(12) STANDARD PATENT (11) Application No. **AU 2015323860 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Bispecific molecules comprising an HIV-1 envelope targeting arm

(51) International Patent Classification(s)
C07K 16/10 (2006.01)

(21) Application No: **2015323860** (22) Date of Filing: **2015.09.29**

(87) WIPO No: **WO16/054101**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/206,586	**2015.08.18**	**US**
62/056,834	**2014.09.29**	**US**

(43) Publication Date: **2016.04.07**
(44) Accepted Journal Date: **2021.05.27**

(71) Applicant(s)
The University of North Carolina at Chapel Hill;Duke University;Macrogenics, Inc.

(72) Inventor(s)
Haynes, Barton F.;Ferrari, Guido;Koenig, Scott;Johnson, Leslie S.;Lam, Chia-Ying Kao;Sung, Julia A.;Margolis, David M.;Liu, Liqin;Nordstrom, Jeffrey Lee

(74) Agent / Attorney
Spruson & Ferguson, GPO Box 3898, Sydney, NSW, 2001, AU

(56) Related Art
WO 2014052620 A1

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 April 2016 (07.04.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/054101 A1

(51) International Patent Classification:
C07K 16/10 (2006.01)

(21) International Application Number:
PCT/US2015/053027

(22) International Filing Date:
29 September 2015 (29.09.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/056,834 29 September 2014 (29.09.2014) US
62/206,586 18 August 2015 (18.08.2015) US

(71) Applicants: **DUKE UNIVERSITY** [US/US]; 2812 Erwin Road, Suite 306, Durham, NC 27705 (US). **MACROGENICS, INC.** [US/US]; 9640 Medical Center Drive, Rockville, MD 20850 (US). **UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL** [US/US]; 100 Europa Drive, Suite 430, Chapel Hill, NC 27517 (US).

(72) Inventors: **HAYNES, Barton, F.**; c/o Duke University, 2812 Erwin Road, Suite 306, Durham, NC 27705 (US). **FERRARI, Guido**; c/o Duke University, 2812 Erwin Road, Suite 306, Durham, NC 27705 (US). **KOENIG, Scott**; c/o MacroGenics, Inc., 9640 Medical Center Drive, Rockville, Maryland 20850 (US). **JOHNSON, Leslie, S.**; c/o MacroGenics, Inc., 9640 Medical Center Drive, Rockville, Maryland 20850 (US). **LAM, Chia-Ying, Kao**; c/o MacroGenics, Inc., 9640 Medical Center Drive, Rockville, Maryland 20850 (US). **SUNG, Julia, A.**; University of North Carolina at Chapel Hill, Department of Medicine, 2060 Genetic Medicine Building, CB#7042, 120 Mason Farm Road, Chapel Hill, NC 27599 (US). **MARGOLIS, David, M.**; University of North Carolina at Chapel Hill, Department of Medicine, 2060 Genetic Medicine Building, CB#7042, 120 Mason Farm Road, Chapel Hill, NC 27599 (US).

(74) Agents: **KIM, William, W.** et al.; Wilmer Cutler Pickering Hale and Dorr LLP, 1875 Pennsylvania Avenue, NW, Washington, DC 20006 (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,

[Continued on next page]

(54) Title: BISPECIFIC MOLECULES COMPRISING AN HIV-1 ENVELOPE TARGETING ARM

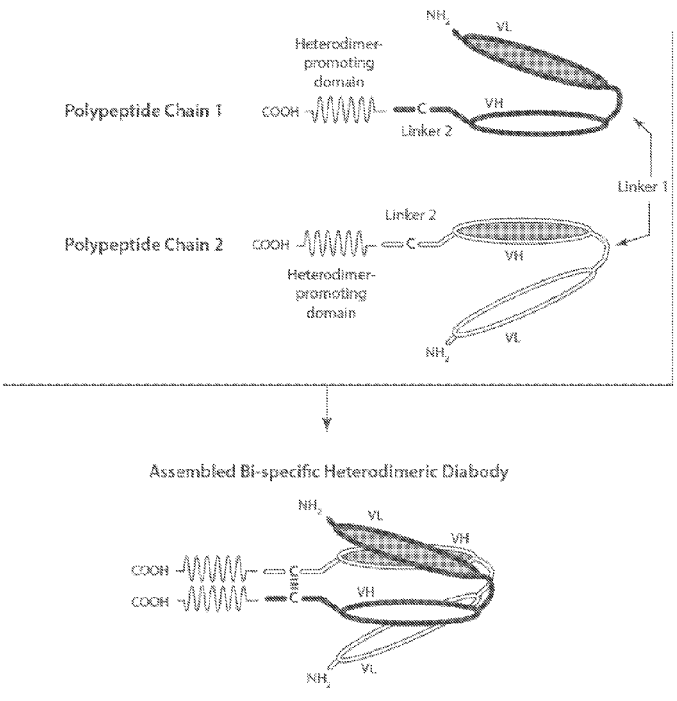


Figure 8A

(57) Abstract: The invention is directed to bispecific molecules comprising an HIV-1 envelope targeting arm and an arm targeting an effector cell, compositions comprising these bispecific molecules and methods of use. In certain aspects, the bispecific molecules of the present invention can bind to two different targets or epitopes on two different cells wherein the first epitope is expressed on a different cell type than the second epitope, such that the bispecific molecules can bring the two cells together. In certain aspects, the bispecific molecules of the present invention can bind to two different cells, wherein the bispecific molecules comprises an arm with the binding specificity of A32, 7B2, CH27, CH28 or CH44.

WO 2016/054101 A1

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).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

Bispecific Molecules Comprising an HIV-1 Envelope Targeting Arm

[0001] This invention claims the benefit of and priority to U.S. Serial No. 62/056,834 filed September 29, 2014, and U.S. Serial No. 62/206,586 filed August 18, 2015, the contents of which are hereby incorporated by reference in their entireties.

[0002] This patent disclosure contains material that is subject to copyright protection. The copyright owner has no objection to the facsimile reproduction by anyone of the patent document or the patent disclosure as it appears in the U.S. Patent and Trademark Office patent file or records, but otherwise reserves any and all copyright rights.

[0003] All patents, patent applications and publications cited herein are hereby incorporated by reference in their entirety. The disclosure of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described herein.

GOVERNMENT SUPPORT

[0004] This invention was made with government support under Grant Nos. U19 AI067854 and UM1 AI100645 awarded by the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

[0005] The invention is directed to HIV-1 antibodies and bispecific molecules comprising an HIV-1 binding domain and an effector cell binding domain, and their uses.

BACKGROUND

[0006] Highly Active Antiretroviral Therapy (HAART) has been effective in reducing the viral burden and ameliorating the effects of HIV-1 infection in infected individuals. However, despite this therapy the virus persists in the individual due to latent reservoir of HIV infected cells which evade this treatment. Thus, there is a need for therapeutic agents for treatment of HIV-1 infected individuals, as well as agents that target virus infected cells and have to potential to reduce the latent reservoir of HIV-1 infected cells.

SUMMARY OF THE INVENTION

[0007] The present invention is directed to bispecific molecules, e.g. covalently linked polypeptide chains to form antibodies, covalent diabodies and/or covalent diabody molecules and their use in the treatment of HIV-1. In certain aspects, the bispecific molecules of the present invention can bind to two different targets or epitopes on two different cells wherein the first epitope is expressed on a different cell type than the second epitope, such that the

bispecific molecules can bring the two cells together. In certain aspects, the bispecific molecules of the present invention can bind to two different cells, wherein the bispecific molecules comprises an arm with the binding specificity of A32, 7B2, CH27, CH28 or CH44, which arm binds to the HIV-1 envelope expressed on a first cell, e.g. HIV infected cell, and a second arm with the binding specificity for an epitope expressed on a different cell type than the first cell, such that the bispecific molecules can bring the two cells together. In certain embodiment, the second cell is in effector cell which expresses CD3 or CD16.

[0008] In certain embodiments an antibody binds specifically to a particular target, even where the specific epitope may not be know, peptide or polysaccharide (such as an antigen present on the surface of a pathogen, for example gpl20, gp41, or CD3) and do not bind in a significant amount to other proteins or polysaccharides present in the sample or subject. Specific binding can be determined by methods known in the art. Various competitive binding assays are known in the art. With reference to an antibody antigen complex, in certain embodiments specific binding of the antigen and antibody has a KD of less than about 10^6 Molar, such as less than about 10^6 Molar, 10^7 Molar, 10^8 Molar, 10^9, or even less than about 10^{10} Molar.

[0009] In certain aspects the invention provides bispecific molecules comprising a first polypeptide chain and a second polypeptide chain, covalently bonded to one another, wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of the A32, 7B2, CH28, or CH44 HIV-1 envelope antibody;

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2), wherein domains (A) and (B) are separated from one another by a peptide linker 1; and

(iii) a domain (C) comprising a heterodimer promoting domain including a K coil or E coil; wherein the heterodimer promoting domain (C) and domain B are separated by a peptide linker 2;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of the A32, 7B2, CH28, or

CH44 HIV-1 antibody, wherein domains (D) and (E) are separated from one another by a peptide linker 1; and

(iii) a domain (F) comprising a heterodimer promoting domain including a K coil or E coil; wherein the heterodimer promoting domain (F) and domain (E) are separated by a peptide linker 2; and wherein:

the domains (A) and (B) do not associate with one another to form an epitope binding site;

the domains (D) and (E) do not associate with one another to form an epitope binding site; and

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like A32, 7B2, CH28, or CH44 antibody (1); and the domains (B) and (D) associate to form a binding site that binds the epitope (2).

[0010] In certain aspects the invention provides bispecific molecules comprising a first polypeptide chain, a second polypeptide chain, and a third polypeptide chain, wherein some of the polypeptides are covalently bonded (See Figure 8), and wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of the A32, 7B2, CH28, or CH44 HIV-1 antibody;

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2), wherein domains (A) and (B) are separated from one another by a peptide linker 1;

(iii) a domain (C) comprising a heterodimer promoting domain including a K coil or E coil; wherein the heterodimer promoting domain (C)and domain B are separated by a peptide linker 2;

(iv) a CH2-CH3 domain, wherein the CH2-CH3 domain and domain (C) are separated by a peptide linker 3 or a spacer-linker 3;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of the A32, 7B2, CH28, or CH44 HIV-1 antibody, wherein domains (D) and (E) are separated from one another by a peptide linker 1;

(iii) a domain (F) comprising a heterodimer promoting domain including a K coil or E coil; wherein the heterodimer promoting domain (F) and domain (E) are separated by a peptide linker 2;

(III) the third polypeptide chain comprises in the N- to C-terminal direction:

(i) a peptide linker 3,

(ii) a CH2-CH3 domain, and wherein:

the domains (A) and (B) do not associate with one another to form an epitope binding site;

the domains (D) and (E) do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope lik A32, 7B2, CH28, or CH44 antibody (1);

the domains (B) and (D) associate to form a binding site that binds the epitope (2); and

the CH2-CH3 domains of the first and third polypeptide form an Fc chain.

[0011] A bispecific molecule comprising a first polypeptide chain, a second polypeptide chain, and a third polypeptide chain, wherein some of the polypeptides are covalently bonded (See Figure 8), and wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

(i) a peptide linker 3 followed by a CH2-CH3 domain;

(ii) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of the A32, 7B2, CH28, or CH44 HIV-1 antibody, wherein the CH2-CH3 domain and domain (A) are separated by a peptide linker 4;

(iii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2), wherein domains (A) and (B) are separated from one another by a peptide linker 1;

(iv) a domain (C) comprising a heterodimer promoting domain including a K coil or E coil; wherein the heterodimer promoting domain (C)and domain B are separated by a peptide linker 2;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of the A32, 7B2, CH28, or CH44

HIV-1 antibody, wherein domains (D) and (E) are separated from one another by a peptide linker 1;

(iii) a domain (F) comprising a heterodimer promoting domain including a K coil or E coil; wherein the heterodimer promoting domain (F) and domain (E) are separated by a peptide linker 2;

(III) the third polypeptide chain comprises in the N- to C-terminal direction:

(i) a peptide linker 3,

(ii) a CH2-CH3 domain, and wherein:

the domains (A) and (B) do not associate with one another to form an epitope binding site;

the domains (D) and (E) do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like A32, 7B2, CH28, or CH44 antibody (1);

the domains (B) and (D) associate to form a binding site that binds the epitope (2); and

the CH2-CH3 domains of the first and third polypeptide form an Fc chain.

[0012] In certain embodiments, the CH2-CH3 domain of polypeptide chain 1 is the of the "knob" design and the CH2-CH3 domain of the third polypeptide chain is of the "hole" design, or vice versa.

[0013] In certain embodiments, the epitope (2) is a CD3 epitope or a CD16 epitope. In certain embodiments, the bispecific molecule binds HIV envelope with the specificity of A32 antibody and also binds CD3. In certain embodiments, the bispecific molecule binds HIV envelope with the specificity of 7B2 antibody and also binds CD3. In certain embodiments, the bispecific molecule binds HIV envelope with the specificity of CH28 antibody and also binds CD3. In certain embodiments, the bispecific molecule binds HIV envelope with the specificity of CH44antibody and also binds CD3. In certain embodiments, the bispecific molecule binds HIV envelope with the specificity of A32 antibody and also binds CD16. In certain embodiments, the bispecific molecule binds HIV envelope with the specificity of 7B2 antibody and also binds CD16. In certain embodiments, the bispecific molecule binds HIV envelope with the specificity of CH28 antibody and also binds CD16. In certain embodiments, the bispecific molecule binds HIV envelope with the specificity of CH44antibody and also binds CD16.

[0014] In certain embodiments, the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope with the binding specificity of the A32, 7B2, CH28, or CH44

antibody. In certain embodiments, the domains (A) and (E) associate to form a binding site that binds the A32, 7B2, CH27, CH28, or CH44 HIV-1 antibody epitope.

[0015] In certain embodiments, the domain (A) binding region of the A32 immunoglobulin (VL1) comprises the VL-A32 CDR3, CDR2, and CDR1. In certain embodiments, wherein the domain (E) binding region of the A32 immunoglobulin (VH1) comprises the VH-A32 CDR3, CDR2, and CDR1. In certain embodiments, the domain (A) binding region of the 7B2 immunoglobulin (VL1) comprises the VL-7B2 CDR3, CDR2, and CDR1. In certain embodiments, the domain (E) binding region of the 7B2 immunoglobulin (VH1) comprises the VH-7B2 CDR3, CDR2, and CDR1. In certain embodiments, the domain (A) binding region of the CH28 immunoglobulin (VL1) comprises the VL-CH28 CDR3, CDR2, and CDR1. In certain embodiments, the domain (E) binding region of the CH28 immunoglobulin (VH1) comprises the VH-CH28 CDR3, CDR2, and CDR1. In certain embodiments, the domain (A) binding region of the CH44 immunoglobulin (VL1) comprises the VL-CH44 CDR3, CDR2, and CDR1. In certain embodiments, the domain (E) binding region of the CH44 immunoglobulin (VH1) comprises the VH-CH44 CDR3, CDR2, and CDR1.

[0016] In certain embodiments, the domain (A) comprises VL-A32, VL-7B2, VL-CH28, or VL-CH44. In certain embodiments, the domain (E) comprises VH-A32, VH-7B2, VH-CH28, VH-CH44.

