from each peak are loaded onto a Lonza IsoGel IEF plate (pH range 7-11) for analysis. Separation of the middle pI heterodimer is achieved in each case, with separation improved when the heterodimer has a larger difference in pI from the homodimers.

[00340] Differential scanning fluorimetry (DSF) can be used to evaluate the stability of antibodies containing isosteric pI substitutions. DSF experiments are performed using a Bio-Rad CFX Connect Real-Time PCR Detection System. Proteins are mixed with SYPRO Orange fluorescent dye and diluted to 0.25 or 0.50 mg/mL in PBS. The final concentration of SYPRO Orange is 10X. After an initial 10 minute incubation period at 25°C, proteins are heated from 25 to 95°C using a heating rate of 1°C/min. A fluorescence measurement waiss taken every 30 sec. Melting temperatures are calculated using the instrument software.

[00341] Example: Trispecifics Production

[00342] Amino acid sequences for anti-CD20 x anti-CD3 x anti-CD8 trispecifics are listed in Figure B. DNA encoding the three chains needed for trispecific expression were generated by gene synthesis (Blue Heron Biotechnology, Bothell, Wash.) and were subcloned using standard molecular biology techniques into the expression vector pTT5. Substitutions were introduced using either site-directed mutagenesis (QuikChange, Stratagene, Cedar Creek, Tex.) or additional gene synthesis and subcloning. DNA was transfected into HEK293E cells for expression and resulting proteins were purified from the supernatant using protein A affinity (GE Healthcare) and cation exchange chromatography. Cation exchange chromatography purification was performed using a HiTrap SP HP column (GE

Healthcare) with a wash/equilibration buffer of 50 mM MES, pH 6.0 and an elution buffer of 50 mM MES, pH 6.0 + 1 M NaCl linear gradient.

[00343] Cell surface binding

[00344] Anti-CD20 x anti-CD3 x anti-CD8 trispecifics were characterized in vitro for differential cell surface binding to purified human T cells (Figure C). XENP18953 exhibited preferential binding to $CD8^+$ T cells over $CD4^+$ T cells. Assay details are indicated in the figure legend.

[00345] Redirected T Cell Cytotoxicity

[00346] Anti-CD20 x anti-CD3 x anti-CD8 trispecifics were characterized in vitro for redirected T cell cytotoxicity (RTCC) of the $CD20^+$ JeKo mantle cell lymphoma cell line using flow cytometry (Figure D). T cell activation, measured by CD69 expression, was also measured by flow cytometry. XENP18953 mediated RTCC activity of the JeKo cell line and preferential activation of $CD8^+$ T cells over $CD4^+$ T cells. Assay details are indicated in the figure legend.

[00347]

[00348]

[00349]

[00350] WHAT IS CLAIMED IS:

1. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein said first and said second variant Fc domains comprise a pair of sets of amino acid substitutions selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen.

1. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and wherein one of said antigen binding domains binds CD3 and another binds CD8.

2. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.30 and a variable light domain having the sequence L1.47.

3. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.32 and a variable light domain having the sequence L1.47.

4. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.89 and a variable light domain having the sequence L1.47.

5. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.90 and a variable light domain having the sequence L1.47.

6. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.33 and a variable light domain having the sequence L1.47.

7. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.31 and a variable light domain having the sequence L1.47.

8. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv

domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.31 and a variable light domain having the sequence L1.47.

9. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD38 and has a variable region comprising a variable heavy domain having the sequence of OTK10 H1.77 and a variable light domain having the sequence L1.24.

10. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD38 and has a variable region comprising a variable heavy domain having the sequence of OTK10 H1 and a variable light domain having the sequence L1.24.

11. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD38 and has a variable region comprising a variable heavy domain having the sequence of OTK10 H1 and a variable light domain having the sequence L1.

12. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD20 and has a variable region comprising a variable heavy domain having the sequence of 2CB8 H1.202 and a variable light domain having the sequence L1.113.

13. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD20 and has a variable region comprising a variable heavy domain having the sequence of 2CB8 H1 and a variable light domain having the sequence L1.

14. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD123 and has a variable region comprising a variable heavy domain having the sequence of 7G3 H1.109 and a variable light domain having the sequence L1.57.

15. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of

said binding domains binds CD8 and has a variable region comprising a variable heavy domain having the sequence of OKT8 H1 and a variable light domain having the sequence L1.

16. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD8 and has a variable region comprising a variable heavy domain having the sequence of OKT8 H2 and a variable light domain having the sequence L1.

17. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD8 and has a variable region comprising a variable heavy domain having the sequence of 51.1 H1 and a variable light domain having the sequence L1.

18. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD8 and has a variable region comprising a variable heavy domain having the sequence ofOKT8 H1 and a variable light domain having the sequence L2.

19. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD3.

20. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD8.

21. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD38.

22. A trispecific antibody comprising:

a) a first monomer comprising:

i) a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

ii) a first scFv domain; and

b) a second monomer comprising:

i) a second heavy chain comprising a second variant Fc domain and said first variable heavy domain;

ii) a second scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein said first variable heavy domain and said variable light domain forms a first antigen binding domain that binds a first antigen, said first scFv domain forms a second antigen binding domain that binds a second antigen, and said second scFv domain forms a third antigen binding domain that binds a third antigen, and one of said binding domains binds CD123.

23. A trispecific antibody according to any of claims 2 to 22 wherein said first and said second variant Fc domains comprise a pair of sets of amino acid substitutions selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q.

24. A trispecific antibody according to any of claims 1 to 23 wherein at least one of said monomers further comprises pI variants.
25. A trispecific antibody according to claim 24 wherein said pI variants are selected from the sets of pI variants shown in Figure 31.
26. A trispecific antibody according to claim 25 wherein said variant sets are selected from the group consisting of I199T/N203D/K274Q/R355Q/N384S/K392N/V397M/Q419E/DEL447 and N208D/Q295E N384D/Q418E/N421D.
27. A trispecific antibody according to any of 1 to 26 wherein said first and second scFv domains are covalently attached to the C-terminus of said first and second heavy chain, respectively.
28. A trispecific antibody according to claim 1 wherein said first and second scFv domains are covalently attached to the N-terminus of said first and second heavy chain, respectively.
29. A trispecific antibody according to claim 1 wherein each of said scFvs is attached between said Fc domain and the CH1 domain of said heavy chain.
30. A trispecific antibody according to any of claims 1 to claim 29 wherein said scFv domains are covalently attached using one or more domain linkers.
31. A trispecific antibody according to any of claims 1 to claim 30 wherein said scFv domains include a scFv linker.
32. A trispecific antibody according to any of claims 1 to claim 31 wherein said scFv linker(s) are charged.
33. A trispecific antibody according to any of claims 1 to claim 32 wherein one of said scFv domains binds CD3.
34. A trispecific antibody according to any of claims 1 to claim 33 wherein one of said scFv domains binds CD8.
35. A trispecific antibody according to any of claims 1 to claim 34 wherein one of said monomers comprises pI variants.
36. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD3 and has a variable region comprising a variable heavy

domain having the sequence of H1.30 and a variable light domain having the sequence L1.47.

37. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.32 and a variable light domain having the sequence L1.47.

38. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.89 and a variable light domain having the sequence L1.47.

39. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.90 and a variable light domain having the sequence L1.47.

40. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.33 and a variable light domain having the sequence L1.47.

41. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.31 and a variable light domain having the sequence L1.47.

42. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.31 and a variable light domain having the sequence L1.47.

43. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD38 and has a variable region comprising a variable heavy domain having the sequence of OTK10 H1.77 and a variable light domain having the sequence L1.24.

44. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD38 and has a variable region comprising a variable heavy domain having the sequence of OTK10 H1 and a variable light domain having the sequence L1.24.
45. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD38 and has a variable region comprising a variable heavy domain having the sequence of OTK10 H1 and a variable light domain having the sequence L1.
46. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD20 and has a variable region comprising a variable heavy domain having the sequence of 2CB8 H1.202 and a variable light domain having the sequence L1.113.
47. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD20 and has a variable region comprising a variable heavy domain having the sequence of 2CB8 H1 and a variable light domain having the sequence L1.
48. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD123 and has a variable region comprising a variable heavy domain having the sequence of 7G3 H1.109 and a variable light domain having the sequence L1.57.
49. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD8 and has a variable region comprising a variable heavy domain having the sequence of OKT8 H1 and a variable light domain having the sequence L1.
50. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD8 and has a variable region comprising a variable heavy domain having the sequence of OKT8 H2 and a variable light domain having the sequence L1.
51. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD8 and has a variable region comprising a variable heavy

domain having the sequence of 51.1 H1 and a variable light domain having the sequence L1.

52. A trispecific antibody according to any of claims 1 to 35, wherein one of said antigen binding domains binds CD8 and has a variable region comprising a variable heavy domain having the sequence ofOKT8 H1 and a variable light domain having the sequence L2.

53. A trispecific antibody according to any of claims 1 to claim 35 wherein said first and/or second Fc domain further comprises amino acid substitution(s) selected from the group consisting of 434A, 434S, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I or V/434S, 436V/428L, 252Y, 252Y/254T/256E, 259I/308F/428L, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L, 236R, 328R, 236R/328R, 236N/267E, 243L, 298A and 299T.

54. A nucleic acid composition comprising:

a) a first nucleic acid encoding said first monomer of any of claims 1 to 53;

b) a second nucleic acid encoding said second monomer of any of claims 1 to 53, respectively; and

c) a third nucleic acid encoding said light chain of any of claims 1 to 53, respectively.

55. An expression vector composition comprising:

a) a first expression vector comprising said first nucleic acid of claim 54;

b) a second expression vector comprising said second nucleic acid of claim 54; and

c) a third expression vector comprising said third nucleic acid of claim 54.

56. A host cell comprising the nucleic acid composition of claim 54.

57. A host cell comprising the expression vector composition of claim 55.

58. A method of making a trispecific antibody according to any of claims 1 to 53 comprising culturing the host cell of claim 56 or 57 under conditions wherein said trispecific antibody is produced and recovering said antibody.

59. A method of treating a patient in need thereof comprising administering a trispecific antibody according to any of claims 1 to 53 to said patient.

60. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.30 and a variable light domain having the sequence L1.47.

61. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD3 and has a variable region comprising a variable

heavy domain having the sequence of H1.32 and a variable light domain having the sequence L1.47.

62. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.89 and a variable light domain having the sequence L1.47.

63. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD3 and has a variable region comprising a variable

heavy domain having the sequence of H1.90 and a variable light domain having the sequence L1.47.

64. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.33 and a variable light domain having the sequence L1.47.

65. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD3 and has a variable region comprising a variable

heavy domain having the sequence of H1.31 and a variable light domain having the sequence L1.47.

66. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD3 and has a variable region comprising a variable heavy domain having the sequence of H1.31 and a variable light domain having the sequence L1.47.

67. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD38 and has a variable region comprising a

variable heavy domain having the sequence of OTK10 H1.77 and a variable light domain having the sequence L1.24.

68. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD38 and has a variable region comprising a variable heavy domain having the sequence of OTK10 H1 and a variable light domain having the sequence L1.24.

69. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD38 and has a variable region comprising a

variable heavy domain having the sequence of OTK10 H1 and a variable light domain having the sequence L1.

70. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD20 and has a variable region comprising a variable heavy domain having the sequence of 2CB8 H1.202 and a variable light domain having the sequence L1.113.

71. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD20 and has a variable region comprising a

variable heavy domain having the sequence of 2CB8 H1 and a variable light domain having the sequence L1.

72. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD123 and has a variable region comprising a variable heavy domain having the sequence of 7G3 H1.109 and a variable light domain having the sequence L1.57.

73. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD8 and has a variable region comprising a variable

heavy domain having the sequence of OKT8 H1 and a variable light domain having the sequence L1.

74. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD8 and has a variable region comprising a variable heavy domain having the sequence of OKT8 H2 and a variable light domain having the sequence L1.

75. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD8 and has a variable region comprising a variable

heavy domain having the sequence of 51.1 H1 and a variable light domain having the sequence L1.

76. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD8 and has a variable region comprising a variable heavy domain having the sequence ofOKT8 H1 and a variable light domain having the sequence L2.

77. A trispecific antibody according to any of claims 60 to 76 wherein said first and said second variant Fc domains comprise a pair of sets of amino acid substitutions selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q.

78. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain;

wherein one of said first and second monomers further comprises a second scFv domain; wherein said first and said second variant Fc domains comprise a pair of sets of amino acid substitutions selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen.

79. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD3.

80. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain

form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD8.

81. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD20.

82. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD38.

83. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD123.

84. A trispecific antibody comprising:

a) a first monomer comprising a first heavy chain comprising a first variant Fc domain and a first variable heavy domain;

b) a second monomer comprising:

i) a second variant Fc domain;

ii) a first scFv domain; and

c) a light chain comprising a constant light domain and a variable light domain; wherein one of said first and second monomers further comprises a second scFv domain; wherein said first variable heavy domain and said variable light domain form a first antigen binding domain that binds a first antigen, said first scFv domain forms a second binding domain that binds a second antigen, and said second scFv domain forms a third binding domain that binds a third antigen, and one of said antigen binding domains binds CD3 and another binds CD8.

85. A trispecific antibody according to any of claims 60 to 84 wherein said second scFv is covalently attached to said first monomer at the C-terminus.

86. A trispecific antibody according to any of claims 60 to 84 wherein said second scFv is covalently attached to said first monomer at the N-terminus.

87. A trispecific antibody according to any of claims 60 tto 84 wherein said second scFv is covalently attached to said second monomer at the N-terminus.
88. A trispecific antibody according to any of claims 60 to 84 wherein said second scFv is covalently attached to said second monomer at the C-terminus.
89. A trispecific antibody according to any of claims 60 to 84 wherein said second scFv is covalently attached to said first monomer between the CH1 and Fc domain of the heavy chain.
90. A trispecific antibody according to any of claims 60 to 89 wherein said scFvs are attached using a domain linker.
91. A trispecific antibody according to any of claims 60 to 90 wherein at least one of said scFvs comprises a charged scFv linker.
92. A trispecific antibody according to any of claims 60 to 91 wherein one of said scFv domains binds CD3.
93. A trispecific antibody according to any of claims 60 to 92 wherein one of said scFv domains binds CD8.
94. A trispecific antibody according to any of claims any of claims 60 to 93 wherein one of said monomers comprises pI variants.
95. A trispecific antibody according to any of claims any of claims 60 to 94 wherein said first and/or second Fc domain further comprises amino acid substitution(s) selected from the group consisting of 434A, 434S, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I or V/434S, 436V/428L, 252Y, 252Y/254T/256E, 259I/308F/428L, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L, 236R, 328R, 236R/328R, 236N/267E, 243L, 298A and 299T.
96. A nucleic acid composition comprising:

a) a first nucleic acid encoding said first monomer of any of claims 60to 95;

b) a second nucleic acid encoding said second monomer of any of claims 60 to 95, respectively; and

c) a third nucleic acid encoding said light chain of any of claims 60 to 95, respectively.