[0017] In certain embodiments, the first polypeptide comprises SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 25, or SEQ ID NO: 44. In certain embodiments, the second polypeptide comprises SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 23, SEQ ID NO: 27, or SEQ ID NO: 45. In certain embodiments, the bispecific molecule comprises the complementary second polypeptide, and wherein the second polypeptide comprises SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 23, SEQ ID NO: 27 or SEQ ID NO: 45.

[0018] In certain embodiments, the bispecific molecule comprises the first polypeptide of SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 25, or SEQ ID NO: 44 and the second polypeptide of SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 23, SEQ ID NO: 27, or SEQ ID NO: 45.

[0019] In certain embodiments, the bispecific molecule comprises the first polypeptide of SEQ ID NO: 9, and the complementary second polypeptide of SEQ ID NO: 11. In certain embodiments, the bispecific molecule comprises the first polypeptide of SEQ ID NO: 13, and the complementary second polypeptide of SEQ ID NO: 15. In certain embodiments, the bispecific molecule comprises the first polypeptide of SEQ ID NO: 17, and the complementary second polypeptide of SEQ ID NO: 19. In certain embodiments, the bispecific molecule comprises the first polypeptide of SEQ ID NO: 21, and the complementary second polypeptide of SEQ ID NO: 23. In certain embodiments, the bispecific molecule comprises the first polypeptide of SEQ ID NO: 25, and the complementary second polypeptide of SEQ ID NO: 27.

[0020] In certain embodiments, the bispecific molecule comprises consisting essentially of the first polypeptide of SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 25, or SEQ ID NO: 44 and the second polypeptide of SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 23, SEQ ID NO: 27, or SEQ ID NO: 45.

[0021] In certain embodiments, the bispecific molecule comprises consisting of the first polypeptide of SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 25, or SEQ ID NO: 44 and the second polypeptide of SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 23, SEQ ID NO: 27, or SEQ ID NO: 45.

[0022] In certain embodiments, the bispecific molecule comprises SEQ ID NO: 46, 47 and 48. In certain embodiments, the bispecific molecule consists essentially of SEQ ID NO: 46, 47 and 48. In certain embodiments, the bispecific molecule consists of SEQ ID NO: 46, 47 and 48. In certain embodiments, the first polypeptide of the bispecific molecule comprises SEQ ID NO: 46, the second polypeptide of the bispecific molecule comprises SEQ ID NO: 47, and the third polypeptide of the bispecific molecule comprises SEQ ID NO: 48.

[0023] In certain aspects, the invention provides a composition comprising any one of the bispecific molecules or any combination thereof. In certain embodiments, the composition comprises a composition comprising a bispecific molecule comprising a first arm with the binding specificity of HIV-1 antibody A32, HIV-1 antibody7B2, HIV-1 antibody CH28, HIV-1 antibody CH44 and a second arm targeting CD3 or CD16. In certain embodiment, the bispecific molecule comprises an Fc portion or any other modification which extends its serum half-life. In certain embodiments, the composition further comprises a second

bispecific molecule comprising a first arm with the binding specificity of the HIV-1 antibody A32, HIV-1 antibody 7B2, HIV-1 antibody CH28, HIV-1 antibody CH44 and a second arm targeting CD3 or CD16, wherein the first and second bispecific molecules are different.

[0024] In certain aspects, the invention provides a method to treat or prevent HIV-1 infection in a subject in need thereof comprising administering to the subject a composition comprising any one of the bispecific molecules of the invention or a combination of any one of the bispecific molecules in a therapeutically effective amount. In certain embodiments, the methods of claim further comprise administering a latency activating agent. In some embodiments, the latency activating agent is vorinostat, romidepsin , panobinostat, disulfiram, JQ1, bryostatin, PMA, inonomycin, or any combination thereof.

[0025] In certain aspects, the invention provides nucleic acids comprising nucleotides encoding the bispecific molecules of the invention. In certain aspects, the invention provides a vector comprising nucleic acids comprising nucleotides encoding the bispecific molecules of the invention. Provided are also compositions comprising a vector comprising a nucleic acid encoding the bispecific molecules. In certain aspects the invention provide a cell line comprising vectors or nucleic acids encoding the bispecific molecules of the invention , wherein the vectors encode polypeptide chains for expression of the bispecific molecules of the invention, e.g., polypeptide chain 1 and polypeptide chain 2, or polypeptide chain 1, polypeptide chain 2 and polypeptide chain 3. In certain embodiments, the vector is suitable for gene delivery and expression. In certain embodiment, the vector is an adenoviral vector, an adeno associated virus based vector, or a combination thereof.

[0026] In certain aspects, the invention provides a bispecific molecule comprising a polypeptide with a dual affinity retargeting reagent (DART), wherein the DART comprises a diabody molecule comprising a first polypeptide chain and a second polypeptide chain, covalently bonded to one another, wherein:

(A) the first polypeptide chain comprises:

(i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) specific for the first epitope (1); wherein the first VL1 comprises, consists essentially of, consists of the VL or VLCDR1, VLCDR2, and VLCDR3 from A32, 7B2, CH27, CH28, or CH44 HIV-1 antibody,

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a second target, e.g an epitope (2), wherein domains (A) and (B) are separated from one another by a peptide linker; and

(iii) a domain (C) comprising a heterodimer promoting domain;

(B) the second polypeptide chain comprises:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for the first epitope (1); wherein the first VH1 comprises, consists essentially of, consists of the VH or VHCDR1, VHCDR2, and VHCDR3 from A32, 7B2, CH27, CH28, or CH44 HIV-1 antibody, wherein domains (D) and (E) are separated from one another by a peptide linker, and

(iii) a domain (F) comprising a heterodimer promoting domain, and

wherein:

the domains (A) and (B) do not associate with one another to form an epitope binding site;

the domains (D) and (E) do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the A32, 7B2, CH27, CH28, or CH44 HIV-1 antibody epitope (1); the domains (B) and (D) associate to form a binding site that binds the second target, e.g., epitope (2).

[0027] In certain embodiments, the invention provides bispecific molecules, wherein the HIV antibodies VH and VL domains, and the CD3 and CD16 VH and VL domains are in a different orientation. For example, in a non-limiting embodiment, the VL1 domain in polypeptide chain 1 is from CD3, and VH2 domain is from an HIV envelope binding antibody. In this embodiment, the VH1 domain of polypeptide 2 is from CD3, and VL2 domain is from is from an HIV envelope binding antibody.

[0028] In certain aspects, the invention provides a bispecific molecule capable of specific binding to HIV-1 envelope and to an epitope of CD3, wherein the bispecific molecule comprises a first polypeptide chain and a second polypeptide chain, covalently bonded to one another, wherein:

A. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

i. a Domain 1, comprising

(1) a sub-Domain (1A), which comprises a VL Domain of a monoclonal antibody capable of binding to CD3 (VLCD3); and

(2) a sub-Domain (1B), which comprises a VH Domain of a monoclonal antibody capable of binding to HIV-1 (VHHIV-1), wherein the sub-Domains 1A and 1B are separated from one another by a peptide linker (e.g. SEQ ID NO:1);

ii. a Domain 2, wherein the Domain 2 is an E-coil Domain (e.g. SEQ ID NO:7) or a K-coil Domain (e.g. SEQ ID NO:8), wherein the Domain 2 is separated from the Domain 1 by a peptide linker (SEQ ID NO:2); and

B. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

i. a Domain 1, comprising

(1) a sub-Domain (1A), which comprises a VL Domain of a monoclonal antibody capable of binding to HIV-1 (VLHIV-1); and

(2) a sub-Domain (1B), which comprises a VH Domain of a monoclonal antibody capable of binding to CD3 (VHCD3), wherein the sub-Domains 1A and 1B are separated from one another by a peptide linker (e.g. SEQ ID NO:1); and

ii. a Domain 2, wherein the Domain 2 is a K-coil Domain (e.g. SEQ ID NO:8) or an E-coil Domain (SEQ ID NO:7), wherein the Domain 2 is separated from the Domain 1 by a peptide linker (SEQ ID NO:2); and wherein the Domain 2 of the first and the second polypeptide chains are not both E-coil Domains or both K-coli Domains

and wherein:

(a) the VL Domain of the first polypeptide chain and the VH Domain of the second polypeptide chain form an Antigen Binding Domain capable of specifically binding to an epitope of CD3; and

(b) the VL Domain of the second polypeptide chain and the VH Domain of the first polypeptide chain form an Antigen Binding Domain capable of specifically binding to HIV-1 envelope.

[0029] A bispecific molecule capable of specific binding to HIV-1envelope and to an epitope of CD16, wherein the bispecific molecule comprises a first polypeptide chain and a second polypeptide chain, covalently bonded to one another, wherein:

A. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

i. a Domain 1, comprising

(1) a sub-Domain (1A), which comprises a VL Domain of a monoclonal antibody capable of binding to CD16 (VLCD16); and

(2) a sub-Domain (1B), which comprises a VH Domain of a monoclonal antibody capable of binding to HIV-1 (VHHIV-1), wherein the sub-Domains 1A and 1B are separated from one another by a peptide linker (e.g. SEQ ID NO:1);

ii. a Domain 2, wherein the Domain 2 is an E-coil Domain (SEQ ID NO:7) or a K-coil Domain (e.g. SEQ ID NO:8), wherein the Domain 2 is separated from the Domain 1 by a peptide linker (SEQ ID NO:2); and

B. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

i. a Domain 1, comprising

(1) a sub-Domain (1A), which comprises a VL Domain of a monoclonal antibody capable of binding to HIV-1 (VLHIV-1); and

(2) a sub-Domain (1B), which comprises a VH Domain of a monoclonal antibody capable of binding to CD16 (VHCD16), wherein the sub-Domains 1A and 1B are separated from one another by a peptide linker (e.g. SEQ ID NO:1); and

ii. a Domain 2, wherein the Domain 2 is a K-coil Domain e.g. SEQ ID NO:8) or an E-coil Domain (e.g. SEQ ID NO:7), wherein the Domain 2 is separated from the Domain 1 by a peptide linker (SEQ ID NO:2); and wherein the Domain 2 of the first and the second polypeptide chains are not both E-coil Domains or both K-coli Domains

and wherein:

(a) the VL Domain of the first polypeptide chain and the VH Domain of the second polypeptide chain form an Antigen Binding Domain capable of specifically binding to an epitope of CD16; and

(b) the VL Domain of the second polypeptide chain and the VH Domain of the first polypeptide chain form an Antigen Binding Domain capable of specifically binding to HIV-1 envelope.

[0030] In certain embodiments, the bispecific molecule binds to the HIV-1 envelope like the HIV antibody from which it is derived. In certain embodiments, the bispecific molecule binds to the A32-HIV-1 envelope epitope, i.e. the bispecific molecule binds to the HIV-1 envelope like the A32 antibody, and CD3, or CD16. In certain embodiments, the bispecific molecule

binds to the 7B2-HIV1 envelope epitope and CD3, or CD16. In certain embodiments, the bispecific molecule binds to the CH27-HIV-1 envelope epitope and CD3, or CD16. In certain embodiments, the bispecific molecule binds to the CH28-HIV-1 envelope epitope and CD3, or CD16. In certain embodiments, the bispecific molecule binds to the CH44-HIV-1 envelope epitope and CD3, or CD16.

[0031] In certain embodiments, the bispecific molecule has the binding specificity of the A32 HIV-1-envelope antibody. In certain embodiments, the bispecific molecule has the binding specificity of the 7B2 HIV-1-envelope antibody. The bispecific molecule has the binding specificity of the CH27 HIV-1-envelope antibody. The bispecific molecule has the binding specificity of the CH28 HIV-1-envelope antibody. In certain embodiments, the bispecific molecule has the binding specificity of the CH44 HIV-1-envelope antibody.

[0032] In certain embodiments a bispecific molecule of the invention comprises, consists essentially of or consists of sequences as described herein, e.g. Table 2 and Table 3)

[0033] In certain embodiments a bispecific molecule of the invention comprises, consists essentially of or consists of SEQ ID NO: 9 and 11; SEQ ID NO: 13 and 15, SEQ ID NO: 17 and 19; SEQ ID NO; 21 and 23; SEQ ID NO: 25 and 27; SEQ ID NO; 44 and 45 (See Table 2 and Table 3).

[0034] In certain aspects the invention provides compositions comprising any of the bispecific molecule described herein, or a combination thereof. In certain embodiments, these compositions are formulated as pharmaceutical composition for therapeutic use.

[0035] In certain aspects the invention is directed to nucleic acids which encode the bispecific molecules of the invention. In certain embodiments, these nucleic acids are comprised in a vector, and are operably linked to a promoter. In certain aspects the invention provides cell lines, or isolated cells, which comprise nucleic acids for the expression of the bispecific molecules of the invention.

[0036] In certain aspects, the invention provides compositions comprising the bispecific molecules of the invention or nucleic acids encoding the same for use in methods of treating or preventing HIV infection. In some embodiments, these methods further comprise administering a Latency Activating Reagent. Non-limiting examples of these include HDAC inhibitors, e,g, vorinostat, romidepsin, panobinostat, disulfiram, JQ1, bryostatin, PMA, inonomycin, or any combination thereof. In some embodiments, this combination therapy targets the pool of latently infected HIV cells.

[0037] In certain aspects, the invention provides methods treating or preventing an HIV infection in a subject, the method comprising administering to the subject a composition

comprising any one of the bispecific molecules of the invention, or a combination thereof in a therapeutically sufficient amount. In certain embodiments, the methods further comprise administering a latency activating agent.

BRIEF DESCRIPTION OF THE DRAWINGS

[0038] Figure 1 shows potency of ADCC-mediating mAbs. The ADCC activity of the 5 CHAVI mAbs against the 22 HIV-1 IMC is reported as maximum percentage of specific killing. Each dot represent the average activity of all the positive results for each group of mAbs against the individual IMCs. The lines represent the mean± standard deviation. The black line represent the cut-off for positive response.

[0039] Figure 2 shows anti-HIV-1-DARTs-mediated cytotoxic activity. Activated CD4+ T cells from a HIV-1 seronegative donor were infected with HIV-1 subtype B BaL, AE CM235, and C 1086.c IMC (top to bottom). The cells were incubated with autologous resting CD8 T cells in the presence of six concentrations of the anti-HIV-1 (A32xCD3 ◆ and 7B2xCD3 ■) and control (4420xCD3 •) DARTs for 6, 24, and 48 hours at an effector to target cell ratio of 33:1. The results are reported as maximum percentage of specific killing observed at each time point.

[0040] Figure 3 shows dose dependence of anti-HIV-1 BaL DARTs-mediated cytotoxic activity. Activated CD4+ T cells from a HIV-1 seronegative donor were infected with HIV-1 subtype B BaL. The cells were incubated with autologous resting CD8 T cells in the presence of six concentrations of the anti-HIV-1 (A32xCD3 ◆ and 7B2xCD3 ■) and control (4420xCD3 •) DARTs for 48 hours at an effector to target cell ratio of 33, 11, and 3:1 (top to bottom). The results are reported as percentage of specific killing.

[0041] Figure 4. shows DART concentration to reach 50% Specific Killing. Activated CD4+ T cells from a HIV-1 seronegative donor were infected with HIV-1 subtype B BaL, AE CM235, and C 1086.c IMC. The cells were incubated with autologous resting CD8 T cells in the presence of six concentrations of the anti-HIV (A32xCD3, red; 7B2xCD3, blue) and control (4420xCD3, black) DARTs for 48 hours at an effector to target cell ratio of 33:1. Each bar represent the concentration required to detect 50% specific killing against each infected target population.