97. An expression vector composition comprising:

a) a first expression vector comprising said first nucleic acid of claim 96;

b) a second expression vector comprising said second nucleic acid of claim 96; and

c) a third expression vector comprising said third nucleic acid of claim 96.

98. A host cell comprising the nucleic acid composition of claim 96.

99. A host cell comprising the expression vector composition of claim 97.

100. A method of making a trispecific antibody according to any of claims 60 to 95 comprising culturing the host cell of claim 98 or 99 under conditions wherein said trispecific antibody is produced and recovering said antibody.

101. A method of treating a patient in need thereof comprising administering a trispecific antibody according to any of claims 60 to 95 to said patient.

102. A trispecific antibody selected from the group consisting of XENP15242, XENP15243, XENP15244, XENP15264, XENP15265, XENP15266; XENP18951, XENP18952, XENP18953 and XENP18954.

Figs. 1A-1D

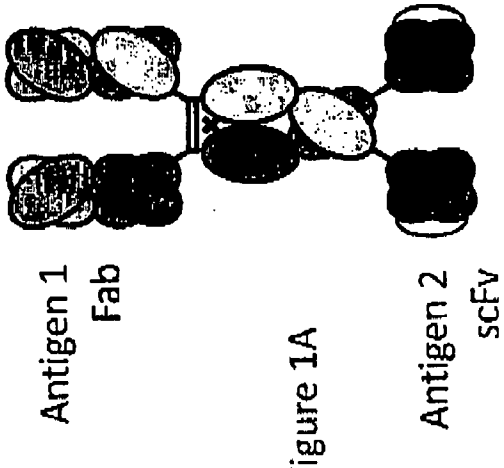


Figure 1A

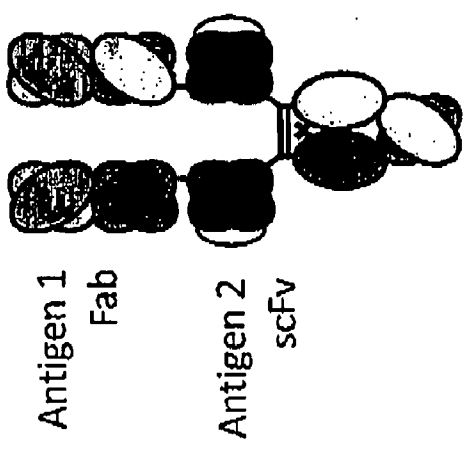


Figure 1B

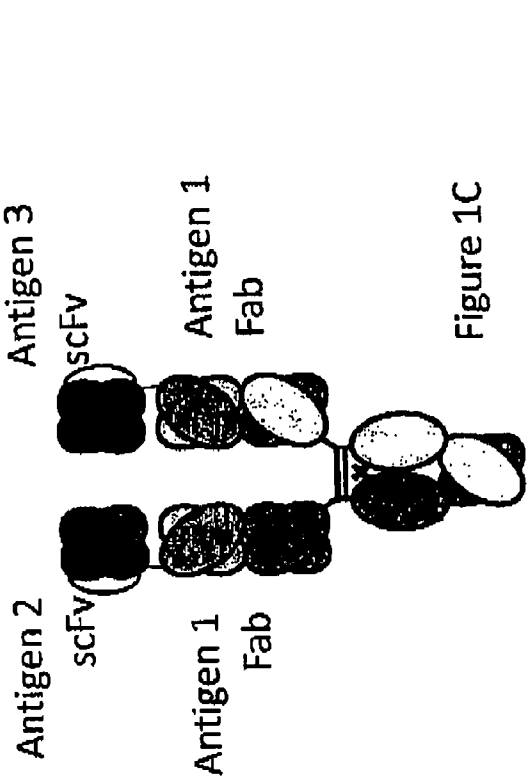


Figure 1C

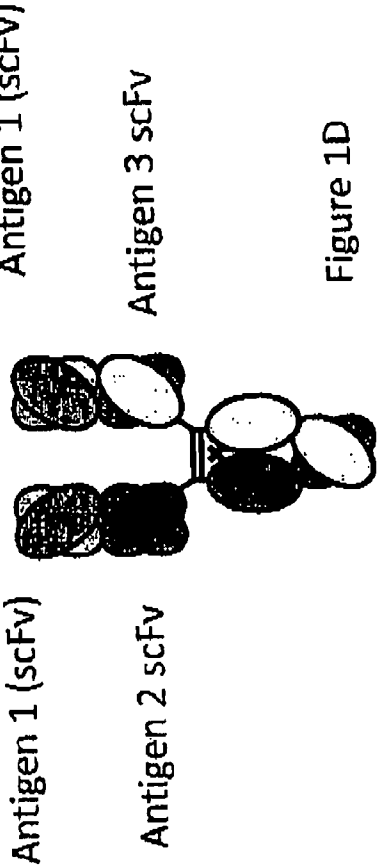


Figure 1D

Figures 2A-2D

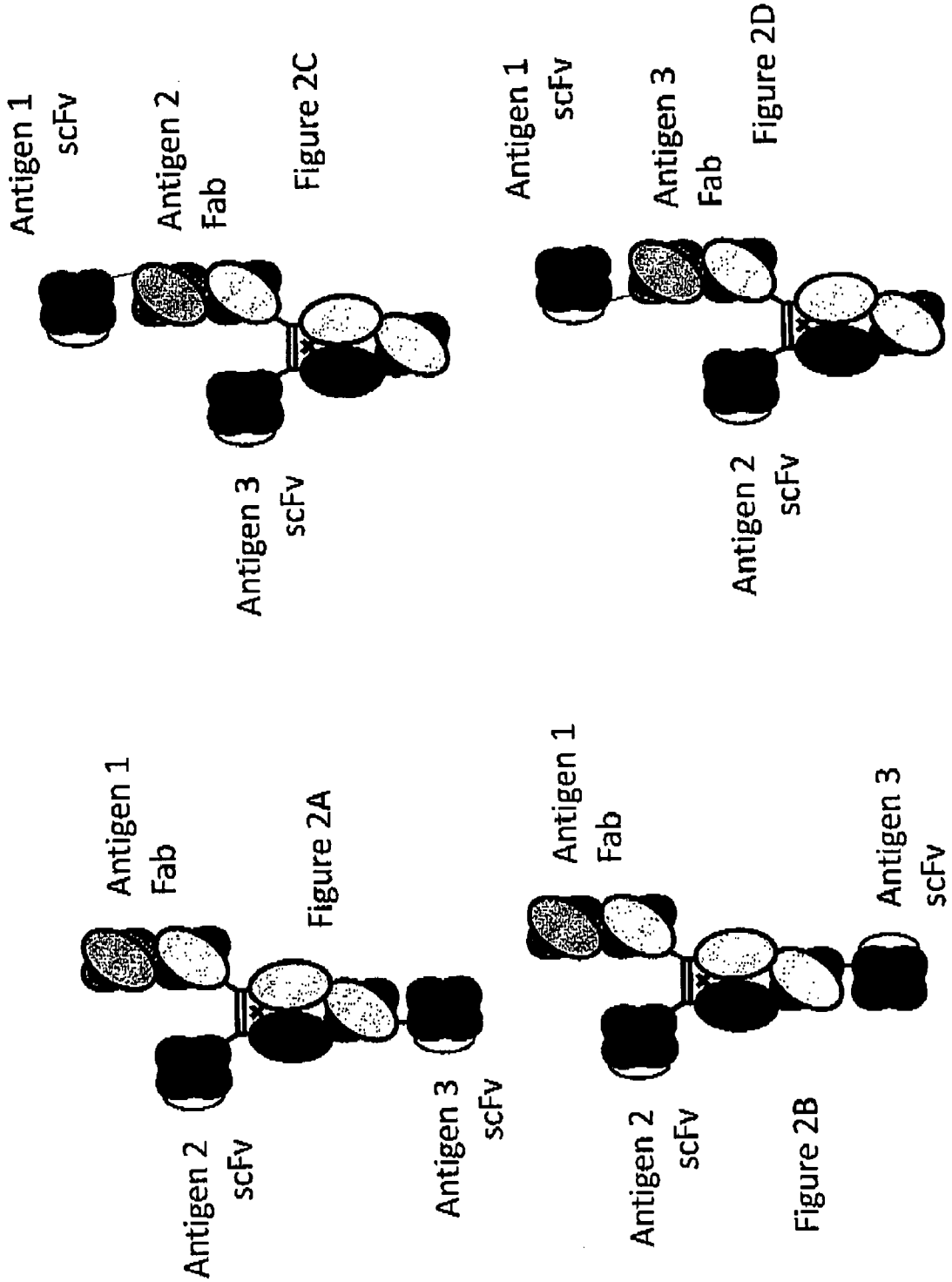
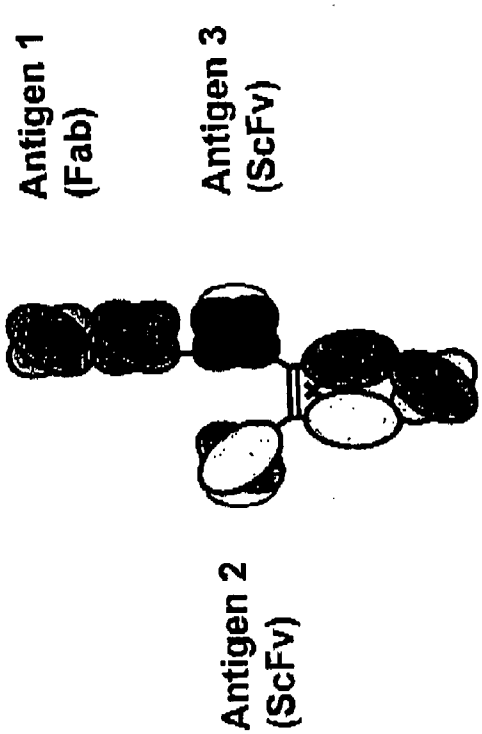


Fig. 2 continued

Fig. 2E



TF Figure 3A-3C
Bivalent anti-tumor X anti-CD3 X anti-CD8 formats

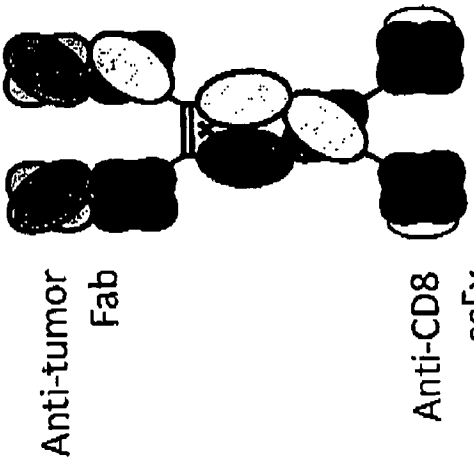


Fig. 3A

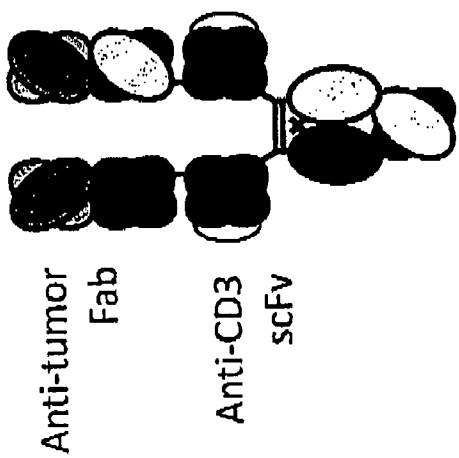
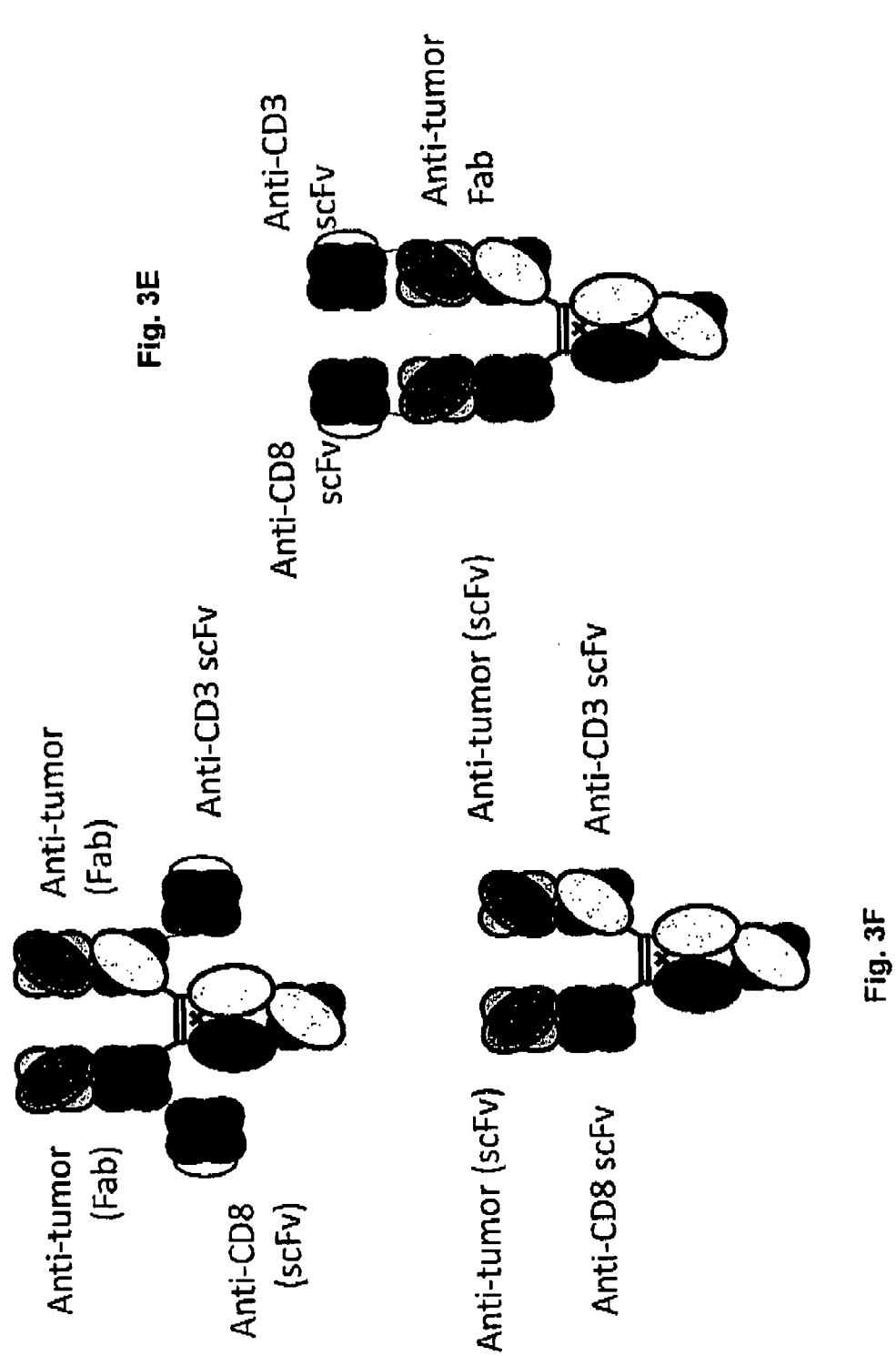


Fig. 3C

Fig. 3B

TF Figure 3, cont.
Bivalent anti-tumor X anti-CD3 X anti-CD8 Formats, cont.