[0042] Figure 5 shows the sequences of CH27, CH28 and CH44 HIV-1 antibodies. CDRs are indicated in the sequences (SEQ ID Nos: 57-74).

[0043] Figure 6 shows the nucleotide sequences encoding VH and VL chains of A32 antibody and amino acid sequences of VH and VL chains of A32 (SEQ ID Nos 75-78 in order of appearance).

[0044] Figure 7 shows nucleotide sequences encoding VH and VK chains of 7B2 antibody and amino acid sequences of VH and VK chains of 7B2 (SEQ ID NO: 79-82 in order of appearance).

[0045] Figure 8A-C show the structures and domains of the bispecific molecules of the present invention. Figure 8A illustrates the structure of a bispecific molecule composed of two polypeptide chains. Figures 8B and 8C illustrate the structures of two versions of the first, second and third polypeptide chains of a three chain bispecific molecule with an Fc domain (Version 1, Figure 8B; Version 2, Figure 8C).

[0046] Figure 9 shows various sequences : Linker 1 (SEQ ID NO; 1); Linker 2 (SEQ ID NO: 2); Heterodimer promoting domain and K-coil and E coil sequences (SEQ ID Nos: 3-6, 7and 8); Linker 3 (DKTHTCPPCP (SEQ ID No: 49); Linker 4--SEQ ID NOS: 39, 40; CH2-CH3 fragments--SEQ ID Nos; 41-43; CH3 VH chain—SEQ ID NO: 51; CD3VL chain --SEQ ID NO:, 52, CD16VH chain—SEQ ID NO 53, CH16 VL chain—SEQ ID NO: 54; 7B2 VL—SEQ IDNO 55; 7b2 VH-SEQ IDNO 56. SEQ ID Nos: 9-38, 44-48 show various bispecific antibodies (See Table 2).

[0047] Figures 10A-10C show HIVxCD3 DART structure. **(Figures 10A-10B)** These DART molecules contain an anti-HIV-1 binding arm (A32 or 7B2) combined with an anti-CD3 binding arm (hXR32). They are composed of two polypeptide chains: one with the VL of anti-CD3 linked to the VH of anti-HIV; the second with the VL of anti-HIV linked to the VH of anti-CD3. The carboxy termini of the chains have an interchain disulfide bond and paired oppositely charged E-coil/K-coil dimerization domains. Control DARTs have one of the arms replaced by an irrelevant one derived from an anti-FITC antibody (4420) or from an anti-RSV antibody, palivizumab (RSV) sequence. **(Figure 10C)** Schematic representation of HIVxCD3 DART binding to two distinct antigens simultaneously and redirecting the cytotoxic T cells (effectors) to lyse the Env-expressing, HIV-1 infected cells (targets).

[0048] Figures 11A-11F show HIVxCD3 DART binding properties. Figures 11A-11C show antigen binding by ELISA. DART binding to human CD3 protein (Figure 11A), to JR-FL gp140 protein (Figure 11B) or simultaneously to both JR-FL gp140 and human CD3 proteins (Figure 11C). **Figures 11D-11F** show cell surface binding by FACS. DART binding to primary human T cells expressing CD3 (Figure 11D), to HEK293-D371 cells expressing HIV-1 Env, CM244, subtype AE (Figure 11E) or to Jurkat 522-F/Y cells expressing CD3 and

HIV-1 Env, HXBC2, subtype B (Figure 11F). Data are reported as mean fluorescence intensity (MFI). CD3 and Env expression characteristics of the cells are reported in parenthesis. A32 and 7B2 are targeting arms that recognize HIV-1 gp120 and gp41, respectively; CD3 is the effector arm that recognizes CD3ε; 4420 is an irrelevant, negative control arm.

[0049] Figures 12A-12H show HIVxCD3 DART redirected T-cell killing of Env^+ target cells. Figure 12A shows DART concentration dependent killing of Env^+ Jurkat 522-F/Y cells in the presence of human T-cells at an E:T ratio of 10:1 for 48 hours with cytolysis measured by LDH release assay; EC_{50} values were 230 and 160 pg/mL for A32xCD3 and 7B2xCD3, respectively. The control DARTs (A32x4420, 7B2x4420, 4420xCD3) were inactive. **Figure 12B shows** lack of DART mediated killing of Env^+ Jurkat 522-F/Y cells in the absence of effector T-cells with cytolysis measured by LDH release assay. **Figure 12C shows** lack of DART redirected T-cell killing of Env^- Jurkat ΔKS cells at an E:T ratio of 10:1 for 48 hours with cytolysis measured by LDH release assay. **Figure 12D shows** DART concentration dependent killing of Env^+ Jurkat 522-F/Y GF cells in the presence of human T-cells at an E:T ratio of 10:1 for 48 hours with cytolysis measured by LUM assay; EC_{50} values were 172 and 147 pg/mL for A32xCD3 and 7B2xCD3, respectively. **Figures 12E-12G show** 7B2xCD3 DART concentration dependent redirected T cell killing of Env^+ Jurkat 522-F/Y GF cells at different E:T ratios (10:1, 5:1, 1:1) and incubation times (24, 48, 72 hours) with cytolysis measured by LUM assay. **Figure 12H shows** time course of maximal cytolytic activity with 7B2xCD3 at different E:T ratios (data from Figures 12E-12G).

[0050] Figures 13A-13F show HIVxCD3 DARTs redirect T-cell cytotoxicity against CD4+ cells infected with HIV-1 IMCs of different subtypes. Figures 13A-13C show DART concentration dependence. Activated CD4+ T cells from a HIV-1 seronegative donor were infected with HIV-1 subtype B BaL (**Figure 13A**), subtype AE CM235 (**Figure 13B**) or subtype C 1086.C (**Figure 13C**) IMC and incubated for 48 hours with A32xCD3 (red circles), 7B2xCD3 (blue squares) or 4420xCD3 (black diamonds) in the presence of autologous resting $CD8^+$ T cells at an E:T ratio of 33:1 (filled symbols) or in the absence of effector cells (E:T ratio of 0:1) (open symbols). The data are reported as percentage of specific lysis (%SL). DART concentrations ranged from 0.001 to 1000 ng/mL. **Figures 13D-13F** show time course. The data represent the maximal %SL observed at 6, 24, and 48 hours for each DART against CD4+ T cells infected infected with HIV-1 subtype B BaL (**Figure 13D**),

subtype AE CM235 (**Figure 13E**) or subtype C 1086.C (**Figure 13F**) IMC and incubated with autologous resting $CD8^+$ T cells at an E:T ratio of 33:1.

[0051] Figures 14A-14H show HIVxCD3 DARTs induce specific degranulation of CD8+ T-cell. **Figures 14A-14D** show schematic of gating strategy to identify Live/CD3+CD8+CD107+ T cells after their incubation with HIV-1 BaL infected target cells in presence of DARTs for 6 hours. **(Figures 14E-14G)** Dot plots represent the percentage of Live/$CD3^+CD8^+CD107^+$ cells observed in presence of 1 ng/mL of 4420xCD3 (**Figure 14E**), 7B2xCD3 (**Figure 14F**) or A32xCD3 (**Figure 14G**). **Figure 14H** shows frequency of the $CD3^+CD4^-CD8^+CD107^+$ T cells observed in each of the five HIV-1 seronegative healthy donors after 6 hours of incubation with the autologous infected $CD4^+$ T cells using the E:T ratio of 33:1. Each symbol represents the average of duplicate stimulations performed for each donor. The lines represent the mean ± standard deviation. * indicates $p<0.05$ after Dunnett's test for multiple comparisons.

[0052] Figures 15A-15C show viral clearance assay to assess HIVxCD3 DART redirected CD8+ T cell killing of autologous JR-CSF-infected CD4+ T cells from healthy HIV seronegative donors. Activated $CD4^+$ T cells from HIV seronegative donors were infected with HIV-1 clone JR-CSF and then incubated with autologous resting $CD8^+$ T effector cells at an E:T ratio of 1:1 in the absence (No DART) or presence of HIVxCD3 or control DARTs at a concentration of 100ng/mL for 7 days. Results are shown for two healthy donors (**Figures 15A-15B**), as well as for healthy donor 2 in the presence of integrase and non-nucleoside reverse transcriptase inhibitors during the co-culture period to inhibit virus replication (**Figure 15C**). Each bar represents the absolute p24 concentration detected in culture supernatants. Error bars represent standard error mean (SEM) of n=3. * indicates $p<0.05$ with Dunnett's test for multiple comparisons.

[0053] Figures 16A-16H show viral clearance assay detects HIVxCD3 DART redirected CD8+ T-cell clearance of JR-CSF or autologous reservoir (AR) virus-infected CD4+ cells using lymphocytes from HIV-infected ART suppressed patients. $CD4^+$ depleted T cells from HIV-infected ART suppressed patients were activated with PHA and infected with HIV-1 subtype B clone JR-CSF (**Figures 16A-16C**) or autologous reservoir (AR) virus isolates (**Figures 16D-16F**) and then incubated without (**Figures 16A, 16D**) or with autologous $CD8^+$ T effector cells at E:T ratios of 1:10 (**Figures 16B, 16E**) or 1:1 (**Figures 16C, 16F**) in the absence (No DART) or presence of HIVxCD3 (A32xCD3, 7B2xCD3) or control (7B2x4420, 4420xCD3) DARTs at a concentration of 100ng/mL for 7 days. 'Combo' indicates a 1:1 cocktail of 7B2xCD3 and A32xCD3 at a total concentration of 100ng/mL. Each bar

represents the log fold reduction of p24 detected in culture supernatants, calculated as the log (p24 of infected target cells only control divided by p24 of the test condition). **Figure 16G** shows schematic of gating strategy to identify Live/CD3+CD4+CD107+ Effector (TFL4-) T cells after their incubation with HIV-1 JR-CSF infected target cells in presence of DARTs for 6 hours. **Figure 16H** shows the % of live/effector cells (TFL4 negative)/CD3+/CD4+/107a+ cells following a 6 hour incubation with the indicated DARTs and JR-CSF infected targets in n=4 patients. Error bars represent SEM of n=8 (**Figures 16A-16C,** except for combo n=5 and 7B2x4420 n=6), n=5 (**Figures 16D-16F**), and n=4 (**Figures 16G-16H**). * indicates $p<0.05$ with Dunnett's test for multiple comparisons.

[0054] Figures 17A-17B show latency clearance assay to assess HIVxCD3 DART redirected $CD8^+$ T-cell activity. Resting $CD4^+$ T cells from HIV-infected, ART suppressed patients were incubated with PHA (**Figure 17A**) or vorinostat (**Figure 17B**), plated in 12-36 replicate wells depending on the size of the patient's latent reservoir, and co-cultured with autologous $CD8^+$ T cells at an E:T ratio of 1:10 in the absence or presence of HIVxCD3 or control DARTs at 100ng/mL for 24 hours (or up to 96 hours where indicated), after which DARTs were washed off and CD8-depleted PBMCs from a seronegative donor were added to amplify residual virus. Wells were assessed for the presence or absence of p24 by ELISA at day 15. 'Combo' indicates a 1:1 cocktail of 7B2xCD3 and A32xCD3 at a total concentration of 100ng/mL. Results are shown as % viral recovery (# of positive wells/total number plated), normalized to a control in which no $CD8^+$ T cells are added. Dashed lines indicate undetectable viral recovery. NT indicated the conditions that were not tested due to low cell availability according to the table shown in Figure 21.

[0055] Figure 18 shows a list of IMC by HIV-1 Subtypes and Neutralization Tier.

[0056] Figure 19 shows Equilibrium Dissociation Constants (K_D) for Binding of A32xCD3 and 7B2xCD3 to Recombinant Env and CD3 Protein.

[0057] Figure 20 shows Clinical Characteristics.

[0058] Figure 21 shows that DARTs redirect patient T cells against JR-CSF infected autologous target cells and absolute p24 concentration.

[0059] Figure 22 shows Absolute # of Positive Wells in Latency Clearance Assay with DARTs.

[0060] Figure 23 shows potency and breadth of ADCC-mediating mAbs. The ADCC activities of the A32 (anti-gp120 C1/C2) mAb (◆) and 7B2 (anti-gp41 cluster I) mAb (■) are reported as maximum percentage of specific lysis (%SL) against each of the 22 HIV-1 IMC . Each dot represents one HIV-1 IMC. The results obtained with plasma from one HIV-1

seropositive (positive control; pos ctrl) and one seronegative (negative control; neg ctrl) donor are also reported. The lines represent the mean ± standard deviation. The black line represents the cut-off for positive response.

[0061] Figure 24 shows conservation of HIV-1 *Env* residues known to influence the binding of 7B2 and A32 mAbs. A linear 7-residue sequence in gp41 (gp160 positions 598-604; immunodominant cluster I) is reported to contain the binding site for 7B2 mAb *(28, 29)*. Discontinuous residues in gp120 C1-C4 known to influence A32 mAb binding (based on point mutagenesis studies) occur at positions 52, 53, 66, 69, 83, 86, 96, 100, 103, 107, 112, 215, 217, 252, 256, 262, 427 and 479 *(37, 39, 68)*. The conservation of these residues in the Los Alamos National Laboratory (LANL) HIV1 Env Amino acid Filtered web alignment, a database consisting of 4556 HIV-1 Env sequences with representation of all subtypes, was assessed by QuickAlign analysis (http://www.hiv.lanl.gov/content/sequence/QUICK_ALIGNv2/QuickAlign.html). The height of the residue at each position of Env is proportional to its frequency of distribution among the HIV-1 isolates. Residues are colored according to hydrophobicity: black, hydrophilic; green, neutral; blue, hydrophobic. Based on a crystal structure of a CD4-stabilized gp120 core complexed with a Fab fragment of N5-i5 (an A32-like mAb), residues at 52, 53, 69, 103, 107 and 217 (located in C1-C2) may be direct epitope contacts *(27)*.

[0062] Figure 25 shows cell surface Env binding of A32x4420 and 7B2x4420 control DARTs. DART binding to HEK293-D371 cells expressing HIV-1 Env, CM244, subtype AE was measured and data are reported as mean fluorescence intensity (MFI). A32 and 7B2 are targeting arms that recognize HIV-1 gp120 and gp41, respectively; 4420 is an irrelevant, negative control arm.

[0063] Figures 26A-26D show HIVxCD3 DART-mediated T-cell activation depends on co-engagement with target cells. Unstimulated $CD4^+$ or $CD8^+$ T-cells from healthy seronegative donors were incubated with (**Figures 26A, 26C**) and without (**Figures 26B, 26D**) Env expressing Jurkat-522 F/Y cell line in the absence or presence of control (RSVxCD3) or HIVxCD3 (A32xCD3, 7B2xCD3) DARTs at 40, 0.32, and 0 ng/mL for 48 hours. $CD8^+$ (**Figures 26A-26B**) and $CD4^+$ (**Figures 26C-26D**) T cell activation was assessed by staining with CD25 Ab cells. The data are reported as frequency (%) of activated ($CD25^+$) T cells. Each bar represent the average of results obtained from 2 different donors.

[0064] Figure 27 shows HIV DARTs bind specifically to HIV-1 IMC infected $CD4^+$ T cells. Activated $CD4^+$ T cells obtained from healthy HIV-1 seronegative donors were infected for 48 hours with HIV-1 IMCs representing the HIV-1 subtype B BaL, AE CM235, and C

1086.C as reported in the methods section. Non-infected $CD4^+$ T cells (mock) were utilized as negative control. The cells were stained using the 7B2x4420 and A32x4420 DART where the CD3 arm was substituted with the irrelevant 4420 protein to avoid binding to the CD3 receptor. After incubation with the DART, the cells were stained with the secondary anti-EK-IgG2a-biotinylated complex to reveal binding of the DARTs. The staining with 7B2 and A32 mAbs, utilizing an indirect staining technique with the secondary mouse anti-human-IgG mAb, was performed as control. The secondary fluoresceinated anti-human IgG Abs and the Palivizumab mAb were utilized as negative controls. The frequency of infected cells was determined by intracellular staining using the anti-p24 mAb as reported in the method section. Each bar represents $CD4^+$ T cells infected with the IMCs and controls as indicated above the graph. The results are reported as frequency (%) of viable infected ($p24^+$) $CD4^+$ T cells that were stained by each of the DARTs, mAbs, and controls as listed on the x-axis.

[0065] **Figures 28A-28D** show lack of HIVxCD3 DART effects on T cell viability or activation status in the absence of added target cells using PBMC from HIV-1 infected donors. Unstimulated $CD4^+$ or $CD8^+$ T-cells from HIV-infected, ART suppressed were incubated in the absence or presence of control (4420xCD3, 7B2x4420, A32x4420) or active (A32xCD3, 7B2xCD3) DARTs at 100ng/mL for 7 days. (**Figures 28A-28B**) T cell viability was assessed by staining cells for Annexin V/7-AAD. Viable cells were identified as those that were Annein V and 7-AAD negative. (**Figures 28C-28D**) T cell activation was assessed by staining cells for HLA-DR and CD25 expression. Data points for both analyses are from n=3 patients performed on 3 independent occasions. Error bars represent standard error mean.

DETAILED DESCRIPTION

[0066] Highly active anti-retroviral therapy (HAART) alone or in combination with latency reversing agents fails to reduce the pool of latently infected cells. This is due to limited ability of the CD8+ T cells to eliminate HIV-1 latently infected cells. Dual Affinity Re-Targeting proteins (DARTs) are bispecific, antibody-based molecules that can bind two distinct antigens simultaneously. HIV-1 DARTs contain an HIV-1 binding arm combined with an effector cell binding arm, and are designed to redirect cytotoxic CD3+ T cells to engage and kill HIV-infected cells. A panel of monoclonal antibodies (mAbs) was studied to determine their magnitude and breadth of mediating ADCC against 22 different isolates. The goals were to: 1) identify mAbs that could be used as the HIV-1 binding arms of DARTs; 2) test the resulting DARTs for their ability to mediate killing of HIV-1 infected cells. Provided herein are data

related to the potency of the different groups of ADCC-mediating mAbs and the resulting DARTs against HIV-1 Infectious Molecular Clones (IMC)-infected target cells.

[0067] Antibodies and Other Binding Molecules

[0068] ***Antibodies***

[0069] The invention provides polyclonal or monoclonal antibodies, variants, fusion proteins comprising an antibody portion with an antigen recognition site of the required specificity, humanized antibodies, and chimeric antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity. Throughout this application, the numbering of amino acid residues of the light and heavy chains of antibodies is according to the EU index as in Kabat *et al.* (1992) SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, National Institutes of Health Publication No. 91-3242. In some embodiments, antigen-binding fragment of an antibody is a portion of an antibody that possesses an at least one antigen recognition site. Fragments include for example but not limited to Fab, Fab', $F(ab')_2$ Fv), and single chain (scFv).

[0070] Monoclonal antibodies are known in the art. In certain embodiments, monoclonal antibody encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', $F(ab')_2$ Fv), single chain (scFv), mutants thereof, fusion proteins comprising an antibody portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity and the ability to bind to an antigen. Monoclonal antibodies are not limited as regards to the source of the antibody or the manner in which it is made (*e.g.*, by hybridoma, phage selection, recombinant expression, transgenic animals, *etc.*).

[0071] Methods of making monoclonal antibodies are known in the art. In certain embodiments, the antibodies are produced recombinantly by any means known in the art. In one embodiment, such an antibody is sequenced and the polynucleotide sequence is then cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use. The polynucleotide sequence of such antibodies may be used for genetic manipulation to generate the bi-specific molecules of the invention as well as a chimeric antibody, a humanized antibody, or a caninized antibody, to improve the affinity, or other characteristics of the antibody. The general principle in humanizing an antibody involves retaining the basic sequence of the antigen-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences. There

are four general steps to humanize a monoclonal antibody. These are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable Domains (2) designing the humanized antibody or caninized antibody, *i.e.*, deciding which antibody framework region to use during the humanizing or canonizing process (3) the actual humanizing or caninizing methodologies/techniques and (4) the transfection and expression of the humanized antibody. See, for example, U.S. Patents Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415.

[0072] Bi-Specific Antibodies, Multi-Specific Diabodies and DART™ Diabodies

[0073] The provision of non-mono-specific "diabodies" provides a significant advantage over antibodies: the capacity to co-ligate and co-localize cells that express different epitopes. Bivalent diabodies thus have wide-ranging applications including therapy and immunodiagnosis. Bi-valency allows for great flexibility in the design and engineering of the diabody in various applications, providing enhanced avidity to multimeric antigens, the cross-linking of differing antigens, and directed targeting to specific cell types relying on the presence of both target antigens. Due to their increased valency, low dissociation rates and rapid clearance from the circulation (for diabodies of small size, at or below ~50 kDa), diabody molecules known in the art have also shown particular use in the field of tumor imaging (Fitzgerald *et al.* (1997) *"Improved Tumour Targeting By Disulphide Stabilized Diabodies Expressed In Pichia pastoris,"* Protein Eng. 10:1221). Of particular importance is the co-ligating of differing cells, for example, the cross-linking of cytotoxic T cells to tumor cells (Staerz *et al.* (1985) *"Hybrid Antibodies Can Target Sites For Attack By T Cells,"* Nature 314:628-631, and Holliger *et al.* (1996) *"Specific Killing Of Lymphoma Cells By Cytotoxic T-Cells Mediated By A Bispecific Diabody,"* Protein Eng. 9:299-305).

[0074] Diabody epitope binding domains may also be directed to a surface determinant of a B cell, such as CD19, CD20, CD22, CD30, CD37, CD40, and CD74 (Moore, P.A. *et al.* (2011) "*Application Of Dual Affinity Retargeting Molecules To Achieve Optimal Redirected T-Cell Killing Of B-Cell Lymphoma*," Blood 117(17):4542-4551; Cheson, B.D. *et al.* (2008) "Monoclonal *Antibody Therapy For B-Cell Non-Hodgkin's Lymphoma*," N. Engl. J. Med. 359(6):613-626; Castillo, J. *et al.* (2008) "Newer monoclonal antibodies for hematological malignancies," Exp. Hematol. 36(7):755-768. In many studies, diabody binding to effector cell determinants, *e.g.*, Fcγ receptors (FcγR), was also found to activate the effector cell (Holliger *et al.* (1996) *"Specific* Killing *Of Lymphoma Cells By Cytotoxic T-Cells Mediated By A Bispecific Diabody,"* Protein Eng. 9:299-305; Holliger *et al.* (1999) *"Carcinoembryonic Antigen (CEA)-Specific T-Cell Activation In Colon Carcinoma Induced By Anti-CD3 x Anti-*

CEA Bispecific Diabodies And B7 x Anti-CEA Bispecific Fusion Proteins," Cancer Res. 59:2909-2916; WO 2006/113665; WO 2008/157379; WO 2010/080538; WO 2012/018687; WO 2012/162068). Normally, effector cell activation is triggered by the binding of an antigen bound antibody to an effector cell via Fc-FcγR interaction; thus, in this regard, diabody molecules may exhibit Ig-like functionality independent of whether they comprise an Fc Domain (*e.g.,* as assayed in any effector function assay known in the art or exemplified herein (*e.g.*, ADCC assay)). By cross-linking tumor and effector cells, the diabody not only brings the effector cell within the proximity of the tumor cells but leads to effective tumor killing (see *e.g.*, Cao *et al.* (2003) *"Bispecific Antibody Conjugates In Therapeutics,"* Adv. Drug. Deliv. Rev. 55:171-197).

[0075] The formation of such non-mono-specific diabodies requires the successful assembly of two or more distinct and different polypeptides (*i.e.*, such formation requires that the diabodies be formed through the heterodimerization of different polypeptide chain species). This fact is in contrast to mono-specific diabodies, which are formed through the homodimerization of identical polypeptide chains. Because at least two dissimilar polypeptides (*i.e.*, two polypeptide species) must be provided in order to form a non-mono-specific diabody, and because homodimerization of such polypeptides leads to inactive molecules (Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588), the production of such polypeptides must be accomplished in such a way as to prevent covalent bonding between polypeptides of the same species (*i.e.*, so as to prevent homodimerization) (Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588). The art has therefore taught the non-covalent association of such polypeptides (see, *e.g.*, Olafsen *et al.* (2004) *"Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications,"* Prot. Engr. Des. Sel. 17:21-27; Asano *et al.* (2004) "*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain*," Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588; Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672).

[0076] The art has recognized that bi-specific diabodies composed of non-covalently associated polypeptides are unstable and readily dissociate into non-functional monomers (see, *e.g.*, Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672).

[0077] In the face of this challenge, the invention provides stable, covalently bonded heterodimeric non-mono-specific diabodies, termed DARTs™ (see, *e.g.*, United States Patent Publications No. 2014-0099318; 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2015/026894; WO2015/026892; WO 2015/021089; WO 2014/159940; WO 2012/162068; WO 2012/018687; WO 2010/080538; Moore, P.A. *et al.* (2011) "*Application Of Dual Affinity Retargeting Molecules To Achieve Optimal Redirected T-Cell Killing Of B-Cell Lymphoma*," Blood 117(17):4542-4551; Veri, M.C. *et al.* (2010) "*Therapeutic Control Of B Cell Activation Via Recruitment Of Fcgamma Receptor IIb (CD32B) Inhibitory Function With A Novel Bispecific Antibody Scaffold*," Arthritis Rheum. 62(7):1933-1943; Johnson, S. *et al.* (2010) "*Effector Cell Recruitment With Novel Fv-Based Dual-Affinity Re-Targeting Protein Leads To Potent Tumor Cytolysis And in vivo B-Cell Depletion*," J. Mol. Biol. 399(3):436-449), the contents of which publications are herein incorporated by reference in their entirety. Such diabodies comprise two or more covalently complexed polypeptides and involve engineering one or more cysteine residues into each of the employed polypeptide species. For example, the addition of a cysteine residue to the c-terminus of such constructs has been shown to allow disulfide bonding between the polypeptide chains, stabilizing the resulting heterodimer without interfering with the binding characteristics of the bivalent molecule.

[0078] In some embodiments, each of the two polypeptides of the DART™ comprises three Domains (**Figure 8A**). The first polypeptide comprises: (i) a Domain that comprises a binding region of a light chain variable Domain of the a first immunoglobulin (VL1), (ii) a second Domain that comprises a binding region of a heavy chain variable Domain of a second immunoglobulin (VH2), and (iii) a third Domain that serves to promote heterodimerization with the second polypeptide and to covalently bond the first polypeptide to the second polypeptide of the diabody. The second polypeptide contains a complementary first Domain (a VL2 Domain), a complementary second Domain (a VH1 Domain) and a third Domain that complexes with the third Domain of the first polypeptide chain in order to promote heterodimerization and covalent bonding with the first polypeptide chain. Such molecules are

stable, potent and have the ability to simultaneously bind two or more antigens. They are able to promote redirected T cell (CD3) or NK (CD16) cell mediated killing of cells expressing target antigens.

[0079] In certain aspects, the present invention is directed to HIV-1 x CD3 and HIV-1 x CD16 bi-specific monovalent diabodies that are capable of simultaneous binding to HIV-1 and CD3 or HIV-1 and CD16, and to the uses of such molecules in the treatment of HIV-1 infection.

[0080] In certain embodiments, the HIV-1 x CD3 and HIV-1 x CD16 bi-specific monovalent diabodies of the present invention are composed of two polypeptide chains which associate with one another to form one binding site specific for an epitope of HIV-1 and one binding site specific for an epitope of CD3 or CD16 (see, **Figure 8**), so as to be capable of simultaneously binding to HIV-1 and to CD3 or CD16. Thus, such diabodies bind to a "first antigen," which may be either CD3 or HIV-1, and a "second antigen," which is HIV-1 when the first epitope is CD3, and is CD3 when the first epitope is HIV-1. Alternatively, such diabodies bind to a "first antigen," which may be either CD16 or HIV-1, and a "second antigen," which is HIV-1 when the first epitope is CD16, and is CD16 when the first epitope is HIV-1.

[0081] In certain embodiments as shown in **Figure 8**, the first of such two polypeptide chains will contain, in the N-terminal to C-terminal direction, an N-terminus, the Antigen-Binding Domain of a Light Chain Variable Domain (VL) of a "first" antigen (either CD3 or HIV-1 envelope), the Antigen-Binding Domain of a Heavy Chain Variable Domain (VH) of a second antigen (HIV-1, if the first antigen was CD3; CD3, if the first antigen was HIV-1), a Heterodimerization-Promoting Domain, and a C-terminus. An intervening linker peptide (Linker 1) separates the Antigen-Binding Domain of the Light Chain Variable Domain from the Antigen-Binding Domain of the Heavy Chain Variable Domain. In certain embodiments the Antigen-Binding Domain of the Heavy Chain Variable Domain is linked to the Heterodimerization-Promoting Domain by an intervening linker peptide (Linker 2). In certain embodiments the first of the two polypeptide chains will thus contain, in the N-terminal to C-terminal direction: $VL_{\text{First Antigen}}$ – Linker 1 – $VH_{\text{Second Antigen}}$ – Linker 2 – Heterodimerization-Promoting Domain.

[0082] In certain embodiments, the second of such two polypeptide chains will contain, in the N-terminal to C-terminal direction, an N-terminus, the Antigen-Binding Domain of a Light Chain Variable Domain (VL) of the second antigen, the Antigen-Binding Domain of a Heavy Chain Variable Domain (VH) of the first antigen, a Heterodimerization-Promoting Domain

and a C-terminus. An intervening linker peptide (Linker 1) separates the Antigen-Binding Domain of the Light Chain Variable Domain from the Antigen-Binding Domain of the Heavy Chain Variable Domain. In certain embodiments, the Antigen-Binding Domain of the Heavy Chain Variable Domain is linked to the Heterodimerization-Promoting Domain by an intervening linker peptide (Linker 2). In certain embodiments the second of the two polypeptide chains will thus contain, in the N-terminal to C-terminal direction: $VL_{\text{Second Antigen}}$ – Linker 1 – $VH_{\text{First Antigen}}$ – Linker 2 – Heterodimerization-Promoting Domain.