TF Figure 4A-4D
Trispecific Formats with different terminal attachments

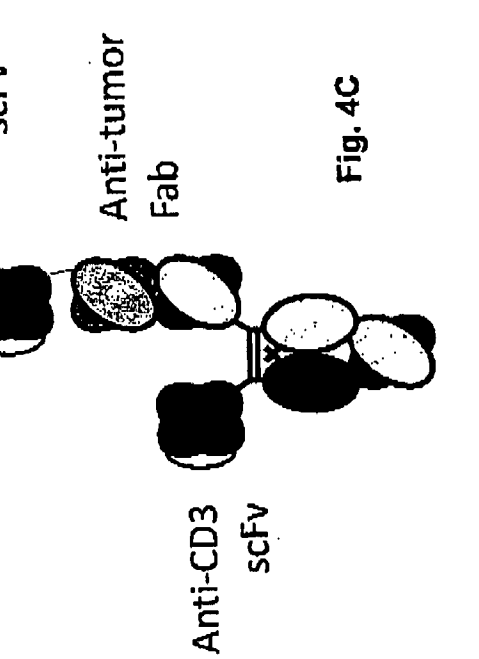


Fig. 4A

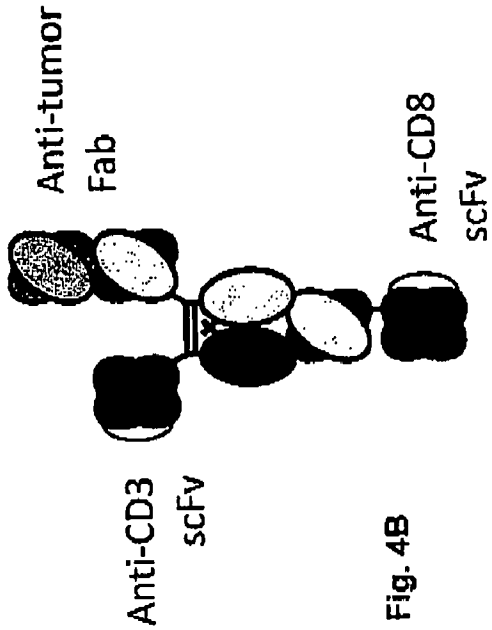


Fig. 4B

Fig. 4C

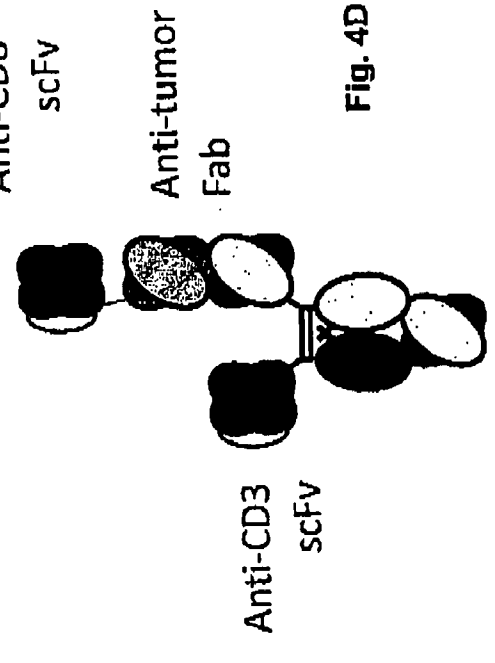
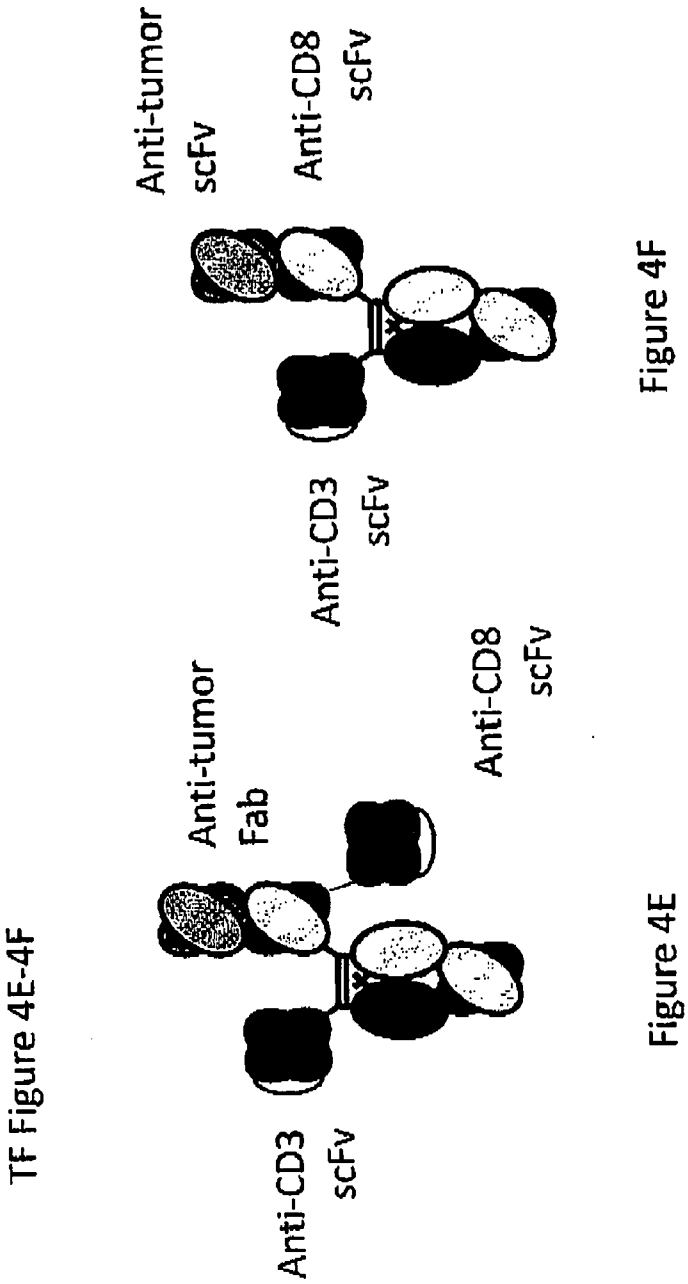


Fig. 4D


TF Figure 4E-4F
Anti-CD3 scFv
Anti-tumor Fab
Anti-CD8 scFv
Figure 4E
Anti-CD3 scFv
Anti-tumor scFv
Anti-CD8 scFv
Figure 4F

TF Figures 5A-5F

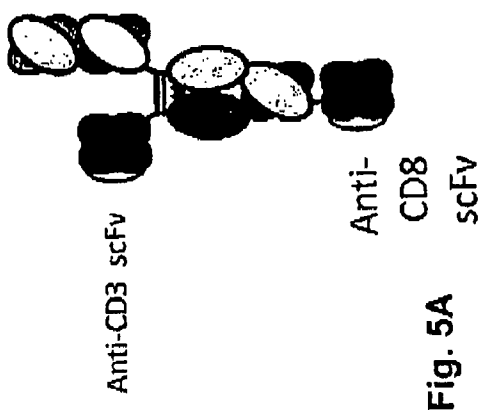


Fig. 5A

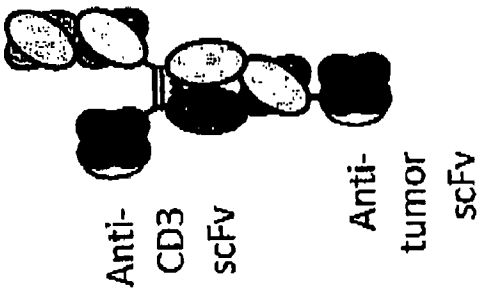


Fig. 5B

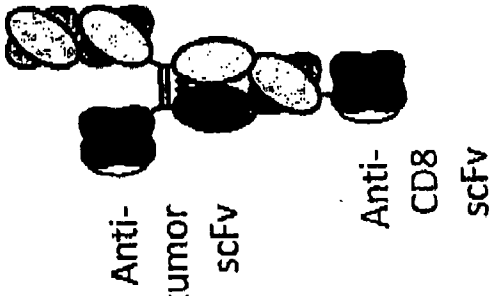


Fig. 5C

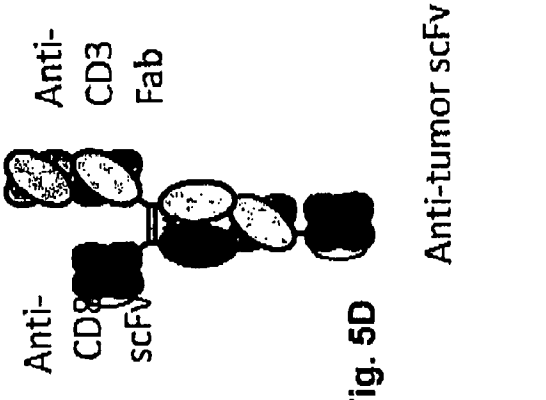


Fig. 5D

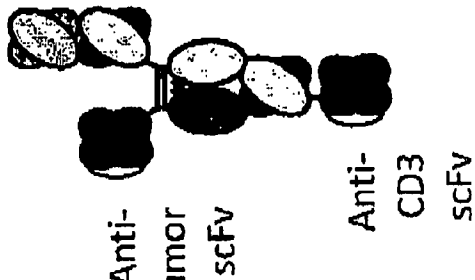


Fig. 5E

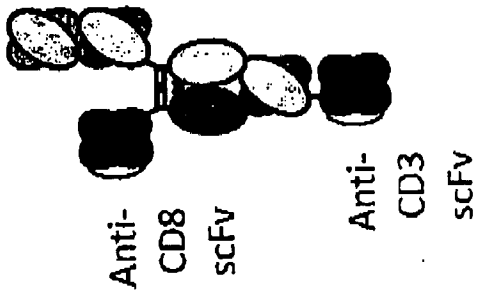
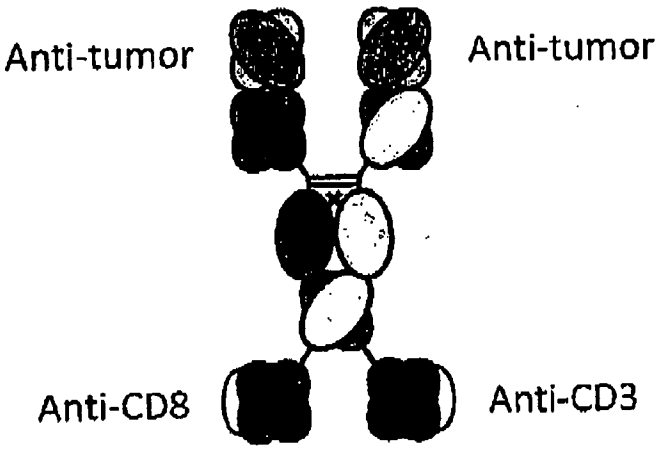
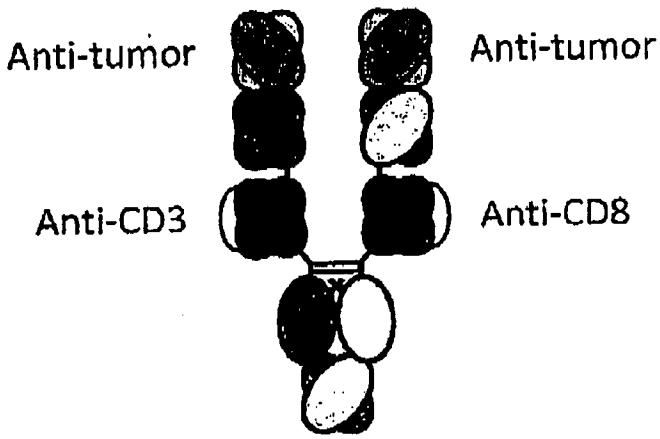