[0083] The Antigen-Binding Domain of the Light Chain Variable Domain of the first polypeptide chain interacts with the Antigen-Binding Domain of the Heavy Chain Variable Domain of the second polypeptide chain in order to form a functional antigen-binding site that is specific for the first antigen (*i.e.*, either HIV-1 envelope or CD3/CD16). Likewise, the Antigen-Binding Domain of the Light Chain Variable Domain of the second polypeptide chain interacts with the Antigen-Binding Domain of the Heavy Chain Variable Domain of the first polypeptide chain in order to form a second functional antigen-binding site that is specific for the second antigen (*i.e.*, either CD3/CD16 or HIV-1 envelope, depending upon the identity of the first antigen). Thus, the selection of the Antigen-Binding Domain of the Light Chain Variable Domain and the Antigen-Binding Domain of the Heavy Chain Variable Domain of the first and second polypeptide chains are coordinated, such that the two polypeptide chains collectively comprise Antigen-Binding Domains of light and Heavy Chain Variable Domains capable of binding to the intended targets, in certain embodiments e.g. HIV-1 envelope and CD3, or CD16.

[0084] In certain embodiments the length of Linker 1, which separates such VL and VH domains of a polypeptide chain is selected to substantially or completely prevent such VL and VH domains from binding to one another. Thus the VL and VH domains of the first polypeptide chain are substantially or completely incapable of binding to one another. Likewise, the VL and VH domains of the second polypeptide chain are substantially or completely incapable of binding to one another. In certain embodiments this is due to the linker which separates the VH and VL domains. In certain embodiments, the linker is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, but no more than 15 amino acids. In certain embodiments an intervening spacer peptide (Linker 1) has the sequence (SEQ ID NO:1): GGGSGGGG.

[0085] Linker 2 separates the VH Domain of a polypeptide chain from the Heterodimer-Promoting Domain of that polypeptide chain. Any of a variety of linkers can be used for the purpose of Linker 2. In certain embodiments a sequence for such Linker 2 has the amino acid

sequence: GGCGGG (SEQ ID NO:2), which has a cysteine residue that may be used to covalently bond the first and second polypeptide chains to one another via a disulfide bond.

[0086] The formation of heterodimers of the first and second polypeptide chains can be driven by the inclusion of Heterodimerization-Promoting Domains. Such domains include GVEPKSC (SEQ ID NO:3) or VEPKSC (SEQ ID NO:4) on one polypeptide chain and GFNRGEC (SEQ ID NO:5) or FNRGEC (SEQ ID NO:6) on the other polypeptide chain (See US2007/0004909 herein incorporated by reference in its entirety).

[0087] In certain embodiments, the Heterodimerization-Promoting Domains of the present invention are formed from one, two, three or four tandemly repeated coil domains of opposing charge that comprise a sequence of at least six, at least seven or at least eight charged amino acid residues (Apostolovic, B. *et al.* (2008) "*pH-Sensitivity of the E3/K3 Heterodimeric Coiled Coil*," Biomacromolecules 9:3173–3180; Arndt, K.M. *et al.* (2001) "*Helix-stabilized Fv (hsFv) Antibody Fragments: Substituting the Constant Domains of a Fab Fragment for a Heterodimeric Coiled-coil Domain*," J. Molec. Biol. 312:221-228; Arndt, K.M. *et al.* (2002) "*Comparison of In Vivo Selection and Rational Design of Heterodimeric Coiled Coils*," Structure 10:1235-1248; Boucher, C. *et al.* (2010) "*Protein Detection By Western Blot Via Coiled–Coil Interactions*," Analytical Biochemistry 399:138-140; Cachia, P.J. *et al.* (2004) "*Synthetic Peptide Vaccine Development: Measurement Of Polyclonal Antibody Affinity And Cross-Reactivity Using A New Peptide Capture And Release System For Surface Plasmon Resonance Spectroscopy*," J. Mol. Recognit. 17:540-557; De Crescenzo, G.D. *et al.* (2003) "*Real-Time Monitoring of the Interactions of Two-Stranded de novo Designed Coiled-Coils: Effect of Chain Length on the Kinetic and Thermodynamic Constants of Binding*," Biochemistry 42:1754-1763; Fernandez-Rodriquez, J. *et al.* (2012) "*Induced Heterodimerization And Purification Of Two Target Proteins By A Synthetic Coiled-Coil Tag*," Protein Science 21:511-519; Ghosh, T.S. *et al.* (2009) "*End-To-End And End-To-Middle Interhelical Interactions: New Classes Of Interacting Helix Pairs In Protein Structures*," Acta Crystallographica D65:1032-1041; Grigoryan, G. *et al.* (2008) "*Structural Specificity In Coiled-Coil Interactions*," Curr. Opin. Struc. Biol. 18:477-483; Litowski, J.R. *et al.* (2002) "*Designing Heterodimeric Two-Stranded α-Helical Coiled-Coils: The Effects Of Hydrophobicity And α-Helical Propensity On Protein Folding, Stability, And Specificity*," J. Biol. Chem. 277:37272-37279; Steinkruger, J.D. *et al.* (2012) "*The d'--d--d' Vertical Triad is Less Discriminating Than the a'--a--a' Vertical Triad in the Antiparallel Coiled-coil Dimer Motif*," J. Amer. Chem. Soc. 134(5):2626–2633; Straussman, R. *et al.* (2007) "*Kinking the Coiled Coil – Negatively Charged Residues at the Coiled-coil Interface*," J. Molec. Biol.

366:1232-1242; Tripet, B. *et al.* (2002) "*Kinetic Analysis of the Interactions between Troponin C and the C-terminal Troponin I Regulatory Region and Validation of a New Peptide Delivery/Capture System used for Surface Plasmon Resonance*," J. Molec. Biol. 323:345–362; Woolfson, D.N. (2005) "*The Design Of Coiled-Coil Structures And Assemblies*," Adv. Prot. Chem. 70:79-112; Zeng, Y. *et al.* (2008) "*A Ligand-Pseudoreceptor System Based On de novo Designed Peptides For The Generation Of Adenoviral Vectors With Altered Tropism*," J. Gene Med. 10:355-367).

[0088] Such repeated coil domains may be exact repeats or may have substitutions. For example, the Heterodimerization-Promoting Domain of the first polypeptide chain may comprise a sequence of eight negatively charged amino acid residues and the Heterodimerization-Promoting Domain of the second polypeptide chain may comprise a sequence of eight negatively charged amino acid residues. It is immaterial which coil is provided to the first or second polypeptide chains, provided that a coil of opposite charge is used for the other polypeptide chain.

[0089] In certain embodiments an HIV-1x CD3 bi-specific monovalent diabody of the present invention has a first polypeptide chain having a negatively charged coil. The positively charged amino acid may be lysine, arginine, histidine, *etc.* and/or the negatively charged amino acid may be glutamic acid, aspartic acid, *etc.* In certain embodiments the positively charged amino acid is lysine and/or the negatively charged amino acid is glutamic acid. It is possible for only a single Heterodimerization-Promoting Domain to be employed (since such domain will inhibit homodimerization and thereby promote heterodimerization). In certain embodiments both the first and second polypeptide chains of the diabodies of the present invention to contain Heterodimerization-Promoting Domains.

[0090] In certain embodiments, one of the Heterodimerization-Promoting Domains will comprise four tandem "E-coil" helical domains (SEQ ID NO:7: EVAALEK-EVAALEK-EVAALEK-EVAALEK), whose glutamate residues will form a negative charge at pH 7, while the other of the Heterodimerization-Promoting Domains will comprise four tandem "K-coil" domains (SEQ ID NO:8: KVAALKE-KVAALKE-KVAALKE-KVAALKE), whose lysine residues will form a positive charge at pH 7. The presence of such charged domains promotes association between the first and second polypeptides, and thus fosters heterodimerization. In some embodiments, the number of K coil and E coil domains can vary and a skilled artisan can readily determine whether a different number of K-coil or E-coil domain lead to heterodimerization.

[0091] In certain embodiments, the HIV-1 x CD3 or HIV-1 x CD16 bi-specific monovalent diabodies of the present invention are engineered so that their first and second polypeptide chains covalently bond to one another via one or more cysteine residues positioned along their length. Such cysteine residues may be introduced into the intervening linker that separates the VL and VH domains of the polypeptides. Alternatively, Linker 2 may contain a cysteine residue.

[0092] The invention also includes variants of the antibodies (and fragments) disclosed herein, including variants that retain the ability to bind to recombinant Env protein, the ability to bind to the surface of virus-infected cells and/or ADCC-mediating properties of the antibodies specifically disclosed, and methods of using same to, for example, reduce HIV-1 infection risk. Combinations of the antibodies, or fragments thereof, disclosed herein can also be used in the methods of the invention.

[0093] In certain embodiments the invention provides a bispecific antibody. A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites (see, e.g., Romain Rouet & Daniel Christ "Bispecific antibodies with native chain structure" Nature Biotechnology 32, 136–137 (2014); Byrne et al. "A tale of two specificities: bispecific antibodies for therapeutic and diagnostic applications" Trends in Biotechnology, Volume 31, Issue 11, November 2013, Pages 621–632 Songsivilai and Lachmann, Clin. Exp. Immunol., 79:315-321 (1990); Kostelny et al., J. Immunol. 148:1547-53 (1992) (and references therein)). In certain embodiments the bispecific antibody is a whole antibody of any isotype. In other embodiments a bispecific fragment, for example but not limited to $F(ab)_2$ fragment. In some embodiments, the bispecific antibodies do not include Fc portion, which makes these diabodies relatively small in size and easy to penetrate tissues.

[0094] In certain embodiments, the bispecific antibodies could include Fc region. Fc bearing diabodies, for example but not limited to Fc bearing DARTs are heavier, and could bind neonatal Fc receptor, increasing their circulating half-life. See Garber "Bispecific antibodies rise again" Nature Reviews Drug Discovery 13, 799–801 (2014), Figure 1a; See US Pub 20130295121, US Pub 20140099318 incorporated by reference in their entirety. In certain embodiments, the invention encompasses diabody molecules comprising an Fc domain or portion thereof (e.g. a CH2 domain, or CH3 domain). The Fc domain or portion thereof may be derived from any immunoglobulin isotype or allotype including, but not limited to, IgA, IgD, IgG, IgE and IgM. In some embodiments, the Fc domain (or portion thereof) is derived from IgG. In some embodiments, the IgG isotype is IgG1, IgG2, IgG3 or IgG4 or an allotype

thereof. In some embodiments, the diabody molecule comprises an Fc domain, which Fc domain comprises a CH2 domain and CH3 domain independently selected from any immunoglobulin isotype (i.e. an Fc domain comprising the CH2 domain derived from IgG and the CH3 domain derived from IgE, or the CH2 domain derived from IgG1 and the CH3 domain derived from IgG2, etc.). In some embodiments, the Fc domain may be engineered into a polypeptide chain comprising the diabody molecule of the invention in any position relative to other domains or portions of the polypeptide chain (e.g., the Fc domain, or portion thereof, may be c-terminal to both the VL and VH domains of the polypeptide of the chain; may be n-terminal to both the VL and VH domains; or may be N-terminal to one domain and c-terminal to another (i.e., between two domains of the polypeptide chain)).

[0095] Other modification s of the bispecific molecules are contemplated to increase the half-life of the bispecific molecules. In some embodiments, these modifications include addition of a polypeptide portion of a serum binding protein. See US20100174053 A1, incorporated by reference.

[0096] In some embodiments, the Fc variants of the bispecific molecules of the invention are expected to have increased serum half-life compared to the non-Fc variants. Skilled artisan can readily carry out various assays, including pharmacokinetic studies, to determine the half-life of these molecules.

[0097] In some embodiments, the invention encompasses polypeptide chains, each of which polypeptide chains comprise a VH and VL domain, comprising CDRs as described herein. In certain embodiments, the VL and VH domains comprising each polypeptide chain have the same specificity, and the multimer molecule is bivalent and monospecific. In other embodiments, the VL and VH domains comprising each polypeptide chain have differing specificity and the multimer is bivalent and bispecific.

[0098] In some embodiments, the polypeptide chains in multimers further comprise an Fc domain. Dimerization of the Fc domains leads to formation of a diabody molecule that exhibits immunoglobulin-like functionality, i.e., Fc mediated function (e.g., Fc-Fc.gamma.R interaction, complement binding, etc.).

[0099] Formation of bispecific molecule as described supra requires the interaction of differing polypeptide chains. Such interactions are difficult to achieve with efficiency within a single cell recombinant production system, due to the many variants of potential chain mispairings. One solution to increase the probability of mispairings, is to engineer "knobs-into-holes" type mutations into the desired polypeptide chain pairs. Such mutations favor heterodimerization over homodimerization. For example, with respect to Fc-Fc-interactions,

an amino acid substitution (preferably a substitution with an amino acid comprising a bulky side group forming a `knob`, e.g., tryptophan) can be introduced into the CH2 or CH3 domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, i.e., `the hole` (e.g., a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides comprising the diabody molecule, and further, engineered into any portion of the polypeptides chains of the pair. Methods of protein engineering to favor heterodimerization over homodimerization are well known in the art, in particular with respect to the engineering of immunoglobulin-like molecules, and are encompassed herein (see e.g., Ridgway et al. (1996) "`Knobs-Into-Holes` Engineering Of Antibody CH3 Domains For Heavy Chain Heterodimerization," Protein Engr. 9:617-621, Atwell et al. (1997) "Stable Heterodimers From Remodeling The Domain Interface Of A Homodimer Using A Phage Display Library," J. Mol. Biol. 270: 26-35, and Xie et al. (2005) "A New Format Of Bispecific Antibody: Highly Efficient Heterodimerization, Expression And Tumor Cell Lysis," J. Immunol. Methods 296:95-101; each of which is hereby incorporated herein by reference in its entirety).

[0100] The invention also encompasses diabody molecules comprising variant Fc or portion thereof), which variant Fc domain comprises at least one amino acid modification (e.g. substitution, insertion deletion) relative to a comparable wild-type Fc domain or hinge-Fc domain (or portion thereof). Molecules comprising variant Fc domains or hinge-Fc domains (or portion thereof) (e.g., antibodies) normally have altered phenotypes relative to molecules comprising wild-type Fc domains or hinge-Fc domains or portions thereof. The variant phenotype may be expressed as altered serum half-life, altered stability, altered susceptibility to cellular enzymes or altered effector function as assayed in an NK dependent or macrophage dependent assay. Fc domain variants identified as altering effector function are known in the art. For example International Application WO04/063351, U.S. Patent Application Publications 2005/0037000 and 2005/0064514.

[0101] The bispecific diabodies of the invention can simultaneously bind two separate and distinct epitopes. In certain embodiments the epitopes are from the same antigen. In other embodiments, the epitopes are from different antigens. In non-limiting embodiments a at least one epitope binding site is specific for a determinant expressed on an immune effector cell (e.g. CD3, CD16, CD32, CD64, etc.) which are expressed on T lymphocytes, natural killer (NK) cells or other mononuclear cells. In one embodiment, the diabody molecule binds to the effector cell determinant and also activates the effector cell. In this regard, diabody molecules

of the invention may exhibit Ig-like functionality independent of whether they further comprise an Fc domain (e.g., as assayed in any effector function assay known in the art or exemplified herein).

[0102] In certain embodiments, the bispecific antibody comprises an HIV envelope binding fragment, for example but not limited to an HIV envelope binding fragment from any of the antibodies described herein. In other embodiments, the bispecific antibody further comprises a second antigen-interaction-site/fragment. In other embodiments, the bispecific antibody further comprises at least one effector domain.

[0103] In certain embodiments the bispecific antibodies engage cells for Antibody-Dependent Cell-mediated Cytotoxicity (ADCC). In certain embodiments the bispecific antibodies engage natural killer cells, neutrophil polymorphonuclear leukocytes, monocytes and macrophages. In certain embodiments the bispecific antibodies are T-cell engagers. In certain embodiments, the bispecific antibody comprises an HIV envelope binding fragment and CD3 binding fragment. Various CD3 antibodies are known in the art. See for example US Patent 8,784,821, and United States Patent Publications No. 2014-0099318 providing various disclosure on various CD3 antibodies, which disclosure in incorporated by reference in its entirety. In certain embodiments, the bispecific antibody comprises an HIV envelope binding fragment and CD16 binding fragment.

[0104] In certain embodiments the invention provides antibodies with dual targeting specificity. In certain aspects the invention provides bi-specific molecules that are capable of localizing an immune effector cell to an HIV-1 envelope expressing cell, so as facilitate the killing of the HIV-1 envelope expressing cell. In this regard, bispecific antibodies bind with one "arm" to a surface antigen on target cells, and with the second "arm" to an activating, invariant component of the T cell receptor (TCR) complex. The simultaneous binding of such an antibody to both of its targets will force a temporary interaction between target cell and T cell, causing activation of any cytotoxic T cell and subsequent lysis of the target cell. Hence, the immune response is re-directed to the target cells and is independent of peptide antigen presentation by the target cell or the specificity of the T cell as would be relevant for normal MHC-restricted activation of CTLs. In this context it is crucial that CTLs are only activated when a target cell is presenting the bispecific antibody to them, i.e. the immunological synapse is mimicked. Particularly desirable are bispecific antibodies that do not require lymphocyte preconditioning or co-stimulation in order to elicit efficient lysis of target cells.

[0105] In certain embodiments, the invention provides antibodies or fragments comprising a CDR(s) of the VH and/or VL chains, or VH and/or VL chains of the inventive antibodies, as

the HIV-1 binding arm(s) of a bispecific molecules, e.g. but not limited to DARTS, or toxin labeled HIV-1 binding molecules.

[0106] In certain embodiments, such bispecific molecules comprise one portion which targets HIV-1 envelope and a second portion which binds a second target. In certain embodiments, the first portion comprises VH and VL sequences, or CDRs from the antibodies described herein. In certain embodiments, the second target could be, for example but not limited to an effector cell. In certain embodiments the second portion is a T-cell engager. In certain embodiments, the second portion comprises a sequence/paratope which targets CD3. In certain embodiments, the second portion is an antigen-binding region derived from a CD3 antibody, optionally a known CD3 antibody. In certain embodiments, the anti-CD antibody induce T cell-mediated killing. In certain embodiments, the bispecific antibodies are whole antibodies. In other embodiments, the dual targeting antibodies consist essentially of Fab fragments. In other embodiments, the dual targeting antibodies comprise a heavy chain constant region (CH1). In certain embodiments, the bispecific antibody does not comprise Fc region. In certain embodiments, the bispecific antibodies have improved effector function. In certain embodiments, the bispecific antibodies have improved cell killing activity. Various methods and platforms for design of bispecific antibodies are known in the art. See for example US Pub. 20140206846, US Pub. 20140170149, 20100174053, US Pub. 20090060910, US Pub 20130295121, US Pub. 20140099318, US Pub. 20140088295 which contents are herein incorporated by reference in their entirety.

[0107] In certain embodiments the invention provides human, humanized and/or chimeric antibodies. Methods to construct such antibodies are well known in the art.

[0108] In certain aspects the invention provides use of the antibodies of the invention, including bispecific antibodies, in methods of treating and preventing HIV-1 infection in an individual, comprising administering to the individual a therapeutically effective amount of a composition comprising the antibodies of the invention in a pharmaceutically acceptable form. In certain embodiment, the methods include a composition which includes more than one HIV-1 targeting antibody. In certain embodiments, the HIV-1 targeting antibodies in such combination bind different epitopes on the HIV-1 envelope. In certain embodiments, such combinations of bispecific antibodies targeting more than one HIV-1 epitope provide increased killing of HIV-1 infected cells. In other embodiments, such combinations of bispecific antibodies targeting more than one HIV-1 epitope provide increased breadth in recognition of different HIV-1 subtypes.

[0109] The invention also includes variants of the antibodies (and fragments) disclosed herein, including variants that retain the ability to bind to recombinant Env protein, the ability to bind to the surface of virus-infected cells and/or ADCC-mediating properties of the antibodies specifically disclosed, and methods of using same to, for example, reduce HIV-1 infection risk. Combinations of the antibodies, or fragments thereof, disclosed herein can also be used in the methods of the invention.

[0110] Homologs and variants of a VL or a VH of an antibody that specifically binds a polypeptide are typically characterized by possession of at least about 75%, for example at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity counted over the full length alignment with the amino acid sequence of interest. Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity. When less than the entire sequence is being compared for sequence identity, homologs and variants will typically possess at least 80% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% or 95% depending on their similarity to the reference sequence. Methods for determining sequence identity over such short windows are available at the NCBI website on the internet. One of skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologs could be obtained that fall outside of the ranges provided.

[0111] In certain embodiments, the invention provides antibodies which are 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% identical to the VH and VL amino acid sequences of the antibodies described herein and still maintain their epitope binding breadth and/or potency. In certain embodiments, the invention provides antibodies which are 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% identical to the CDR1, 2, and/or 3 of VH and CDR1, 2, and/or 3 VL amino acid sequences of the antibodies described herein and still maintain their epitope binding breadth and/or potency.

[0112] In another aspect, the invention provides Fc bearing bispecific molecules. In some embodiments, the third Domain of one or both of the polypeptides may additionally comprises the sequence of a CH2-CH3 Domain, such that complexing of the diabody polypeptides forms an Fc Domain that is capable of binding to the Fc receptor of cells (such as B lymphocytes, dendritic cells, natural killer cells, macrophages, neutrophils, eosinophils,

basophils and mast cells) (**Figures 8B-8C**). Many variations of such molecules have been described (see, *e.g.*, United States Patent Publications No. 2014-0099318; 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2015/026894; WO 2015/026892; WO 2015/021089; WO 2014/159940; WO 2012/162068; WO 2012/018687; WO 2010/080538), the content of each of these publications in herein incorporated by reference in its entirety.

[0113] In some embodiments, these Fc-bearing DARTs may comprise three polypeptide chains. The first polypeptide of such a diabody contains three Domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody's first polypeptide chain and (iv) a Domain containing a CH2-CH3 sequence. The second polypeptide of such DART™ contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody's first polypeptide chain. The third polypeptide of such DART™ comprises a CH2-CH3 sequence. Thus, the first and second polypeptide chains of such DART™ associate together to form a VL1/VH1 binding site that is capable of binding to the epitope, as well as a VL2/VH2 binding site that is capable of binding to the second epitope. The first and second polypeptides are bonded to one another through a disulfide bond involving cysteine residues in their respective third Domains. Notably, the first and third polypeptide chains complex with one another to form an Fc Domain that is stabilized via a disulfide bond. Such diabodies have enhanced potency. Such Fc-bearing **DARTs™** may have either of two orientations (**Table 1**):

Table 1

First Orientation	3rd Chain	NH_2-CH2-CH3-COOH
	1st Chain	NH_2-VL1-VH2-Heterodimer Promoting Domain-CH2-CH3-COOH
	2nd Chain	NH_2-VL2-VH1-Heterodimer Promoting Domain-COOH
Second Orientation	3rd Chain	NH_2-CH2-CH3-COOH
	1st Chain	NH_2-CH2-CH3- VL1-VH2-Heterodimer Promoting Domain-COOH
	2nd Chain	NH_2-VL2-VH1-Heterodimer Promoting Domain-COOH

[0114] HIV x CD3 bi-specific monovalent Fc diabodies that are composed of three polypeptide chains which associate with one another to form one binding site specific for an epitope of HIV and one binding site specific for an epitope of CD3 (see, **Figure 8B-8C**), so as to be capable of simultaneously binding to HIV and to CD3. Thus, such diabodies bind to a "first antigen," which may be either CD3 or HIV, and a "second antigen," which is HIV when the first epitope is CD3, and is CD3 when the first epitope is HIV.

[0115] As shown in **Figure 8B**, the first of such three polypeptide chains will contain, in the N-terminal to C-terminal direction, an N-terminus, the Antigen-Binding Domain of a Light Chain Variable Domain (**VL**) of a "first" antigen (either CD3 or HIV), the Antigen-Binding Domain of a Heavy Chain Variable Domain (**VH**) of a second antigen (HIV, if the first antigen was CD3; CD3, if the first antigen was HIV), a Heterodimerization-Promoting Domain, and a C-terminus. An intervening linker peptide (**Linker 1**) separates the Antigen-Binding Domain of the Light Chain Variable Domain from the Antigen-Binding Domain of the Heavy Chain Variable Domain. In non-limiting embodiments, the Antigen-Binding Domain of the Heavy Chain Variable Domain is linked to the Heterodimerization-Promoting Domain by an intervening linker peptide (**Linker 2**). In the case of an HIV x CD3 bi-specific monovalent Fc diabody, the C-terminus of the Heterodimerization-Promoting Domain is linked to the CH2-CH3 domains of an Fc region ("Fc Domain") by an intervening linker peptide (**Linker 3**) or by an intervening spacer-linker peptide (**Spacer-Linker 3**). In non-limiting embodiments, the first of the three polypeptide chains will thus contain, in the N-terminal to C-terminal direction: $VL_{\text{First Antigen}}$ – Linker 1 – $VH_{\text{Second Antigen}}$ – Linker 2 – Heterodimerization-Promoting Domain – Spacer-Linker 3 – Fc Domain.

[0116] Alternatively, as shown in **Figure 8C**, the first of such three polypeptide chains will contain, in the N-terminal to C-terminal direction, an N-terminus, **Linker 3**, the CH2-CH3 domains of an Fc region ("Fc Domain"), an intervening spacer peptide (**Linker 4**), having, for example the amino acid sequence: APSSS (**SEQ ID NO:39**) or the amino acid sequence APSSSPME (**SEQ ID NO:40**), the Antigen-Binding Domain of a Light Chain Variable Domain (**VL**) of the first antigen (either CD3 or HIV), the Antigen-Binding Domain of a Heavy Chain Variable Domain (**VH**) of the second antigen (HIV, if the first antigen was CD3; CD3, if the first antigen was HIV), a Heterodimerization-Promoting Domain, and a C-terminus. An intervening linker peptide (**Linker 1**) separates the Antigen-Binding Domain of the Light Chain Variable Domain from the Antigen-Binding Domain of the Heavy Chain Variable Domain. In non-limiting embodiments, the Antigen-Binding Domain of the Heavy Chain Variable Domain is linked to the Heterodimerization-Promoting Domain by an

intervening linker peptide (**Linker 2**). In non-limiting embodiments, the first of the three polypeptide chains will thus contain, in the N-terminal to C-terminal direction: **Linker 3** – Fc Domain – **Linker 4** – $VL_{\text{First Antigen}}$ – Linker 1 – $VH_{\text{Second Antigen}}$ – Linker 2 – Heterodimerization-Promoting Domain.

[0117] In non-limiting embodiments, the second of such three polypeptide chains will contain, in the N-terminal to C-terminal direction, an N-terminus, the Antigen-Binding Domain of a Light Chain Variable Domain (**VL**) of the second antigen, the Antigen-Binding Domain of a Heavy Chain Variable Domain (**VH**) of the first antigen, a Heterodimerization-Promoting Domain and a C-terminus. An intervening linker peptide (**Linker 1**) separates the Antigen-Binding Domain of the Light Chain Variable Domain from the Antigen-Binding Domain of the Heavy Chain Variable Domain. In non-limiting embodiments, the Antigen-Binding Domain of the Heavy Chain Variable Domain is linked to the Heterodimerization-Promoting Domain by an intervening linker peptide (**Linker 2**). In non-limiting embodiments, the second of the three polypeptide chains will thus contain, in the N-terminal to C-terminal direction: $VL_{\text{Second Antigen}}$ – Linker 1 – $VH_{\text{First Antigen}}$ – Linker 2 – Heterodimerization-Promoting Domain.

[0118] In non-limiting embodiments, the third of such three polypeptide chains will contain the linker peptide (**Linker 3**) and the CH2-CH3 domains of an Fc region ("Fc Domain").

[0119] The bispecific molecules of the invention contemplate designs with various linkers to separate the different domain comprised in the polypeptide chains. Specific non-limiting embodiemts of the linkers are disclosed herein. Other linkers can be readily determined. Some additional examples of linkers are disclosed in US Pub 20100174053, incorporated by reference in its entirety.

[0120] The Antigen-Binding Domain of the Light Chain Variable Domain of the first polypeptide chain interacts with the Antigen-Binding Domain of the Heavy Chain Variable Domain of the second polypeptide chain in order to form a functional antigen-binding site that is specific for the first antigen (*i.e.*, either HIV or CD3). Likewise, the Antigen-Binding Domain of the Light Chain Variable Domain of the second polypeptide chain interacts with the Antigen-Binding Domain of the Heavy Chain Variable Domain of the first polypeptide chain in order to form a second functional antigen-binding site that is specific for the second antigen (*i.e.*, either CD3 or HIV, depending upon the identity of the first antigen). Thus, the selection of the Antigen-Binding Domain of the Light Chain Variable Domain and the Antigen-Binding Domain of the Heavy Chain Variable Domain of the first and second polypeptide chains are coordinated, such that the two polypeptide chains collectively

comprise Antigen-Binding Domains of light and Heavy Chain Variable Domains capable of binding to HIV and CD3.

[0121] The Fc Domain of the HIV x CD3 bi-specific monovalent Fc diabodies of the present invention may be either a complete Fc region (*e.g.*, a complete IgG Fc region) or only a fragment of a complete Fc region. Although the Fc Domain of the bi-specific monovalent Fc diabodies of the present invention may possess the ability to bind to one or more Fc receptors (*e.g.*, FcγR(s)), In non-limiting embodiments such Fc Domain will cause reduced binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by a wild-type Fc region) or will substantially eliminate the ability of such Fc Domain to bind to such receptor(s). The Fc Domain of the bi-specific monovalent Fc diabodies of the present invention may include some or all of the CH2 Domain and/or some or all of the CH3 Domain of a complete Fc region, or may comprise a variant CH2 and/or a variant CH3 sequence (that may include, for example, one or more insertions and/or one or more deletions with respect to the CH2 or CH3 domains of a complete Fc region). The Fc Domain of the bi-specific monovalent Fc diabodies of the present invention may comprise non-Fc polypeptide portions, or may comprise portions of non-naturally complete Fc regions, or may comprise non-naturally occurring orientations of CH2 and/or CH3 domains (such as, for example, two CH2 domains or two CH3 domains, or in the N-terminal to C-terminal direction, a CH3 Domain linked to a CH2 Domain, etc.).

[0122] In non-limiting embodiments the first and third polypeptide chains of the HIV x CD3 bi-specific monovalent Fc diabodies of the present invention each comprise CH2-CH3 domains that complex together to form an immunoglobulin (IgG) Fc Domain. The amino acid sequence of the CH2-CH3 domain of human IgG1 is (**SEQ ID NO:41**):

APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL
TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK

[0123] Thus the CH2 and/or CH3 Domains of the first and third polypeptide chains may both be composed of **SEQ ID NO:41**, or a variant thereof.