Fig. 5F

Fig. 6



TF Fig. 7



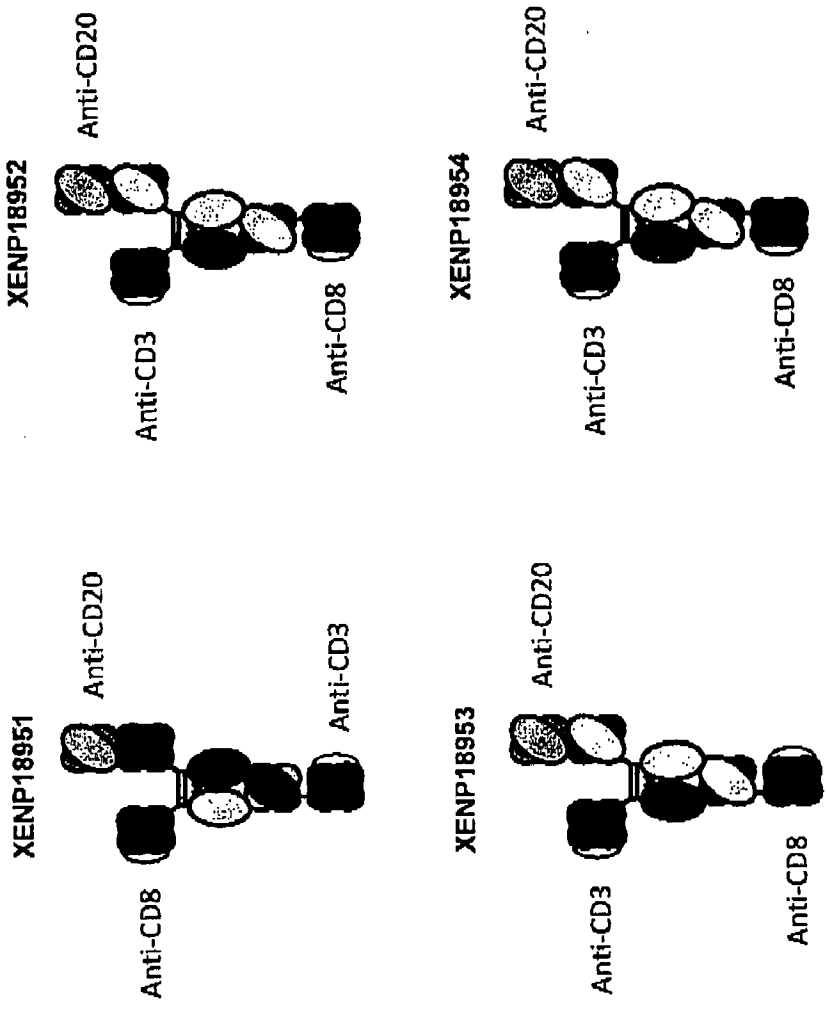

XENP18952
Anti-CD20
Anti-CD3
Anti-CD8
XENP18954
Anti-CD20
Anti-CD3
Anti-CD8
XENP18951
Anti-CD20
Anti-CD8
Anti-CD3
XENP18953
Anti-CD20
Anti-CD3
Anti-CD8

Figure 8

Figure 9

XENP18951 Heavy Chain 1 (SEQ ID NO: 228)

QIQLVQSGAEVKKPGASVKVSCKASGYSFTNFGMIWVRQAPGQGLEWMGWINTYTGEPTYADGFTGRFVFSLDTSVNTAYLQISSLKAEDTAVYFCARKDYAGFFDY
WGQGTLVTVSSGGGGSGGGGSGGGGSDILMTQSPSSLSASVGDRVTITCQASQDIGSNMGWLQQKPGKSFKALIYHGTNLEYGVPSRFSGSGSGADYTLTISSLQPED
FATYYCVQFAQFPYTFGGGTKVEIK/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKL
TVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

XENP18951 Heavy Chain 2 (SEQ ID NO: 229)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQRLEWMGAIYPGNGATSYSQKFQGRVTITADTSASTAYMELSSLRSEDTAVYYCARSYYMGGD
WYFDVWGAGTLVTVSS/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV
DKKVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGS/EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTIS
RDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFDYWGQGTLVTVSSGKPGSGKPGSGKPGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYA
NWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

XENP18951 Light Chain (SEQ ID NO: 230)

QIVLTQSPSSLSASVGDRVTITCRASWSVSYIHWFQQKPGKSPKPLIYATSNLASGVPVRFSGSGSGTDYTLTISSLQPEDFATYYCQQWTHNPPTFGGGTKVEIK/RTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 10

XENP18952 Heavy Chain 1 (SEQ ID NO: 231)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQRLEWMGAIYPGNGATSYSQKFQGRVTITADTSASTAYMELSSLRSEDTAVYYCARSYYMGGD
WYFDVWGAGTLVTVSS/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTKV
DKKVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWEQGDVFSCSVMHE
ALHNHYTQKSLSLSPGK

XENP18952 Heavy Chain 2 (SEQ ID NO: 232)

EVQLQQSGAEVKKPGASVKVSCKASGFNIKDTYIHWVRQAPGKGLEWMGRIDPANDNTLYASKFQGRVTITADTSTNTAYMELSSLRSEDTAVYYCGRGYGYYVFDH
WGQGTTVTVSSGKPGSGKPGSGKPGSGKPGSDIKMTQSPSSLSASVGDRVTITCRTSRSISQYLAWYQEKPGKTNKLLIYSGSTLQSGIPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQHNENPLTFGAGTKLEIK/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK
LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGS/EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRI
RSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFDYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSQAVVTQEPSLTVS
PGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

XENP18952 Light Chain (SEQ ID NO: 233)

QIVLTQSPSSLSASVGDRVTITCRASWSVSYIHWFQQKPGKSPKPLIYATSNLASGVPVRFSGSGSGTDYTLTISSLQPEDFATYYCQQWTHNPPTFGGGTKVEIK/RTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 11 XENP18953 Heavy Chain 1 (SEQ ID NO: 234)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQRLEWMGAIYPGNGATSYSQKFQGRVTITADTSASTAYMELSSLRSEDTAVYYCARSYYMGGD
WYFDVWGAGTLVTVSS/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTKV
DKKVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHE
ALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGS/QIQLVQSGAEVKKPGASVKVSCKASGYSFTNFGMIWVRQAPGQGLEWMGWINTYTGEPTYADGFTGRFVFSLD
TSVNTAYLQISSLKAEDTAVYFCARKDYAGFFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDILMTQSPSSLSASVGDRVTITCQASQDIGSNMGWLQQKPGKSFKALI
YHGTNLEYGVPSRFSGSGSGADYTLTISSLQPEDFATYYCVQFAQFPYTFGGGTKVEIK

XENP18953 Heavy Chain 2 (SEQ ID NO: 235)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDS
YVSWFDYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPG5QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLL
GGKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

XENP18953 Light Chain (SEQ ID NO: 236)

QIVLTQSPSSLSASVGDRVTITCRASWSVSYIHWFQQKPGKSPKPLIYATSNLASGVPVRFSGSGSGTDYTLTISSLQPEDFATYYCQQWTHNPPTFGGGTKVEIK/RTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 12

XENP18954 Heavy Chain 1 (SEQ ID NO: 237)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQRLEWMGAIYPGNGATSYSQKFQGRVTITADTSASTAYMELSSLRSEDTAVYYCARSYYMGGD
WYFDVWGAGTLVTVSS/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTKV
DKKVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHE
ALHNHYTQKSLSLSPGK

XENP18954 Heavy Chain 2 (SEQ ID NO: 238)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDS
YVSWFDYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLL
GGKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGS/EVQLQQSGAEVKKPGASVKVSCKASGFNIKDTYIHW
VRQAPGKGLEWMGRIDPANDNTLYASKFQGRVTITADTSTNTAYMELSSLRSEDTAVYYCGRGYGYYVFDHWGQGTTVTVSSGGGGSGGGGSGGGGSDIKMTQSPS
SLSASVGDRVTITCRTSRSISQYLAWYQEKPGKTNKLLIYSGSTLQSGIPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQHNENPLTFGAGTKLEIK

XENP18954 Light Chain (SEQ ID NO: 239)

QIVLTQSPSSLSASVGDRVTITCRASWSVSYIHWFQQKPGKSPKPLIYATSNLASGVPVRFSGSGSGTDYTLTISSLQPEDFATYYCQQWTHNPPTFGGGTKVEIK/RTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 13

High CD3: Anti-CD3_H1.30_L1.47

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLY LQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS	1
vhCDR1	TYAMN	2
vhCDR2	RIRSKYNNYATYYADSVKG	3
vhCDR3	HGNFGDSYVSWFAY	4
Variable light (vl) domain	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPE DEADYYCALWYSNHWVFGGGTKLTVL	5
vlCDR1	GSSTGAVTTSNYAN	6
vlCDR2	GTNKRAP	7
vlCDR3	ALWYSNHWV	8
scFv (including charged linker)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLY LQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSQAVVTQEPSLTVSPGGT VTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYSNHW VFGGGTKLTVL	9

Figure 14

High-Int #1 CD3: Anti-CD3_H1.32_L1.47

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKANNYATYYADSVKGRFTISRDDSKNTLY LQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS	10
vhCDR1	TYAMN	11
vhCDR2	RIRSKANNYATYYADSVKG	12
vhCDR3	HGNFGDSYVSWFAY	13
Variable light (vl) domain	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPE DEADYYCALWYSNHWVFGGGTKLTVL	14
vlCDR1	GSSTGAVTTSNYAN	15
vlCDR2	GTNKRAP	16
vlCDR3	ALWYSNHWV	17
scFv (including charged linker)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKANNYATYYADSVKGRFTISRDDSKNTLY LQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSQAVVTQEPSLTVSPGGT VTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYSNHW VFGGGTKLTVL	18

Figure 14

Figure 15

High-Int #2 CD3: Anti-CD3_H1.89_L1.47

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLY LQMNSLRAEDTAVYYCVRHGNFGDEYVSWFAYWGQGTLVTVSS	19
vhCDR1	TYAMN	20
vhCDR2	RIRSKYNNYATYYADSVKG	21
vhCDR3	HGNFGDEYVSWFAY	22
Variable light (vl) domain	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPE DEADYYCALWYSNHWVFGGGTKLTVL	23
vlCDR1	GSSTGAVTTSNYAN	24
vlCDR2	GTNKRAP	25
vlCDR3	ALWYSNHWV	26
scFv (including charged linker)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLY LQMNSLRAEDTAVYYCVRHGNFGDEYVSWFAYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSQAVVTQEPSLTVSPGGT VTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYSNHW VFGGGTKLTVL	27

Figure 16

High-Int #3 CD3: Anti-CD3_H1.90_L1.47

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLY LQMNSLRAEDTAVYYCVRHGNFGDPYVSWFAYWGQGTLVTVSS	28
vhCDR1	TYAMN	29
vhCDR2	RIRSKYNNYATYYADSVKG	30
vhCDR3	HGNFGDPYVSWFAY	31
Variable light (vl) domain	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPE DEADYYCALWYSNHWVFGGGTKLTVL	32
vlCDR1	GSSTGAVTTSNYAN	33
vlCDR2	GTNKRAP	34
vlCDR3	ALWYSNHWV	35
scFv (including charged linker)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLY LQMNSLRAEDTAVYYCVRHGNFGDPYVSWFAYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSQAVVTQEPSLTVSPGGT VTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYSNHW VFGGGTKLTVL	36

Figure 17

Intermediate CD3: Anti-CD3_H1.33_L1.47

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLY LQMNSLRAEDTAVYYCVRHGNFGDSYVSWFDYWGQGTLVTVSS	37
vhCDR1	TYAMN	38
vhCDR2	RIRSKYNNYATYYADSVKG	39
vhCDR3	HGNFGDSYVSWFDY	40
Variable light (vl) domain	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPE DEADYYCALWYSNHWVFGGGTKLTVL	41
vlCDR1	GSSTGAVTTSNYAN	42
vlCDR2	GTNKRAP	43
vlCDR3	ALWYSNHWV	44
scFv (including charged linker)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLY LQMNSLRAEDTAVYYCVRHGNFGDSYVSWFDYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSQAVVTQEPSLTVSPGGT VTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYSNHW VFGGGTKLTVL	45

Figure 18

Low CD3: Anti-CD3_H1.31_L1.47

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYL QMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS	46
vhCDR1	TYAMS	47
vhCDR2	RIRSKYNNYATYYADSVKG	48
vhCDR3	HGNFGDSYVSWFAY	49
Variable light (vl) domain	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPE DEADYYCALWYSNHWVFGGGTKLTVL	50
vlCDR1	GSSTGAVTTSNYAN	51
vlCDR2	GTNKRAP	52
vlCDR3	ALWYSNHWV	53
scFv (including charged linker)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYL QMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSQAVVTQEPSLTVSPGGTV TLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYSNHWV FGGGTKLTVL	54

Figure 19

High CD38: OKT10_H1.77_L1.24

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYSWMNWVRQAPGKGLEWVSEINPQSSTINYATSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCARYGNWFPYWGQGTLVTVSS	55
vhCDR1	YSWMN	56
vhCDR2	EINPQSSTINYATSVKG	57
vhCDR3	YGNWFPY	58
Variable light (vl) domain	DIVMTQSPSSLSASVGDRVTITCRASQNVDTWVAWYQQKPGQSPKALIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFA TYFCQQYDSYPLTFGGGTKLEIK	59
vlCDR1	RASQNVDTWVA	60
vlCDR2	SASYRYS	61
vlCDR3	QQYDSYPLT	62
scFv (including charged linker)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYSWMNWVRQAPGKGLEWVSEINPQSSTINYATSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCARYGNWFPYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSDIVMTQSPSSLSASVGDRVTITCRASQ NVDTWVAWYQQKPGQSPKALIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFATYFCQQYDSYPLTFGGGTKLEIK	63

Figure 20

Intermediate CD38: OKT10_H1L1.24

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGFDFSRSWMNWVRQAPGKGLEWVSEINPDSSTINYATSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCARYGNWFPYWGQGTLVTVSS	64
vhCDR1	RSWMN	65
vhCDR2	EINPDSSTINYATSVKG	66
vhCDR3	YGNWFPY	67
Variable light (vl) domain	DIVMTQSPSSLSASVGDRVTITCRASQNVDTWVAWYQQKPGQSPKALIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFA TYFCQQYDSYPLTFGGGTKLEIK	68
vlCDR1	RASQNVDTWVA	69
vlCDR2	SASYRYS	70
vlCDR3	QQYDSYPLT	71
scFv (including charged linker)	EVQLVESGGGLVQPGGSLRLSCAASGFDFSRSWMNWVRQAPGKGLEWVSEINPDSSTINYATSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCARYGNWFPYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSDIVMTQSPSSLSASVGDRVTITCRASQ NVDTWVAWYQQKPGQSPKALIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFATYFCQQYDSYPLTFGGGTKLEIK	72

Figure 21

Low CD38: OKT10_H1L1

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGFDFSRSWMNWVRQAPGKGLEWVSEINPDSSTINYATSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCARYGNWFPYWGQGTLVTVSS	73
vhCDR1	RSWMN	74
vhCDR2	EINPDSSTINYATSVKG	75
vhCDR3	YGNWFPY	76
Variable light (vl) domain	EVQLVESGGGLVQPGGSLRLSCAASGFDFSRSWMNWVRQAPGKGLEWVSEINPDSSTINYATSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCARYGNWFPYWGQGTLVTVSS	77
vlCDR1	RASQNVDTNVA	78
vlCDR2	SASYRYS	79
vlCDR3	QQYDSYPLT	80
scFv (including charged linker)	EVQLVESGGGLVQPGGSLRLSCAASGFDFSRSWMNWVRQAPGKGLEWVSEINPDSSTINYATSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCARYGNWFPYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSEVQLVESGGGLVQPGGSLRLSCAASGF DFSRSWMNWVRQAPGKGLEWVSEINPDSSTINYATSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYGNWFPYWG QGTLVTVSS	81

Figure 22

High CD20 C2B8_H1.202_L1.113

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTWVRQAPGQRLEWMGAIYPGNGATSYSQKFQGRVTITADTSASTAYMELSSL RSEDTAVYYCARSYYMGGDWYFDVWGAGTLVTVSS	82
vhCDR1	SYNMH	83
vhCDR2	AIYPGNGATSYSQKFQG	84
vhCDR3	SYYMGGDWYFDV	85
Variable light (vl) domain	QIVLTQSPSSLSASVGDRVTITCRASWSVSYIHWFQQKPGKSPKPLIYATSNLASGVPVRFSGSGSGTDYTLTISSLQPEDFATYY CQQWTHNPPTFGGGTKVEIK	86
vlCDR1	RASWSVSYIH	87
vlCDR2	ATSNLAS	88
vlCDR3	QQWTHNPPT	89
scFv (including charged linker)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTWVRQAPGQRLEWMGAIYPGNGATSYSQKFQGRVTITADTSASTAYMELSSL RSEDTAVYYCARSYYMGGDWYFDVWGAGTLVTVSSGKPGSGKPGSGKPGSGKPGSQIVLTQSPSSLSASVGDRVTITCRASW SVSYIHWFQQKPGKSPKPLIYATSNLASGVPVRFSGSGSGTDYTLTISSLQPEDFATYYCQQWTHNPPTFGGGTKVEIK	90

Figure 23

Low CD20 C2B8_H1L1

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWMGAIYPGNGDTSYNQKFQGRVTITADKSISTAY MELSSLRSEDTAVYYCARSTYYGGDWYFNVWGAGTLVTVSS	91
vhCDR1	SYNMH	92
vhCDR2	AIYPGNGDTSYNQKFQG	93
vhCDR3	STYYGGDWYFNV	94
Variable light (vl) domain	QIVLTQSPSSLSASVGDRVTITCRASSSVSYIHWFQQKPGKSPKPLIYATSNLASGVPVRFSGSGSGTDYTLTISSLQPEDFATYYC QQWTSNPPTFGGGTKVEIK	95
vlCDR1	RASSSVSYIH	96
vlCDR2	ATSNLAS	97
vlCDR3	QQWTSNPPT	98
scFv (including charged linker)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWMGAIYPGNGDTSYNQKFQGRVTITADKSISTAY MELSSLRSEDTAVYYCARSTYYGGDWYFNVWGAGTLVTVSSGKPGSGKPGSGKPGSGKPGSQIVLTQSPSSLSASVGDRVTIT CRASSSVSYIHWFQQKPGKSPKPLIYATSNLASGVPVRFSGSGSGTDYTLTISSLQPEDFATYYCQQWTSNPPTFGGGTKVEIK	99

Figure 24

CD123 7G3_H1.109_L1.57

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGAEVKKPGASVKVSCKASGYTFTDYYMKWVKQSHGKSLEWMGDIIPSNGATFYNQKFKGKATLTVDRSTSTAY MELSSLRSEDTAVYYCARSHLLRASWFAYWGQGTLVTVSS	100
vhCDR1	DYYMK	101
vhCDR2	DIIPSNGATFYNQKFKG	102
vhCDR3	SHLLRASWFAY	103
Variable light (vl) domain	DFVMTQSPDSLAVSLGERATINCKSSQSLLNTGNQKNYLTWYQQKPGQPPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSL QAEDVAVYYCQNDYSYPYTFGGGTKLEIK	104
vlCDR1	KSSQSLLNTGNQKNYLT	105
vlCDR2	WASTRES	106
vlCDR3	QNDYSYPYT	107
scFv (including charged linker)	QVQLQQSGAEVKKPGASVKVSCKASGYTFTDYYMKWVKQSHGKSLEWMGDIIPSNGATFYNQKFKGKATLTVDRSTSTAY MELSSLRSEDTAVYYCARSHLLRASWFAYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSDFVMTQSPDSLAVSLGERATIN CKSSQSLLNTGNQKNYLTWYQQKPGQPPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSLQAEDVAVYYCQNDYSYPYTFG GGTKLEIK	108

Figure 25

CD8 OKT8_H1L1

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLQQSGAEVKKPGASVKVSCKASGFNIKDTYIHWVRQAPGKGLEWMGRIDPANDNTLYASKFQGRVTITADTSTNTAYM ELSSLRSEDTAVYYCGRGYGYYVFDHWGQGTTVTVSS	109
vhCDR1	DTYIH	110
vhCDR2	RIDPANDNTLYASKFQG	111
vhCDR3	GYGYYVFDH	112
Variable light (vl) domain	DIKMTQSPSSLSASVGDRVTITCRTSRSISQYLAWYQEKPGKTNKLLIYSGSTLQSGIPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQHNENPLTFGAGTKLEIK	113
vlCDR1	RTSRSISQYLA	114
vlCDR2	SGSTLQS	115
vlCDR3	QQHNENPLT	116
scFv (including charged linker)	EVQLQQSGAEVKKPGASVKVSCKASGFNIKDTYIHWVRQAPGKGLEWMGRIDPANDNTLYASKFQGRVTITADTSTNTAYM ELSSLRSEDTAVYYCGRGYGYYVFDHWGQGTTVTVSSGKPGSGKPGSGKPGSDIKMTQSPSSLSASVGDRVTITCRTS RSISQYLAWYQEKPGKTNKLLIYSGSTLQSGIPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQHNENPLTFGAGTKLEIK	117

Figure 26

CD8 OKT8_H2L1

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWVRQAPGKGLEWMGRIDPANDNTLYASKFQGRVTITADTSINTAYM ELSRLRSDDTAVYYCGRGYGYYVFDHWGQGTTVTVSS	118
vhCDR1	DTYIH	119
vhCDR2	RIDPANDNTLYASKFQG	120
vhCDR3	GYGYYVFDH	121
Variable light (vl) domain	DIKMTQSPSSLSASVGDRVTITCRTSRSISQYLAWYQEKPGKTNKLLIYSGSTLQSGIPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQHNENPLTFGAGTKLEIK	122
vlCDR1	RTSRSISQYLA	123
vlCDR2	SGSTLQS	124
vlCDR3	QQHNENPLT	125
scFv (including charged linker)	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWVRQAPGKGLEWMGRIDPANDNTLYASKFQGRVTITADTSINTAYM ELSRLRSDDTAVYYCGRGYGYYVFDHWGQGTTVTVSSGKPGSGKPGSGKPGSGKPGSDIKMTQSPSSLSASVGDRVTITCRTS RSISQYLAWYQEKPGKTNKLLIYSGSTLQSGIPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQHNENPLTFGAGTKLEIK	126

Figure 27

CD8 51.1_H1L1

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QIQLVQSGAEVKKPGASVKVSCKASGYSFTNFGMIWVRQAPGQGLEWMGWINTYTGEPTYADGFTGRFVFSLDTSVNTAYL QISSLKAEDTAVYFCARKDYAGFFDYWGQGTLVTVSS	127
vhCDR1	NFGMI	128
vhCDR2	WINTYTGEPTYADGFTG	129
vhCDR3	KDYAGFFDY	130
Variable light (vl) domain	DILMTQSPSSLSASVGDRVTITCQASQDIGSNMGWLQQKPGKSFKALIYHGTNLEYGVPSRFSGSGSGADYTLTISSLQPEDFA TYYCVQFAQFPYTFGGGTKVEIK	131
vlCDR1	QASQDIGSNMG	132
vlCDR2	HGTNLEY	133
vlCDR3	VQFAQFPYT	134
scFv (including uncharged linker)	QIQLVQSGAEVKKPGASVKVSCKASGYSFTNFGMIWVRQAPGQGLEWMGWINTYTGEPTYADGFTGRFVFSLDTSVNTAYL QISSLKAEDTAVYFCARKDYAGFFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDILMTQSPSSLSASVGDRVTITCQASQDIG SNMGWLQQKPGKSFKALIYHGTNLEYGVPSRFSGSGSGADYTLTISSLQPEDFATYYCVQFAQFPYTFGGGTKVEIK	135

Figure 28

CD8 51.1_H1L2

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QIQLVQSGAEVKKPGASVKVSCKASGYSFTNFGMIWVRQAPGQGLEWMGWINTYTGEPTYADGFTGRFVFSLDTSVNTAYL QISSLKAEDTAVYFCARKDYAGFFDYWGQGTLVTVSS	136
vhCDR1	NFGMI	137
vhCDR2	WINTYTGEPTYADGFTG	138
vhCDR3	KDYAGFFDY	139
Variable light (vl) domain	DILMTQSPSSLSASVGDRVTITCQASQDIGSNMGWLQQKPGKSFKALIYHGTNLEYGVPSRFSGSGSGADYTLTISSLQPEDFA TYYCVQFAQFPYTFGGGTKVEIK	140
vlCDR1	QASQDIGSNMG	141
vlCDR2	HGTNLEY	142
vlCDR3	VQFAQFPYT	143
scFv (including charged linker)	QIQLVQSGAEVKKPGASVKVSCKASGYSFTNFGMIWVRQAPGQGLEWMGWINTYTGEPTYADGFTGRFVFSLDTSVNTAYL QISSLKAEDTAVYFCARKDYAGFFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDILMTQSPSSLSASVGDRVTITCQASQDIG SNMGWLQQKPGKSFKALIYHGTNLEYGVPSRFSGSGSGADYTLTISSLQPEDFATYYCVQFAQFPYTFGGGTKVEIK	144

Figure 29

>sp|P11836|CD20_HUMAN B-lymphocyte antigen CD20 (SEQ ID NO: 145)

MTTPRNSVNGTFPAEPMKGPIAMQSGPKPLFRRMSSLVGPTQSFFMRESKTLGAVQIMNGLFHIALGGLLMIPAGIYAPICVTVWYPLWGGIMYIISGSLLAATEKNSR
KCLVKGKMIMNSLSLFAAISGMILSIMDILNIKISHFLKMESLNFIRAHTPYINIYNCEPANPSEKNSPSTQYCYSIQSLFLGILSVMLIFAFFQELVIAGIVENEWKRTCSRPKS
NIVLLSAEEKKEQTIEIKEEVVGLTETSSQPKNEEDIEIIPIQEEEEEETETNFPEPPQDQESSPIENDSSP

>sp|P26951|IL3RA_HUMAN Interleukin-3 receptor subunit alpha (CD123) (SEQ ID NO: 146)

MVLLWLTLLLIALPCLLQTKEDPNPPITNLRMKAKAQQLTWDLNRNVTDIECVKDADYSMPAVNNSYCQFGAISLCEVTNYTVRVANPPFSTWILFPENSGKPWAGAE
NLTCWIHDVDFLSCSWAVGPGAPADVQYDLYLNVANRRQQYECLHYKTDAQGTRIGCRFDDISRLSSGSQSSHILVRGRSAAFGIPCTDKFVVFSQIEILTPPNMTAKCN
KTHSFMHWKMRSHFNRKFRYELQIQKRMQPVITEQVRDRTSFQLLNPGTYTVQIRARERVYEFLSAWSTPQRFECDQEEGANTRAWRTSLLIALGTLLALVCVFVICRR
YLVMQRLFPRIPHMKDPIGDSFQNDKLVVWEAGKAGLEECLVTEVQVVQKT

>sp|P07766|CD3E_HUMAN T-cell surface glycoprotein CD3 epsilon chain (SEQ ID NO: 147)

MQSGTHWRVLGLCLLSVGVWGQDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDA
NFYLYLRARVCENCMEMDVMSVATIVIVDICITGGLLLLVYYWSKNRKAKAKPVTRGAGAGGRQRGQNKERPPPVPNPDYEPIRKGQRDLYSGLNQRRI

Human CD38 sequence, "/" indicates the junction with the extracellular domain (ECD) (SEQ ID NO: 148)

MANCEFSPVSGDKPCCRLSRRAQLCLGVSILVLILVVVLAVV/VPRWRQQWSGPGTTKRFPETVLARCVKYTEIHPEMRHVDCQSVWDAFKGAFISKHPCNITEEDYQP
LMKLGTQTVPCNKILLWSRIKDLAHQFTQVQRDMFTLEDTLLGYLADDLTWCGEFNTSKINYQSCPDWRKDCSNNPVSVFWKTVSRRFAEAACDVVHVMLNGSRSKI
FDKNSTFGSVEVHNLQPEKVQTLEAWVIHGGREDSRDLCQDPTIKELESIISKRNIQFSCKNIYRPDKFLQCVKNPEDSSCTSEI

ECD domain (SEQ ID NO: 149)

VPRWRQQWSGPGTTKRFPETVLARCVKYTEIHPEMRHVDCQSVWDAFKGAFISKHPCNITEEDYQPLMKLGTQTVPCNKILLWSRIKDLAHQFTQVQRDMFTLEDTL
LGYLADDLTWCGEFNTSKINYQSCPDWRKDCSNNPVSVFWKTVSRRFAEAACDVVHVMLNGSRSKIFDKNSTFGSVEVHNLQPEKVQTLEAWVIHGGREDSRDLCQD
PTIKELESIISKRNIQFSCKNIYRPDKFLQCVKNPEDSSCTSEI