[0124] In non-limiting embodiments the CH2-CH3 domains of the first and third polypeptide chains of the HIV x CD3 bi-specific monovalent Fc diabodies of the present invention to exhibit decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc region (**SEQ ID NO:41**). Fc variants and mutant forms capable of

mediating such altered binding are well known in the art and include amino acid substitutions at positions 234 and 235, a substitution at position 265 or a substitution at position 297 (see, for example, US Patent No. 5,624,821, herein incorporated by reference). In non-limiting embodiments the CH2-CH3 Domain of the first and/or third polypeptide chains of the HIV x CD3 bi-specific monovalent Fc diabodies of the present invention include a substitution at position 234 with alanine and 235 with alanine.

[0125] The CH2 and/or CH3 Domains of the first and third polypeptide chains need not be identical in sequence, and advantageously are modified to foster complexing between the two polypeptide chains. For example, an amino acid substitution (for example a substitution with an amino acid comprising a bulky side group forming a 'knob', *e.g.,* tryptophan) can be introduced into the CH2 or CH3 Domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, *i.e.*, 'the hole' (*e.g.*, a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides comprising the bi-specific monovalent Fc diabody molecule, and further, engineered into any portion of the polypeptides chains of the pair. Methods of protein engineering to favor heterodimerization over homodimerization are well known in the art, in particular with respect to the engineering of immunoglobulin-like molecules, and are encompassed herein (see *e.g.,* Ridgway *et al.* (1996) *"'Knobs-Into-Holes' Engineering Of Antibody CH3 Domains For Heavy Chain Heterodimerization,"* Protein Engr. 9:617-621, Atwell *et al.* (1997) *"Stable Heterodimers From Remodeling The Domain Interface Of A Homodimer Using A Phage Display Library,"* J. Mol. Biol. 270: 26-35, and Xie *et al.* (2005) *"A New Format Of Bispecific Antibody: Highly Efficient Heterodimerization, Expression And Tumor Cell Lysis,"* J. Immunol. Methods 296:95-101; each of which is hereby incorporated herein by reference in its entirety). In non-limiting embodiments the 'knob' is engineered into the CH2-CH3 Domains of the first polypeptide chain and the 'hole' is engineered into the CH2-CH3 Domains of the third polypeptide chain. Thus, the 'knob' will help in preventing the first polypeptide chain from homodimerizing via its CH2 and/or CH3 Domains. In non-limiting embodiments , as the third polypeptide chain contains the 'hole' substitution it will heterodimerize with the first polypeptide chain as well as homodimerize with itself. In non-limiting embodiments a knob is created by modifying a native IgG Fc Domain to contain the modification T366W. In non-limiting embodiments a hole is created by modifying a native IgG Fc Domain to contain the modification T366S, L368A and Y407V. To aid in purifying the third polypeptide chain homodimer from the final bi-specific monovalent Fc diabody

comprising the first, second and third polypeptide chains, the protein A binding site of the CH2 and CH3 Domains of the third polypeptide chain is mutated by amino acid substitution at position 435 (H435R). Thus, the third polypeptide chain homodimer will not bind to protein A, whereas the bi-specific monovalent Fc diabody will retain its ability to bind protein A via the protein A binding site on the first polypeptide chain.

[0126] In non-limiting embodiments a sequence for the CH2 and CH3 Domains of the first polypeptide chain of the HIV x effector (e.g.CD3) bi-specific monovalent Fc diabodies of the present invention will have the "**knob-bearing**" sequence (**SEQ ID NO:42**):

APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
LPPSREEMTK NQVSL**W**C**L**VK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL
TVDKSRWQQG NVFSCSVMHE ALHN**H**YTQKS LSLSPGK

[0127] In non-limiting embodiments a sequence for the CH2 and CH3 Domains of the third polypeptide chain of the HIV x effector (e.g. CD3) bi-specific monovalent Fc diabodies of the present invention will have the "**hole-bearing**" sequence (**SEQ ID NO:43**):

APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
LPPSREEMTK NQVSL**S**C**A**VK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFL**V**SKL
TVDKSRWQQG NVFSCSVMHE ALHN**R**YTQKS LSLSPGK

[0128] As will be noted, the CH2-CH3 Domains of **SEQ ID NO:42** and **SEQ ID NO:43** include a substitution at position 234 with alanine and 235 with alanine, and thus form an Fc Domain exhibit decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc region (**SEQ ID NO:41**).

[0129] In non-limiting embodiments a the first polypeptide chain will have a "knob-bearing" CH2-CH3 sequence, such as that of **SEQ ID NO:42**. However, as will be recognized, a "hole-bearing" CH2-CH3 Domain (*e.g.*, **SEQ ID NO:43**) could be employed in the first polypeptide chain, in which case, a "knob-bearing" CH2-CH3 Domain (*e.g.*, **SEQ ID NO:42**) would be employed in the third polypeptide chain.

[0130] In non-limiting embodiments, the Fc domain can be modified by amino acid substitution to increase binding to the neonatal Fc receptor and therefore the half-life of the antibody when administered to a subject. The Fc domain can be an IgA, IgM, IgD, IgE or IgG Fc domain. The Fc domain can be an optimized Fc domain, as described in U.S. Published Patent Application No. 20100093979, incorporated herein by reference. In certain embodiments the antibodies comprise amino acid alterations, or combinations thereof, for example in the Fc region outside of epitope binding, which alterations can improve their

properties. Various Fc modifications are known in the art. Amino acid numbering is according to the EU Index in Kabat. In some embodiments, the invention contemplates antibodies comprising mutations that affect neonatal Fc receptor (FcRn) binding, antibody half-life, and localization and persistence of antibodies at mucosal sites. See e.g. Ko SY et al., Nature 514: 642-45, 2014, at Figure 1a and citations therein; Kuo, T. and and Averson, V., mAbs 3(5): 422-430, 2011, at Table 1, US Pub 20110081347 (an aspartic acid at Kabat residue 288 and/or a lysine at Kabat residue 435), US Pub 20150152183 for various Fc region mutation, incorporated by reference in their entirety.

[0131] In certain embodiments, the antibodies comprise AAAA substitution in and around the Fc region of the antibody that has been reported to enhance ADCC via NK cells (AAA mutations) containing the Fc region aa of S298A as well as E333A and K334A (Shields RI et al JBC , 276: 6591-6604, 2001) and the 4th A (N434A) is to enhance FcR neonatal mediated transport of the IgG to mucosal sites (Shields RI et al. ibid). Other antibody mutations have been reported to improve antibody half-life or function or both and can be incorporated in sequences of the antibodies. These include the DLE set of mutations (Romain G, et al. Blood 124: 3241, 2014), the LS mutations M428L/N434S, alone or in a combination with other Fc region mutations, (Ko SY et al. Nature 514: 642-45, 2014, at Figure 1a and citations therein; Zlevsky et al., Nature Biotechnology, 28(2): 157-159, 2010; US Pub 20150152183); the YTE Fc mutations (Robbie G et al Antimicrobial Agents and Chemotherapy 12: 6147-53, 2013) as well as other engineered mutations to the antibody such as QL mutations, IHH mutations (Ko SY et al. Nature 514: 642-45, 2014, at Figure 1a and relevant citations; See also Rudicell R et al. J. Virol 88: 12669-82, 201). In some embodiments, modifications, such as but not limited to antibody fucosylation, may affect interaction with Fc receptors (See e.g. Moldt, et al. JVI 86(11): 66189-6196, 2012). In some embodiments, the antibodies can comprise modifications, for example but not limited to glycosylation, which reduce or eliminate polyreactivity of an antibody. See e.g. Chuang, et al. Protein Science 24: 1019-1030, 2015. In some embodiments the antibodies can comprise modifications in the Fc domain such that the Fc domain exhibits, as compared to an unmodified Fc domain enhanced antibody dependent cell mediated cytotoxicity (ADCC); increased binding to Fc.gamma.RIIA or to Fc.gamma.RIIIA; decreased binding to Fc.gamma.RIIB; or increased binding to Fc.gamma.RIIB. See e.g. US Pub 20140328836.

[0132] The antibodies, and fragments thereof, described above can be formulated as a composition (e.g., a pharmaceutical composition). Suitable compositions can comprise the ADCC-mediating antibody (or antibody fragment) dissolved or dispersed in a

pharmaceutically acceptable carrier (e.g., an aqueous medium). The compositions can be sterile and can be in an injectable form (e.g. but not limited to a form suitable for intravenous injection, or intramascular injection). The antibodies (and fragments thereof) can also be formulated as a composition appropriate for topical administration to the skin or mucosa. Such compositions can take the form of liquids, ointments, creams, gels and pastes. The antibodies (and fragments thereof) can also be formulated as a composition appropriate for intranasal administration. The antibodies (and fragments thereof) can be formulated so as to be administered as a post-coital douche or with a condom. Standard formulation techniques can be used in preparing suitable compositions.

[0133] The antibody (and fragments thereof), for example the ADCC-mediating antibodies, described herein have utility, for example, in settings including but not limited to the following:

i) in the setting of anticipated known exposure to HIV-1 infection, the antibodies described herein (or fragments thereof) and be administered prophylactically (e.g., IV, topically or intranasally) as a microbiocide,

ii) in the setting of known or suspected exposure, such as occurs in the setting of rape victims, or commercial sex workers, or in any homosexual or heterosexual transmission without condom protection, the antibodies described herein (or fragments thereof) can be administered as post-exposure prophylaxis, e.g., IV or topically, and

iii) in the setting of Acute HIV-1 infection (AHI), the antibodies described herein (or fragments thereof) can be administered as a treatment for AHI to control the initial viral load, or for the elimination of virus-infected CD4 T cells.

[0134] In accordance with the invention, the ADCC-mediating antibody (or antibody fragments) described herein can be administered prior to contact of the subject or the subject's immune system/cells with HIV-1 or within about 48 hours of such contact. Administration within this time frame can maximize inhibition of infection of vulnerable cells of the subject with HIV-1.

In addition, various forms of the antibodies described herein can be administered to chronically or acutely infected HIV-1 patients and used to kill remaining virus infected cells by virtue of these antibodies binding to the surface of virus infected cells and being able to deliver a toxin to these reservoir cells. In certain embodiments, the antibodies of the invention can be administered in combination with latency activating agents, so as to activate latent reservoir of HIV-infected cells. The expectation is that by activating latent proviral HIV DNA in resting cells, once inactive cells will start producing new virus and they will be

recognized and eliminated by the immune system. Non-limiting examples of latency activating agents are HDAC inhibitors, e,g, vorinostat, romidepsin, panobinostat, disulfiram, JQ1, bryostatin, PMA, inonomycin, or any combination thereof. See Bullen et al. Nature Medicine 20, 425–429 (2014).

[0135] Suitable dose ranges can depend on the antibody (or fragment) and on the nature of the formulation and route of administration. Optimum doses can be determined by one skilled in the art without undue experimentation. For example, doses of antibodies in the range of 1-50 mg/kg of unlabeled or labeled antibody (with toxins or radioactive moieties) can be used. If antibody fragments, with or without toxins are used or antibodies are used that can be targeted to specific CD4 infected T cells, then less antibody can be used (e.g., from 5 mg/kg to 0.01 mg/kg).

[0136] Antibodies of the invention and fragments thereof can be produced recombinantly using nucleic acids comprising nucleotide sequences encoding VH and VL sequences selected from those shown in the figures and examples.

[0137] In certain embodiments the invention provides intact/whole antibodies. In certain embodiments the invention provides antigen binding fragments thereof. Typically, fragments compete with the intact antibody from which they were derived for specific binding to the target including separate heavy chains, light chains Fab, Fab', F(ab').sub.2, F(ab)c, diabodies, Dabs, nanobodies, and Fv. Fragments can be produced by recombinant DNA techniques, or by enzymatic or chemical separation of intact immunoglobulins.

[0138] Nucleic acid sequences encoding polypeptides for the production of bispecific antibodies with specificities as described herein can be used to produce plasmids for stable expression of recombinant antibodies. Methods for recombinant expression and purification are known in the art. In certain embodiments of Fc, the plasmids also comprise any of the changes to the Fc portion described herein. In some embodiemts, these are AAAA substitution in and around the Fc region of the antibody that has been reported to enhance ADCC via NK cells (AAA mutations) containing the Fc region aa of S298A as well as E333A and K334A (Shields RI et al JBC , 276: 6591-6604, 2001) and the 4th A (N434A) is to enhance FcR neonatal mediated transport of the IgG to mucosal sites (Shields RI et al. ibid).

[0139] In certain embodiments, the nucleic acids are optimized for recombinant expression in a suitable host cell. In certain embodiments, the vector is suitable for gene delivery and expression. There are numerous expression systems available for expression of proteins including E. coli, other bacterial hosts, yeast, and various higher eukaryotic cells such as the COS, CHO, HeLa and myeloma cell lines.

[0140] Any suitable cell line can be used for expression of the polypeptides of the invention, including but not limited to CHO cells, 293T cells. In some aspects, the invention provides nucleic acids encoding these antibodies, expression cassettes and vectors including these nucleic acids, and isolated cells that express the nucleic acids which encode the antibodies of the invention are also provided.. The polypeptides of the invention can be purified by any suitable method for purification of polypeptides and/or antibodies.

[0141] The contents of the various publications cited throughout the specification are incorporated by reference in their entirety.

EXAMPLES

[0142] Example 1A: Construction of an HIV-1 x CD3 or HIV-1 x CD16 Bispecific Molecules and Control Bispecific Molecules

[0143] **Table 2**contains a list of bi-specific diabodies that were designed, expressed and purified. The bi-specific diabodies are heterodimers, or heterotrimer of the recited amino acid sequences. Methods for forming bi-specific diabodies are provided in WO 2006/113665, WO 2008/157379, WO 2010/080538, WO 2012/018687, WO 2012/162068, WO 2012/162067, WO 2014/159940, WO 2015/021089, WO 2015/026892 and WO 2015/026894.