Figure 30A

Monomer 1	Monomer 2
F405A	T394F
S364D	Y349K
S364E	L368K
S364E	Y349K
S364F	K370G
S364H	Y349K
S364H	Y349T
S364Y	K370G
T411K	K370E
V397S/F405A	T394F
K370R/T411K	K370E/T411E
L351E/S364D	Y349K/L351K
L351E/S364E	Y349K/L351K
L351E/T366D	L351K/T366K
P395T/V397S/F405A	T394F
S364D/K370G	S364Y/K370R
S364D/T394F	Y349K/F405A
S364E/F405A	Y349K/T394F
S364E/F405S	Y349K/T394Y
S364E/T411E	Y349K/D401K
S364H/D401K	Y349T/T411E
S364H/F405A	Y349T/T394F
S364H/T394F	Y349T/F405A
Y349C/S364E	Y349K/S354C
L351E/S364D/F405A	Y349K/L351K/T394F
L351K/S364H/D401K	Y349T/L351E/T411E
S364E/T411E/F405A	Y349K/T394F/D401K
S364H/D401K/F405A	Y349T/T394F/T411E
S364H/F405A/T411E	Y349T/T394F/D401K

Figure 30B

Monomer 1	Monomer 2
K370E/T411D	T411K
L368E/K409E	L368K
Y349T/T394F/S354C	S364H/F405A/Y349C
T411E	D401K
T411E	D401R/T411R
Q347E/K360E	Q347R
L368E	S364K
L368E/K370S	S364K
L368E/K370T	S364K
L368E/D401R	S364K
L368E/D401N	S364K
L368E	E357S/S364K
L368E	S364K/K409E
L368E	S364K/K409V
L368D	S364K
L368D/K370S	S364K
L368D/K370S	S364K/E357L
L368D/K370S	S364K/E357Q
T411E/K360E/Q362E	D401K
K370S	S364K
L368E/K370S	S364K/E357Q
K370S	S364K/E357Q
T411E/K360D	D401K
T411E/K360E	D401K
T411E/Q362E	D401K
T411E/N390D	D401K
T411E	D401K/Q347K
T411E	D401K/Q347R
T411E/K360D/Q362E	D401K

Figure 30C

Monomer 1	Monomer 2
T411E/K360E/N390D	D401K
T411E/Q362E/N390D	D401K
T411E/Q347R	D401K/K360D
T411E/Q347R	D401K/K360E
T411E/K360	D401K/Q347K
T411E/K360D	D401K/Q347R
T411E/K360E	D401K/Q347K
T411E/K360E	D401K/Q347R
T411E/S364K	D401K/K370S
T411E/K370S	D401K/S364K
Q347E	E357Q
Q347E	E357Q/Q362K
K360D/Q362E	Q347R
K360D/Q362E	D401K
K360D/Q362E	Q347R/D401K
K360E/Q362E	Q347R
K360E/Q362E	D401K
K360E/Q362E	Q347R/D401K
Q362E/N390D	D401K
Q347E/K360D	D401N
K360D	Q347R/N390K
K360D	N390K/D401N
K360E	Y349H
K370S/Q347E	S364K
K370S/E357L	S364K
K370S/E357Q	S364K
K370S/Q347E/E357L	S364K
K370S/Q347E/E357Q	S364K

Figure 30D

Monomer 1	Monomer 2
L368D/K370S/Q347E	S364K
L368D/K370S/E357L	S364K
L368D/K370S/E357Q	S364K
L368D/K370S/Q347E/E357L	S364K
L368D/K370S/Q347E/E357Q	S364K
L368E/K370S/Q347E	S364K
L368E/K370S/E357L	S364K
L368E/K370S/E357Q	S364K
L368E/K370S/Q347E/E357L	S364K
L368E/K370S/Q347E/E357Q	S364K
L368D/K370T/Q347E	S364K
L368D/K370T/E357L	S364K
L368D/K370T/E357Q	S364K
L368D/K370T/Q347E/E357L	S364K
L368D/K370T/Q347E/E357Q	S364K
L368E/K370T/Q347E	S364K
L368E/K370T/E357L	S364K
L368E/K370T/E357Q	S364K
L368E/K370T/Q347E/E357L	S364K
L368E/K370T/Q347E/E357Q	S364K
T411E/Q362E	D401K/T411K
T411E/N390D	D401K/T411K
T411E/Q362E	D401R/T411R
T411E/N390D	D401R/T411R
Y407T	T366Y
F405A	T394W
T366Y/F405A	T394W/Y407T
Y407A	T366W
T366S/L368A/Y407V	T366W
T366S/L368A/Y407V/Y349C	T366W/S354C

Figure 30E

Monomer 1	Monomer 2
K392D/K409D	E356K/D399K
K370D/K392D/K409D	E356K/E357K/D399K
I199T/N203D/K247Q/R355Q/N384S/K392N/V397M/Q419E/K447_	Q196K/I199T/P217R/P228R/N276K
I199T/N203D/K247Q/R355Q/N384S/K392N/V397M/Q419E/K447_	Q196K/I199T/N276K
N384S/K392N/V397M/Q419E	N276K
D221E/P228E/L368E	D221R/P228R/K409R
C220E/P228E/L368E	C220R/E224R/P228R/K409R
F405L	K409R
T366I/K392M/T394W	F405A/Y407V
T366V/K409F	L351Y/Y407A
T366A/K392E/K409F/T411E	D399R/S400R/Y407A
L351K	L351E
I199T/N203D/K247Q/R355Q/Q419E/K447_	Q196K/I199T/P217R/P228R/N276K
I199T/N203D/K247Q/R355Q/Q419E/K447_	Q196K/I199T/N276K
I199T N203D K274Q R355Q N384S K392N V397M Q419E DEL447	
N208D Q295E N384D Q418E N421D	
N208D Q295E Q418E N421D	
Q196K I199T P217R P228R N276K	
Q196K I199T N276K	
E269Q E272Q E283Q E357Q	
E269Q E272Q E283Q	
E269Q E272Q	
E269Q E283Q	
E272Q E283Q	
E269Q	

Figure 31 pI variants

Variant constant region	Substitutions
pI_ISO(-)	I199T N203D K274Q R355Q N384S K392N V397M Q419E DEL447
pI_(-)_Isosteric_A	N208D Q295E N384D Q418E N421D
pI_(-)_Isosteric_B	N208D Q295E Q418E N421D
pI_ISO(+RR)	Q196K I199T P217R P228R N276K
pI_ISO(+)	Q196K I199T N276K
pI_(+)_Isosteric_A	E269Q E272Q E283Q E357Q
pI_(+)_Isosteric_B	E269Q E272Q E283Q
pI_(+)_isosteric_E269Q/E272Q	E269Q E272Q
pI_(+)_isosteric_E269Q/E283Q	E269Q E283Q
pI_(+)_isosteric_E272Q/E283Q	E272Q E283Q
pI_(+)_isosteric_E269Q	E269Q

Figure 32 Ablation variants

Variant	Variant(s), cont.
G236R	P329K
S239G	A330L
S239K	A330S/P331S
S239Q	I332K
S239R	I332R
V266D	V266D/A327Q
S267K	V266D/P329K
S267R	S267R/A327Q
H268K	S267R/P329K
E269R	G236R/L328R
299R	E233P/L234V/L235A/G236del/S239K
299K	E233P/L234V/L235A/G236del/S267K
K322A	E233P/L234V/L235A/G236del/S239K/A327G
A327G	E233P/L234V/L235A/G236del/S267K/A327G
A327L	E233P/L234V/L235A/G236del
A327N	S239K/S267K
A327Q	267K/P329K
L328E	
L328R	
P329A	
P329H	

Figure 33

Positive charged scFv linkers

Name	Sequence	Length	Charge	SEQ ID NO:
Gly-Ser 15	GGGGSGGGGSGGGGS	15	0	150
Whitlow linker	GSTSGSGKPGSGEGSTKG	18	+1	151
6paxA_1 (+A)	IRPRAIGGSKPRVA	14	+4	152
+B	GKGGSGKGGSGKGGS	15	+3	153
+C	GGKGSGGKGSGGKGS	15	+3	154
+D	GGGKSGGGKSGGGKS	15	+3	155
+E	GKGKSGKGKSGKGKS	15	+6	156
+F	GGGKSGGKGSGKGGS	15	+3	157
+G	GKPGSGKPGSGKPGS	15	+3	158
+H	GKPGSGKPGSGKPGSGKPGS	20	+4	159
+I	GKGKSGKGKSGKGKSGKGKS	20	+8	160

Negative charged scFv linkers

Name	Sequence	Length	Charge	SEQ ID NO:
Gly-Ser 15	GGGGSGGGGSGGGGSGGGGS	20	0	161
3hsc_2 (-A)	STAGDTHLGGEDFD	14	-4	162
-B	GEGGSGEGGSGEGGS	15	-3	163
-C	GGEGSGGEGSGGEGS	15	-3	164
-D	GGGESGGGESGGGES	15	-3	165
-E	GEGESGEGESGEGES	15	-6	166
-F	GGGESGGEGSGEGGS	15	-3	167
-G	GEGESGEGESGEGESGEGES	20	-8	168

Figure 33 continued

scFv Linkers

GGGGSGGGGSGGGGS	(SEQ ID NO: 169)
GGGGSGGGGSGGGGSGGGGS	(SEQ ID NO: 170)
GSTSGSGKPGSGEGSTKG	(SEQ ID NO: 171)
PRGASKSGSASQTGSAPGS	(SEQ ID NO: 172)
GTAAAGAGAAGGAAAGAAG	(SEQ ID NO: 173)
GTSGSSGSGSGGSGSGGGG	(SEQ ID NO: 174)
GKPGSGKPGSGKPGSGKPGS	(SEQ IS NO: 175)

Figure 34

XENP	Heterodimer-skewing variant, Chain 1	Heterodimer-skewing variant, Chain 2	Heterodimer Yield (%)	CH3 Tm (°C)
12757	none	none	52.7	83.1
12758	L368D/K370S	S364K	94.4	76.6
12759	L368D/K370S	S364K/E357L	90.2	77.2
12760	L368D/K370S	S364K/E357Q	95.2	77.5
12761	T411E/K360E/Q362E	D401K	85.6	80.6
12496	L368E/K370S	S364K	91.5	n.d.
12511	K370S	S364K	59.9	n.d.
12840	L368E/K370S	S364K/E357Q	59.5	n.d.
12841	K370S	S364K/E357Q	90.4	n.d.
12894	L368E/K370S	S364K	41.0	n.d.
12895	K370S	S364K	49.3	n.d.
12896	L368E/K370S	S364K/E357Q	73.9	n.d.
12901	K370S	S364K/E357Q	87.9	n.d.

Figure 35

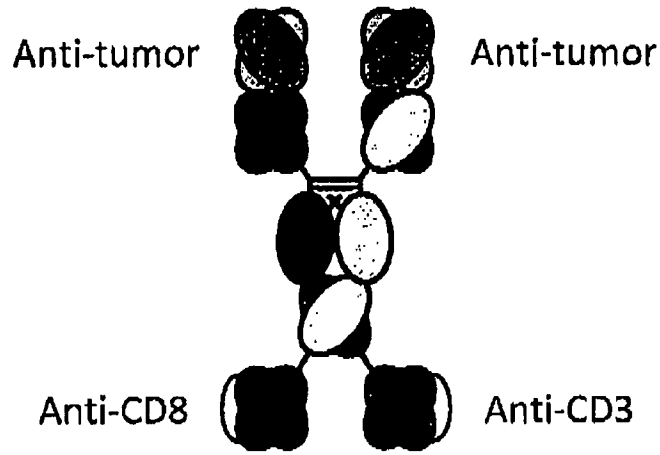


Figure 36

XENP15242 Anti-CD19 x Anti-CD8 x Anti-CD3 Heavy Chain 1 (Anti-CD19-Anti-CD8 scFv (4G7_H1.227/51.1_H1L1)) (SEQ ID NO: 176)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGGGLEWIGYINPYNDGTKYNEKFKGRVTISSDKSKST
AYMELSSLRSEDTAVYYCARGTYYYGTRVFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTKVDKKVEPKSCDKTHTCPPCPAPPV
AGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSQIQLVQS
GAEVKKPGASVKVSCKASGYSFTNFGMIWVRQAPGQGLEWMGWINTYTGEPTYADGFTGRFVFSLDTSVNTAYLQIS
SLKAEDTAVYFCARKDYAGFFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDILMTQSPSSLSASVGDRVTITCQASQDI
GSNMGWLQQKPGKSFKALIYHGTNLEYGVPSRFSGSGSGADYTLTISSLQPEDFATYYCVQFAQFPYTFGGGTKVEIK

XENP15242 Anti-CD19 x Anti-CD8 x Anti-CD3 Heavy Chain 2 (Anti-CD19-Anti-CD3 scFv (4G7_H1.227/αCD3_H1.31_L1.47)) (SEQ ID NO: 177)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGGGLEWIGYINPYNDGTKYNEKFKGRVTISSDKSKST
AYMELSSLRSEDTAVYYCARGTYYYGTRVFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPPV
AGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSEVQLV
ESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYL
QMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSQAVVTQEPSLTVSP
GGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCAL
WYSNHWVFGGGTKLTVL

XENP15242 Anti-CD19 x Anti-CD8 x Anti-CD3 Light Chain (Anti-CD19 (4G7_L1.199)) (SEQ ID NO: 178)

DIVMTQSPATLSLSPGERATISCRSSKSLQNVNGNTYLYWFQQKPGQSPKLLIYRASNLNSGVPDRFSGSGSGTEFTLTIS
SLEPEDFAVYYCMQHLEYPITFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS
GNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 37

XENP15243 Anti-CD38 x Anti-CD8 x Anti-CD3 Heavy Chain 1 (Anti-CD38-Anti-CD8 scFv (OKT10_H1/51.1_H1L1)) (SEQ ID NO: 179)

EVQLVESGGGLVQPGGSLRLSCAASGFDFSRSWMNWVRQAPGKGLEWVSEINPDSSTINYATSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCARYGNWFPYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTKVDKKVEPKSCDKTHTCPPCPAPPVAG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSQIQLVQSGAE
VKKPGASVKVSCKASGYSFTNFGMIWVRQAPGQGLEWMGWINTYTGEPTYADGFTGRFVFSLDTSVNTAYLQISSLK
AEDTAVYFCARKDYAGFFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDILMTQSPSSLSASVGDRVTITCQASQDIGS
NMGWLQQKPGKSFKALIYHGTNLEYGVPSRFSGSGSGADYTLTISSLQPEDFATYYCVQFAQFPYTFGGGTKVEIK

XENP15243 Anti-CD38 x Anti-CD8 x Anti-CD3 Heavy Chain 2 (Anti-CD38-Anti-CD3 scFv (OKT10_H1/αCD3_H1.31_L1.47)) (SEQ ID NO: 180)

EVQLVESGGGLVQPGGSLRLSCAASGFDFSRSWMNWVRQAPGKGLEWVSEINPDSSTINYATSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCARYGNWFPYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPPVAG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSEVQLVESG
GGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQM
NSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSQAVVTQEPSLTVSPGGT
VTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYS
NHWVFGGGTKLTVL

XENP15243 Anti-CD38 x Anti-CD8 x Anti-CD3 Light Chain (Anti-CD38 (OKT10_L1)) (SEQ ID NO: 181)

DIVMTQSPSSLSASVGDRVTITCRASQNVDTNVAWYQQKPGQSPKALIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQP
EDFATYFCQQYDSYPLTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 38

XENP15244 Anti-CD20 x Anti-CD8 x Anti-CD3 Heavy Chain 1 (Anti-CD20-Anti-CD8 scFv (C2B8_H1/S1.1_H1L1)) (SEQ ID NO: 182)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWMGAIYPGNGDTSYNQKFQGRVTITADKSI
STAYMELSSLRSEDTAVYYCARSTYYGGDWYFNVWGAGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTKVDKKVEPKSCDKTHTCPPCPA
PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSQIQL
VQSGAEVKKPGASVKVSCKASGYSFTNFGMIWVRQAPGQGLEWMGWINTYTGEPTYADGFTGRFVFSLDTSVNTAY
LQISSLKAEDTAVYFCARKDYAGFFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDILMTQSPSSLSASVGDRVTITCQA
SQDIGSNMGWLQQKPGKSFKALIYHGTNLEYGVPSRFSGSGSGADYTLTISSLQPEDFATYYCVQFAQFPYTFGGGTKV
EIK

XENP15244 Anti-CD20 x Anti-CD8 x Anti-CD3 Heavy Chain 2 (Anti-CD20-Anti-CD3 scFv (C2B8_H1/αCD3_H1.31_L1.47)) (SEQ ID NO: 183)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWMGAIYPGNGDTSYNQKFQGRVTITADKSI
STAYMELSSLRSEDTAVYYCARSTYYGGDWYFNVWGAGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSEV
QLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNT
LYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSQAVVTQEPSLT
VSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYY
CALWYSNHWVFGGGTKLTVL

XENP15244 Anti-CD20 x Anti-CD8 x Anti-CD3 Light Chain (Anti-CD20 (C2B8_L1)) (SEQ ID NO: 184)

QIVLTQSPSSLSASVGDRVTITCRASSSVSYIHWFQQKPGKSPKPLIYATSNLASGVPVRFSGSGSGTDYTLTISSLQPEDF
ATYYCQQWTSNPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE
SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 39

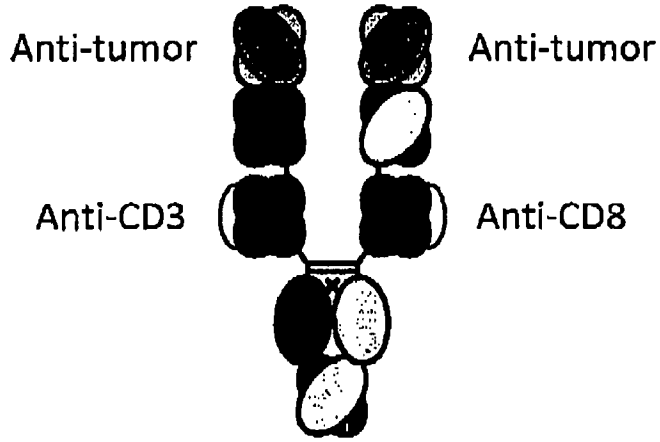


Figure 40

XENP15264 Anti-CD19 x Anti-CD8 x Anti-CD3 Heavy Chain 1 (Anti-CD19 Fab-Anti-CD8 scFv-Fc (4G7_H1.227/51.1_H1L1)) (SEQ ID NO: 185)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGGGLEWIGYINPYNDGTKYNEKFKGRVTISSDKSKST
AYMELSSLRSEDTAVYYCARGTYYYGTRVFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTKVDKKVEPKSCGGGGSGGGGSQIQ
LVQSGAEVKKPGASVKVSCKASGYSFTNFGMIWVRQAPGQGLEWMGWINTYTGEPTYADGFTGRFVFSLDTSVNTA
YLQISSLKAEDTAVYFCARKDYAGFFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDILMTQSPSSLSASVGDRVTITCQ
ASQDIGSNMGWLQQKPGKSFKALIYHGTNLEYGVPSRFSGSGSGADYTLTISSLQPEDFATYYCVQFAQFPYTFGGGTK
VEIKGGGGSGGGGSKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHN
AKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLS
PGK

XENP15264 Anti-CD19 x Anti-CD8 x Anti-CD3 Heavy Chain 2 (Anti-CD19 Fab-Anti-CD3 scFv-Fc (4G7_H1.227/αCD3_H1.31_L1.47)) (SEQ ID NO: 186)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGGGLEWIGYINPYNDGTKYNEKFKGRVTISSDKSKST
AYMELSSLRSEDTAVYYCARGTYYYGTRVFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGGGGSGGGGSEV
QLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNT
LYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSQAVVTQEPSLT
VSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYY
CALWYSNHWVFGGGTKLTVLGGGGSGGGGSKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC
SVMHEALHNHYTQKSLSLSPGK

XENP15264 Anti-CD19 x Anti-CD8 x Anti-CD3 Light Chain (Anti-CD19 (4G7_L1.199)) (SEQ ID NO: 187)

DIVMTQSPATLSLSPGERATISCRSSKSLQNVNGNTYLYWFQQKPGQSPKLLIYRASNLNSGVPDRFSGSGSGTEFTLTIS
SLEPEDFAVYYCMQHLEYPITFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS
GNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 41

XENP15265 Anti-CD20 x Anti-CD8 x Anti-CD3 Heavy Chain 1 (Anti-CD20 Fab-Anti-CD8 scFv-Fc (C2B8_H1/51.1_H1L1)) (SEQ ID NO: 188)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWMGAIYPGNGDTSYNQKFQGRVTITADKSI
STAYMELSSLRSEDTAVYYCARSTYYGGDWYFNVWGAGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTKVDKKVEPKSCGGGGSGGGGS
QIQLVQSGAEVKKPGASVKVSCKASGYSFTNFGMIWVRQAPGQGLEWMGWINTYTGEPTYADGFTGRFVFSLDTSV
NTAYLQISSLKAEDTAVYFCARKDYAGFFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDILMTQSPSSLSASVGDRVTI
TCQASQDIGSNMGWLQQKPGKSFKALIYHGTNLEYGVPSRFSGSGSGADYTLTISSLQPEDFATYYCVQFAQFPYTFGG
GTKVEIKGGGGSGGGGSKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVE
VHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKS
LSLSPGK

XENP15265 Anti-CD20 x Anti-CD8 x Anti-CD3 Heavy Chain 2 (Anti-CD20 Fab-Anti-CD3 scFv-Fc (C2B8_H1/αCD3_H1.31_L1.47)) (SEQ ID NO: 189)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWMGAIYPGNGDTSYNQKFQGRVTITADKSI
STAYMELSSLRSEDTAVYYCARSTYYGGDWYFNVWGAGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGGGGSGGGGS
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSK
NTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSQAVVTQEPS
LTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEAD
YYCALWYSNHWVFGGGTKLTVLGGGGSGGGGSKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVK
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

XENP15265 Anti-CD20 x Anti-CD8 x Anti-CD3 Light Chain (Anti-CD20 (C2B8_L1)) (SEQ ID NO: 190)

QIVLTQSPSSLSASVGDRVTITCRASSSVSYIHWFQQKPGKSPKPLIYATSNLASGVPVRFSGSGSGTDYTLTISSLQPEDF
ATYYCQQWTSNPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE
SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 42

XENP15266 Anti-CD38 x Anti-CD8 x Anti-CD3 Heavy Chain 1 (Anti-CD38 Fab-Anti-CD8 scFv-Fc (OKT10_H1/51.1_H1L1)) (SEQ ID NO: 191)

EVQLVESGGGLVQPGGSLRLSCAASGFDFSRSWMNWVRQAPGKGLEWVSEINPDSSTINYATSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCARYGNWFPYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTKVDKKVEPKSCGGGGSGGGGSQIQLVQ
SGAEVKKPGASVKVSCKASGYSFTNFGMIWVRQAPGQGLEWMGWINTYTGEPTYADGFTGRFVFSLDTSVNTAYLQI
SSLKAEDTAVYFCARKDYAGFFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDILMTQSPSSLSASVGDRVTITCQASQ
DIGSNMGWLQQKPGKSFKALIYHGTNLEYGVPSRFSGSGSGADYTLTISSLQPEDFATYYCVQFAQFPYTFGGGTKVEIK
GGGGSGGGGSKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKT
KPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDV
SGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

XENP15266 Anti-CD38 x Anti-CD8 x Anti-CD3 Heavy Chain 2 (Anti-CD38 Fab-Anti-CD3 scFv-Fc (OKT10_H1/αCD3_H1.31_L1.47)) (SEQ ID NO: 192)

EVQLVESGGGLVQPGGSLRLSCAASGFDFSRSWMNWVRQAPGKGLEWVSEINPDSSTINYATSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCARYGNWFPYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGGGGSGGGGSEVQLVE
SGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQ
MNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGKPGSGKPGSGKPGSGKPGSQAVVTQEPSLTVSPG
GTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALW
YSNHWVFGGGTKLTVLGGGGSGGGGSKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGK

XENP15266 Anti-CD38 x Anti-CD8 x Anti-CD3 Light Chain (Anti-CD38 (OKT10_L1)) (SEQ ID NO: 193)

DIVMTQSPSSLSASVGDRVTITCRASQNVDTNVAWYQQKPGQSPKALIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQP
EDFATYFCQQYDSYPLTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 43

OKT8_H1 Anti-CD8 VH (SEQ ID NO: 194)

EVQLQQSGAEVKKPGASVKVSCKASGFNIKDTYIHWVRQAPGKGLEWMGRIDPANDNTLYASKFQGRVTITADTSTN
TAYMELSSLRSEDTAVYYCGRGYGYYVFDHWGQGTTVTVSS

OKT8_L1 Anti-CD8 VL (SEQ ID NO: 195)

DIKMTQSPSSLSASVGDRVTITCRTSRSISQYLAWYQEKPGKTNKLLIYSGSTLQSGIPSRFSGSGSGTDFTLTISSLQPEDF
ATYYCQQHNENPLTFGAGTKLEIK

OKT8_H2 Anti-CD8 VH (SEQ ID NO: 196)

QVQLVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWVRQAPGKGLEWMGRIDPANDNTLYASKFQGRVTITADTSINT
AYMELSRLRSDDTAVYYCGRGYGYYVFDHWGQGTTVTVSS

51.1_H0 Anti-CD8 VH (SEQ ID NO: 197)

QIQLVQSGPELRKPGETVRISCKASGYSFTNFGMIWVKQAPGKGLKWLGWINTYTGEPTYADDLKGRFAFSLETSANTA
YLKINNFKNEDMATYFCARKDYAGFFDYWGQGTTLTVSS

51.1_L0 Anti-CD8 VL (SEQ ID NO: 198)

DILMTQSPSSMSVSLGDTVSITCHASQDIGSNMGWLQQKPGKSFKALIYHGTNLEYGVPSRFSGSGSGADYSLSISSLES
EDFADYYCVQFAQFPYTFGGGTSLEIK

51.1_H1 Anti-CD8 VH (SEQ ID NO: 199)

QIQLVQSGAEVKKPGASVKVSCKASGYSFTNFGMIWVRQAPGQGLEWMGWINTYTGEPTYADGFTGRFVFSLDTSV
NTAYLQISSLKAEDTAVYFCARKDYAGFFDYWGQGTLVTVSS

51.1_L1 Anti-CD8 VL (SEQ ID NO: 200)

DILMTQSPSSLSASVGDRVTITCQASQDIGSNMGWLQQKPGKSFKALIYHGTNLEYGVPSRFSGSGSGADYTLTISSLQP
EDFATYYCVQFAQFPYTFGGGTKVEIK

Figure 44

Kappa constant light chain (CK) (SEQ ID NO: 201)

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC

IgG1 constant heavy chain (CH1-hinge-CH2-CH3) (SEQ ID NO: 202)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY
ICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSLSLSPGK

IgG2 constant heavy chain (CH1-hinge-CH2-CH3) (SEQ ID NO: 203)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTY
TCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVD
GVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK

IgG3 constant heavy chain (CH1-hinge-CH2-CH3) (SEQ ID NO: 204)

ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY
TCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPEN
NYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK

IgG4 constant heavy chain (CH1-hinge-CH2-CH3) (SEQ ID NO: 205)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYT
CNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVD
GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
QKSLSLSLGK

IgG1/2 constant heavy chain (CH1-hinge-CH2-CH3) (SEQ ID NO: 206)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY
ICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSLSLSPGK

Figure 45

XENP13760 7G3_H0L0_Fab_Heavy chain (SEQ ID NO: 207)

EVQLQQSGPELVKPGASVKMSCKASGYTFTDYYMKWVKQSHGKSLEWIGDIIPSNGATFYNQKFKGKATLTVDRSSST
AYMHLNSLTSEDSAVYYCTRSHLLRASWFAYWGQGTLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGS

XENP13760 7G3_H0L0_Fab_Light chain (SEQ ID NO: 208)

DFVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYLQKPGQPPKLLIYWASTRESGVPDRFTGSGSGTDFT
LTISSVQAEDLAVYYCQNDYSYPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

XENP13761 7G3_H1L1_Fab_Heavy chain (SEQ ID NO: 209)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMKWVRQAPGQSLEWMGDIIPSNGATFYNQKFQGRVTMTVDRS
TSTAYMELSSLRSEDTAVYYCTRSHLLRASWFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGS

XENP13761 7G3_H1L1_Fab_Light chain (SEQ ID NO: 210)

DFVMTQSPDSLAVSLGERATINCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYWASTRESGVPDRFTGSGSGTDFTL
TISSLQAEDVAVYYCQNDYSYPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

XENP13961 7G3_H1.107_L1_Fab_Heavy chain (SEQ ID NO: 211)

QVQLQQSGAEVKKPGASVKVSCKASGYTFTDYYMKWVRQAPGQSLEWMGDIIPSNGATFYNQKFKGKATLTVDRST
STAYMELSSLRSEDTAVYYCARSHLLRASWFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGS

XENP13961 7G3_H1.107_L1_Fab_Light chain (SEQ ID NO: 212)

DFVMTQSPDSLAVSLGERATINCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYWASTRESGVPDRFTGSGSGTDFTL
TISSLQAEDVAVYYCQNDYSYPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 45 continued

XENP13963 7G3_H1.109_L1_Fab_Heavy chain (SEQ ID NO: 213)

QVQLQQSGAEVKKPGASVKVSCKASGYTFTDYYMKWVKQSHGKSLEWMGDIIPSNGATFYNQKFKGKATLTVDRSTS
TAYMELSSLRSEDTAVYYCARSHLLRASWFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGS

XENP13963 7G3_H1.109_L1_Fab_Hs Light chain (SEQ ID NO: 214)

DFVMTQSPDSLAVSLGERATINCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYWASTRESGVPDRFTGSGSGTDFTL
TISSLQAEDVAVYYCQNDYSYPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

XENP13965 7G3_H1.107_L1.57_Fab_His Heavy chain (SEQ ID NO: 215)

QVQLQQSGAEVKKPGASVKVSCKASGYTFTDYYMKWVRQAPGQSLEWMGDIIPSNGATFYNQKFKGKATLTVDRST
STAYMELSSLRSEDTAVYYCARSHLLRASWFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGS

XENP13965 7G3_H1.107_L1.57_Fab_His Light chain (SEQ ID NO: 216)

DFVMTQSPDSLAVSLGERATINCKSSQSLLNTGNQKNYLTWYQQKPGQPPKLLIYWASTRESGVPDRFTGSGSGTDFT
LTISSLQAEDVAVYYCQNDYSYPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

XENP13967 7G3_H1.109_L1.57_Fab_His Heavy chain (SEQ ID NO: 217)

QVQLQQSGAEVKKPGASVKVSCKASGYTFTDYYMKWVKQSHGKSLEWMGDIIPSNGATFYNQKFKGKATLTVDRSTS
TAYMELSSLRSEDTAVYYCARSHLLRASWFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGS

XENP13967 7G3_H1.109_L1.57_Fab_His Light chain (SEQ ID NO: 218)

DFVMTQSPDSLAVSLGERATINCKSSQSLLNTGNQKNYLTWYQQKPGQPPKLLIYWASTRESGVPDRFTGSGSGTDFT
LTISSLQAEDVAVYYCQNDYSYPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 46

XENP Number	Variant	Substitution (VH)	Substitution (VL)	Fold improvement in off-rate vs. H1L1	Tm (°C)
13760	7G3_H0L0_Fab_His	H0	L0	7.21	68.0
13761	7G3_H1L1_Fab_His	H1	L1	-----	70.0
13253	7G3_H1.1_L1_Fab_His	D31S	L1	0.88	69.5
13254	7G3_H1.2_L1_Fab_His	D31N	L1	0.95	69.5
13255	7G3_H1.3_L1_Fab_His	D31E	L1	0.83	70.0
13256	7G3_H1.4_L1_Fab_His	D31H	L1	0.81	69.5
13257	7G3_H1.5_L1_Fab_His	D31R	L1	0.47	70.0
13278	7G3_H1.26_L1_Fab_His	S56N	L1	1.12	69.5
13279	7G3_H1.27_L1_Fab_His	S56G	L1	2.05	69.5
13281	7G3_H1.29_L1_Fab_His	S56E	L1	1.32	70.0
13283	7G3_H1.31_L1_Fab_His	S56H	L1	1.43	69.5
13288	7G3_H1.36_L1_Fab_His	N59G	L1	1.69	68.5
13291	7G3_H1.39_L1_Fab_His	N59R	L1	0.45	68.0
13292	7G3_H1.40_L1_Fab_His	N59Y	L1	0.64	68.5
13294	7G3_H1.42_L1_Fab_His	T101A	L1	3.30	71.5
13318	7G3_H1.62_L1_Fab_His	L109Y	L1	1.38	69.0
13331	7G3_H1.75_L1_Fab_His	A111Q	L1	1.00	70.0
13735	7G3_H1.86_L1_Fab_His	Q69K R71K V72A M74L	L1	4.61	68.5
13736	7G3_H1.87_L1_Fab_His	K12V	L1	0.94	68.5
13737	7G3_H1.88_L1_Fab_His	P43H	L1	0.96	70.0
13738	7G3_H1.89_L1_Fab_His	M50I	L1	1.29	70.0
13740	7G3_H1.91_L1_Fab_His	E86H	L1	1.04	70.0
13741	7G3_H1.92_L1_Fab_His	R91T	L1	1.89	70.0

Figure 46 continued

13742	7G3_H1.93_L1_Fab_His	Q1E V5Q A9P V11L K12V	L1	1.04	72.5
13743	7G3_H1.94_L1_Fab_His	R40K A42S P43H Q45K	L1	0.65	72.0
13744	7G3_H1.95_L1_Fab_His	V5Q	L1	1.24	69.0
13344	7G3_H1_L1.3_Fab_His	H1	N31S	2.77	68.5
13347	7G3_H1_L1.6_Fab_His	H1	N31Q	2.15	67.0
13854	7G3_H1_L1.56_Fab_His	H1	S32A	0.68	70.0
13855	7G3_H1_L1.57_Fab_His	H1	S32T	0.84	70.5
13349	7G3_H1_L1.8_Fab_His	H1	S32Q	N.D.	70.5
13350	7G3_H1_L1.9_Fab_His	H1	S32V	0.98	70.5
13351	7G3_H1_L1.10_Fab_His	H1	S32E	0.98	70.8
13352	7G3_H1_L1.11_Fab_His	H1	S32K	1.00	70.5
13353	7G3_H1_L1.12_Fab_His	H1	S32Y	0.86	69.5

Figure 47

XENP Number	Variant	Substitution (VH)	Substitution (VL)	k_{on} ($M^{-1}s^{-1}$)	k_{off}(s^{-1})	K_A (1/M)	K_D (M)	Tm (°C)
13760	7G3_H0L0_Fab_His	H0	L0	6.74E+05	1.08E-04	6.25E+09	1.60E-10	68.0
13761	7G3_H1L1_Fab_His	H1	L1	3.95E+05	8.20E-04	4.81E+08	2.08E-09	70.0
13961	7G3_H1.107_L1_Fab_His	H1 - V5Q R40K A42S P43H Q45K Q69K R71K V72A M74L T101A	L1	6.00E+05	8.84E-05	6.79E+09	1.47E-10	71.0
13963	7G3_H1.109_L1_Fab_His	H1 - V5Q R40K A42S P43H Q45K Q69K R71K V72A M74L T101A	L1	7.45E+05	5.53E-05	1.35E+10	7.42E-11	73.0
13965	7G3_H1.107_L1.57_Fab_His	H1 - V5Q Q69K R71K V72A M74L T101A	S32T	6.12E+05	3.82E-05	1.60E+10	6.24E-11	71.5
13967	7G3_H1.109_L1.57_Fab_His	H1 - V5Q R40K A42S P43H Q45K Q69K R71K V72A M74L T101A	S32T	6.55E+05	6.25E-05	1.05E+10	9.54E-11	73.5

Figure 48

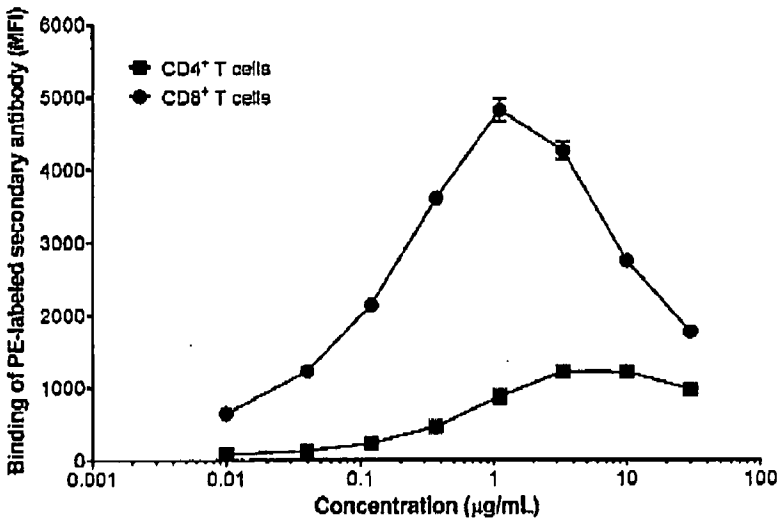
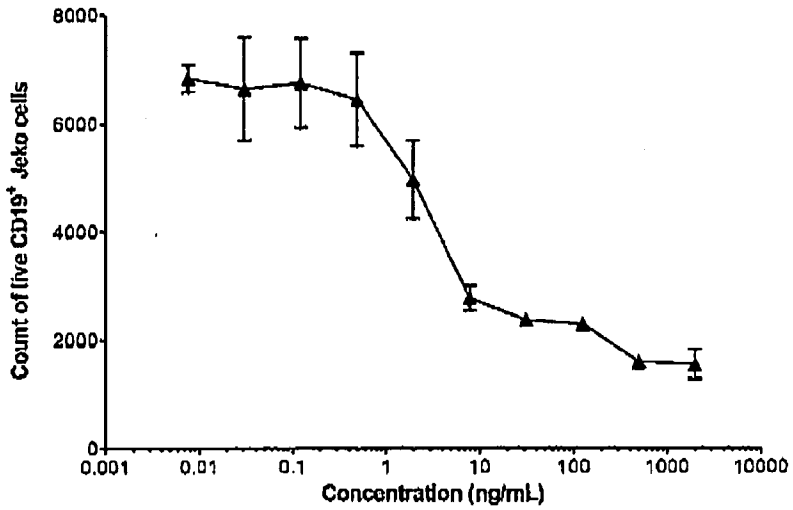

Binding of PE-labeled secondary antibody (MFI)
6000
5000
4000
3000
2000
1000
0
CD4+ T cells
CD8+ T cells
0.001
0.01
0.1
1
10
100
Concentration (µg/mL)

Figure 49


8000
6000
4000
2000
0
Count of live CD19+ Jeko cells
0.001
0.01
0.1
1
10
100
1000
10000
Concentration (ng/mL)

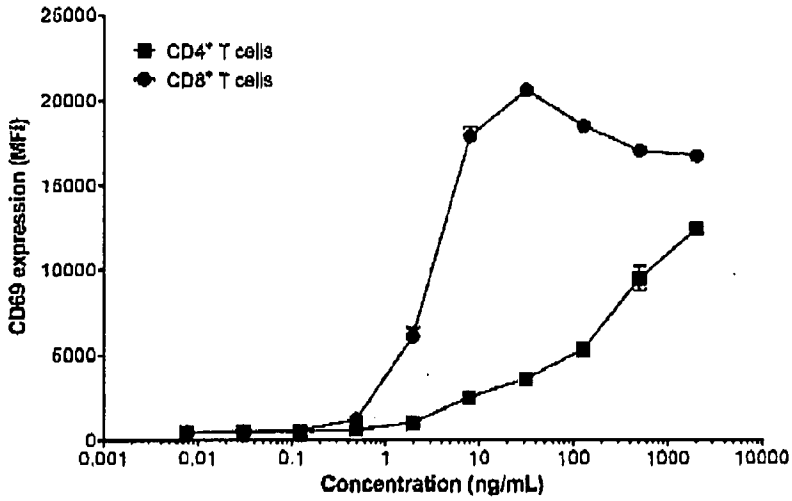

25000
20000
15000
10000
5000
0
CD69 expression (MFI)
CD4+ T cells
CD8+ T cells
0.001
0.01
0.1
1
10
100
1000
10000
Concentration (ng/mL)

Figure 50

		High CD3	High-Int #1 CD3	High-Int #2 CD3	High-Int #3 CD3	Int. CD3	Low CD3
		Anti-CD3 H1.30_L1.47	Anti-CD3 H1.32_L1.47	Anti-CD3 H1.89_L1.47	Anti-CD3 H1.90_L1.47	Anti-CD3 H1.33_L1.47	Anti-CD3 H1.31_L1.47
High CD38	OKT10 H1.77_L1.24	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F
Int CD38	OKT10 H1L1.24	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F
Low CD38	OKT10 H1L1	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F
High CD20	C2B8_H1.202_L1.113	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F
Low CD20	C2B8_H1L1	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F
CD123	7G3_H1.109_L1.57	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F

		High CD3	High-Int #1 CD3	High-Int #2 CD3	High-Int #3 CD3	Int. CD3	Low CD3
		Anti-CD3 H1.30_L1.47	Anti-CD3 H1.32_L1.47	Anti-CD3 H1.89_L1.47	Anti-CD3 H1.90_L1.47	Anti-CD3 H1.33_L1.47	Anti-CD3 H1.31_L1.47
CD8 1	OKT8 H1L1	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F
CD8 2	OKT8 H2L1	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F
CD8 3	51.1 H1L1	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F
CD8 4	51.1H1L2	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F	A, B, C, D, E. F

Figure 51

XENP14484 PMSA Fv region

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVSCKTSGYTFTEYTIHWVRQAPG QSLEWMGNINPNNGGTTYNQKFQGRVTITVDKSTSTAYME LSSLRSEDTAVYYCAAGWNFDYWGQGTLVTVSS	219
vhCDR1	EYTIH	220
vhCDR2	NINPNNGGTTYNQKFQG	221
vhCDR3	GWNFDY	222
Variable light (vl) domain	DIVMTQSPDSLAVSLGERATLSCRASQDVGTAVDWYQQKPD QSPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSLQAEDVA VYFCQQYNSYPLTFGAGTKVEIK	223
vlCDR1	RASQDVGTAVD	224
vlCDR2	WASTRHT	225
vlCDR3	QQYNSYPLT	226
scFv (including charged linker)	EVQLVQSGAEVKKPGASVKVSCKTSGYTFTEYTIHWVRQAPG QSLEWMGNINPNNGGTTYNQKFQGRVTITVDKSTSTAYME LSSLRSEDTAVYYCAAGWNFDYWGQGTLVTVSSGKPGSGKP GSGKPGSGKPGSDIVMTQSPDSLAVSLGERATLSCRASQDVG TAVDWYQQKPDQSPKLLIYWASTRHTGVPDRFTGSGSGTDF TLTISSLQAEDVAVYFCQQYNSYPLTFGAGTKVEIK	227