Table 2

Bi-Specific Molecule	Polypeptide Chain Amino Acid Sequences	Nucleic Acid Encoding Sequences
HIV-1 x CD3 Bi-Specific Diabody (A32 x CD3) (Variable domain from A32, binds to HIV-1 gp120)	**SEQ ID NO:9** **SEQ ID NO:11**	**SEQ ID NO:10** **SEQ ID NO:12**
HIV-1 x CD3 Bi-Specific Diabody (7B2 x CD3) (Variable domain from 7B2, binds to HIV-1 gp41)	**SEQ ID NO:13** **SEQ ID NO:15**	**SEQ ID NO:14** **SEQ ID NO:16**
HIV-1 x CD3 Bi-Specific Diabody (CH28 x CD3) (Variable domain from CH28, binds to HIV-1 gp)	**SEQ ID NO:17** **SEQ ID NO:19**	**SEQ ID NO:18** **SEQ ID NO:20**

Table 2		
Bi-Specific Molecule	**Polypeptide Chain Amino Acid Sequences**	**Nucleic Acid Encoding Sequences**
HIV-1 x CD3 Bi-Specific Diabody **(CH44 x CD3)** (Variable domain from CH44, binds to HIV-1 gp)	**SEQ ID NO:21** **SEQ ID NO:23**	**SEQ ID NO:22** **SEQ ID NO:24**
HIV-1 x CD16 Bi-Specific Diabody **(7B2 x CD16)** (Variable domain from 7B2, binds to HIV-1 gp41)	**SEQ ID NO:25** **SEQ ID NO:27**	**SEQ ID NO:26** **SEQ ID NO:28**
Fluorescein x CD3 Bi-Specific Diabody **(4420 x CD3)**	**SEQ ID NO:29** **SEQ ID NO:30**	
Fluorescein x CD16 Bi-Specific Diabody **(4420 x CD16)**	**SEQ ID NO:31** **SEQ ID NO:32**	
HIV-1 x Fluorescein Bi-Specific Diabody **(7B2 x 4420)** (Variable domain from 7B2, binds to HIV-1 gp41)	**SEQ ID NO:33** **SEQ ID NO:34**	
HIV-1 x Fluorescein Bi-Specific Diabody **(A32 x 4420)** (Variable domain from A32, binds to HIV-1 gp120)	**SEQ ID NO:35** **SEQ ID NO:36**	
Palivizumab x CD3 Bi-Specific Diabody **(Palivizumab x CD3)**	**SEQ ID NO:37** **SEQ ID NO:38**	
HIV-1 x CD16 Bi-Specific Diabody **(A32 x CD16)** (Variable domain from A32, binds to	**SEQ ID NO: 44** **SEQ ID NO: 45**	

Table 2		
Bi-Specific Molecule	**Polypeptide Chain Amino Acid Sequences**	**Nucleic Acid Encoding Sequences**
HIV-1 gp120)		
HIV-1 x CD3 Bi-Specific Diabody with Fc Domain V1 **(7B2 x CD3)** (Variable domain from 7B2, binds to HIV-1 gp41)	**SEQ ID NO: 46** **SEQ ID NO: 47** **SEQ ID NO: 48**	

[0144] HIV-1 x CD3 bi-specific diabodies are capable of simultaneously binding to HIV-1 and CD3. HIV-1 x CD16 bi-specific diabodies are capable of simultaneously binding to HIV-1 and CD16. The control bi-specific diabody (4420 x CD3) is capable of simultaneously binding to FITC and CD3. The control bi-specific diabody (4420 x CD16) is capable of simultaneously binding to FITC and CD16.The control bi-specific diabody (7B2 x 4420) is capable of simultaneously binding to HIV-1 and FITC. The control bi-specific diabody (A32 x 4420) is capable of simultaneously binding to HIV-1 and FITC. The control bi-specific diabody (Palivizumab x CD3) is capable of simultaneously binding to RSV and CD3.

[0145] **Table 3** shows a summary of some embodiments of bispecific molecules. The information in the specification can be readily used for alternative designs of the listed bispecific molecules, and for design of other bispecific molecules, for example 7B2, CH27, Ch28, CH44 using CDRs, or VH and VL chains from these antibodies.

HIVXCD3		**HIVXCD3 Fc V1**		**HIVXCD3 FcV2**	
One embodiment	**A32xCD3** **One embodiment SEQ ID Nos: 9 and 11 Together**	**One embodiment**	**A32xCD3 Fc V1**	**One embodiment**	**A32xCD3 Fc V2**
Polypeptide Chain 1	**SEQ ID NO:9 is one embodiment**	**Polypeptide Chain 1**		**Polypeptide Chain 1**	
NH2-VL(HIV)	SEQ ID NO:78(Figure 6) -	NH2-VL(HIV)	SEQ ID NO:78	NH2-Linker3	SEQ ID NO:49
Linker 1	SEQ ID NO: 1	Linker 1	SEQ ID NO: 1	CH2-CH3	SEQ ID NO: 42 (knob bearing) or

HIVXCD3		HIVXCD3 Fc V1		HIVXCD3 FcV2	
					SEQ ID NO: 43 (hole bearing)
VH(CD3)	SEQ ID NO: 51	VH(CD3)	SEQ ID NO: 51	Linker 4	SEQ ID NO:39, 40
Linker 2	SEQ ID NO: 2	Linker 2	SEQ ID NO: 2	VL(HIV)	SEQ ID NO:78
Heterodimer promoting domain K coil or E coil	SEQ ID Nos: 3-6 SEQ ID Nos: 7 or 8	Heterodimer promoting domain K coil or E coil	SEQ ID Nos: 3-6 SEQ ID Nos: 7 or 8	Linker 1	SEQ ID NO: 1
		Linker 3 or Spacer Linker 3	SEQ ID NO:49 SEQ ID NO:50	VH (CD3)	SEQ ID NO:51
		CH2-CH3	SEQ ID NO: 42 (knob bearing) or SEQ ID NO: 43 (hole bearing)	Linker 2	SEQ ID NO: 2
				Heterodimer promoting domain K coil or E coil	SEQ ID Nos: 3-6 SEQ ID Nos: 7 or 8
Polypeptide Chain 2	**SEQ ID NO: 11 is one embodiment**	**Polypeptide Chain 2**		**Polypeptide Chain 2**	
NH2-VL(CD3)	SEQ ID NO: 52	NH2-VL(CD3)	SEQ ID NO: 52	NH2-VL(CD3)	SEQ ID NO: 52
Linker 1	SEQ ID NO: 1	Linker 1	SEQ ID NO: 1	Linker 1	SEQ ID NO: 1
VH(HIV1)	SEQ ID NO: 77 (Figure 6)	VH(HIV1)	SEQ ID NO:77	VH(HIV)	SEQ ID NO:77
Linker 2	SEQ ID NO: 2	Linker 2	SEQ ID NO: 2	Linker 2	SEQ ID NO: 2
Heterodimer promoting	SEQ ID Nos: 3-6	Heterodimer promoting	SEQ ID Nos: 3-6	Heterodimer promoting	SEQ ID Nos: 3-

HIVXCD3		HIVXCD3 Fc V1		HIVXCD3 FcV2	
domain K coil or E coil	SEQ ID Nos: 7 or 8	domain K coil or E coil	SEQ ID Nos: 7 or 8	domain K coil or E coil	6 SEQ ID Nos: 7 or 8
Polypeptide Chain 3	NONE	**Polypeptide Chain 3**		**Polypeptide Chain 3**	
		NH2- Linker 3	SEQ ID NO:49	NH2- Linker 3	SEQ ID NO: 49
		CH2-CH3	SEQ ID NO: 42 (knob bearing) or SEQ ID NO: 43 (hole bearing)	CH2-CH3	SEQ ID NO: 42 (knob bearing) or SEQ ID NO: 43 (hole bearing)

[0146] **Example 1C**: HIV-1 Antibodies with ADCC Activity

[0147] Monoclonal antibodies. Five mAbs representing those directed against the HIV-1 env gp120 constant region 1 (C1; n= 1), CD4 binding site (CD4bs; n= 3), and the gp41 Cluster 1 [Pollara J. *Curr. HIV Res.* 2013; 11(8):378–3870]. All the mAbs are listed in **Table 1**. All but mAbs were generated with a sequence for the Fc region that included amino acid substitutions according to Shields et al to optimize the binding to the Fcγ-Receptor (Fcγ-R) IIIa [Shields RL *J Biol Chem* 2001; 276(9): 6591–6604].

[0148] A32 mAb recognizes a conformational epitope in the C1 region of HIV-1 Env gp120 (Wyatt et al, J. Virol. 69:5723-5733 (1995)), could mediate potent ADCC activity and could block a significant proportion of ADCC-mediating Ab activity detectable in HIV-1 infected individuals (Ferrari et al, J. Virol. 85:7029-7036 (2011)).

[0149] CH28 or CH44 are HIV-1 CD4 bs neutralizing antibodies. .

Table 4. List of mAbs tested for ADCC

gp120		**gp41**
C1	**CD4bs**	**Cluster I**
A32	CH27 CH28	7B2

	CH44	

All mAbs were produced in the 3A version to optimize the binding to the Fcγ-Receptor IIIa, but those identified by the symbol (*).

[0150] Infectious Molecular Clones (IMC). The HIV-1 IMCs represented 22 isolates to represent those with various degree of susceptibility to neutralization based on testing with the A3R5 cell line. The list of the IMCs is reported in **Table 5**.

Table 5. List of IMC by HIV-1 subtype used to generate infected target cells.

A	AE	B	C
Q23.17	C1080.C03	SF162	MW96.5
	427299	BaL	CAP45
	92TH023	CH058	245-F3-C10
	CM235	CH040	TV-1
	CM244	SUMA	CH0505
	816763	WITO	DU151
		YU2	DU422
			1086.c

[0151] All IMCs were generated on backbone derived from NHL4-3 isolate as previously described [Edmonds TG. *Virology*. 2010;408(1):1–13; Adachi A. *J Virol*. 1986;59(2):284–291] but for the subtype AE 92TH023 that was generated utilizing the backbone from the 40021 AE HIV-1 Isolate. All IMCs expressed the *Renilla* luciferase reporter gene and preserved all nine viral open reading frames. The *Renilla* luciferase reporter gene was expressed under the control of the HIV-1 Tat gene. Upon HIV-1 infection of the CD4+ T cells, expression of Tat during HIV-1 replication will induce expression of the luciferase and infected cells can be easily quantified by measure of Relative Luminescence Units.

[0152] Antibody Dependent Cellular Cytotoxicity (ADCC) assay. The assay was performed according to our previously published methods using a luciferase based platform as read-out

for the cytotoxicity mediated by the mAbs [Pollara J. *J Virol.* 2014;88(14):7715–7726]. The effector cells populations were all derived from a single donor with the characterized heterozygous phenotype F/V for the amino acid in position 158 of the Fcγ-R IIIa. The effector to target ratio was 30:1 in each assay. The plasma from a HIV-1 infected individuals (A300) and the Palivizumab (anti-RSV) mAb were used as positive and negative control in each assay. All the mAbs were tested together against each IMC. The percentage of specific killing (%SK) was calculated as previously reported. The results were considered positive if the %SK was >20%.

[0153] Potency and breadth of ADCC-mediating mAbs. Each mAbs listed in **Table 1** was tested individually against each of the 22 IMCs listed in **Table 2**. The results have been evaluated to identify the maximum ADCC activity as %SK independently from the concentration at which the activity was observed. The mAbs were grouped based on the env gp120 and gp41 regions recognized. The average of the positive responses for each mAb are reported in **Figure 1.** The magnitude and breadth of the mAbs is summarized in **Table 3.** The non-neutralizing Abs directed against gp120 C1 and gp41 cluster 1 provided the broadest spectrum of ADCC by recognizing 21 (95%) and 20 (91%) HIV-1 isolates, respectively. The average % of specific killing (%SK) was 37% for the C1 mAbs and 34% for the gp41 cluster I mAbs. The averages of the maximum %SK of A32 and 7B2 were 45 and 42, respectively. Cumulatively, the CH44 mAb recognized <60% of the isolates tested with a range of activity between 21 and 60%SK.

Table 6. Magnitude and breadth of ADCC-mediating mAbs

	A32	7B2	CH27	CH28	CH44
Average Max %SK	45	42	31	36	13
Range	23-86	21-74	21-53	23-48	21-60
# IMCs recognized	21	20	8	7	14
(%)	(95%)	(91%)	(36%)	(31%)	(59%)

[0154] Example 2: Cell Killing by Dual Affinity Re-Targeting (DART) molecules A32/CD3 and 7B2/CD3

[0155] Dual affinity Re-Targeting molecules A32/CD3 (SEQ ID NOs: 9 and 11) and 7B2/CD3 (SEQ ID NOs: 13 and 15) were designed and expressed. These molecules include an HIV-1 binding arm generated based on the Fab of anti-HIV-1 monoclonal antibodies (mAbs) (mAbs that have the property of binding to the surface of tier 2 transmitted/founder (T/F) virus infected CD4 T cells (i.e. A32 or 7B2) [Ferrari G, *J Virol.* 2011; 85(14):7029–7036; Pollara J. *Curr. HIV Res.* 2013; 11(8):378–3870], and an effector cell binding arm that can bind the CD3 (αCD3ε arm) or CD16 (αCD16 h3G8 arm) receptors. Appropriate negative controls with an irrelevant binding arm [αfluorescein (4420) or αRSV] instead of the HIV-1 or effector arm have also been developed. The results presented in this example are from experiments with the CD3-DARTs.

[0156] Luciferase-based Cytotoxicity Assay. We optimized a method to quantify the elimination of HIV-1-infected cells by cytotoxic CD8 T cells recruited by the DARTs that is based on the detection of luciferase activity as final readout as previously reported [Pollara J. *J Virol.* 2014; 88(14):7715–7726]. Cryopreserved resting PBMC from normal healthy HIV-1 seronegative donors were activated for 72 hours with anti-human CD3 (clone OKT3; eBioscience)/anti-human CD28(clone CD28.2; BD Pharmingen). Subsequently, a CD4+ enriched cell population was obtained using magnetic beads, spinoculated in presence of the IMC representing the HIV-1 subtype AE (CM235), B (BaL), and C (1086.c) and cultured, for 72 hours. CD4+ infected target cells were then plated along with resting CD8+ effector cells at 33:1, 11:1, 3:1, and 0:1 effector to target ratios. DARTs (4420xCD3, 7B2xCD3, or A32xCD3) were added to combined cells at concentrations ranging from 0.001 to 1000ng/ml and incubated for 6, 24, and 48-hour time points. Combined effector and target cells without DARTs, uninfected cells, and target cells alone were included on each plate for control conditions. At the end of each incubation time, Viviren substrate was added to each well and cells were analyzed on a luminometer to measure RLU values through luciferase readout. In presence of the cytotoxic cells of interest the elimination of infected target cells was evaluated using the appropriate already published formula [Pollara J. *J Virol.* 2014; 88(14):7715–7726]. The results are reported as %SK as described for the ADCC assay.

[0157] Anti-HIV-1 DARTs-mediated cytotoxic activity. Based on the results described above, two DARTs were generated whose anti-HIV-1 arm was the A32 and 7B2 Fab region and the effector cell binding arm the αCD3ε arm. We studied these two DART molecules for their ability to recognize and mediate the killing of infected CD4+ T cells. Leukapheresis samples obtained from HIV-1 seronegative donor were infected *in vitro* to generate the target

cells as described in the material and method section using our previously described ADCC Luciferase-based assay to detect the cytotoxic effects of the DARTs. We tested the two CD3-DART molecules (7B2xCD3 and A32xCD3) for their ability to redirect the cytotoxicity of resting CD8+ T cells against subtype B BaL, AE CM235, and C 1086.c HIV-1 IMC infected autologous CD4+ T cells. We evaluated DART-mediated cytotoxicity at 6, 24, and 48 hours after incubation of effector and target cells at the effector-to-target ratios of 33, 11, and 3 to 1. Although cytotoxic activity was already observed after 6 hours incubation, the peak cytotoxic activity (>70%SK) was detected at 48 hours using the 33:1 E:T ratio against each HIV-1 IMC (**Figure 2**). The activity of the two HIV-1 DARTs was always greater than the background maximum killing observed with the 4420xCD3 control DART. We also observed a dose dependent potency of the two DARTs against each HIV-1 IMC-infected target cell population that is also reflected at the level of each E:T ratio, as illustrated for the BaL IMC (**Figure 3**).

[0158] The difference in the potency of the two DARTs was also analyzed as the DART concentration at which 50% of specific killing (Killing Concentration$_{50}$ or KC_{50}) was detected at 48 hours with E:T of 33:1. The A32xCD3 DART KC_{50} was always approximately one log lower than the 7B2xCD3 DART KC_{50} (**Figure 4**) against each HIV-1 IMC.

[0159] These results indicated that DARTs can effectively recruit CD8+ T cells and direct their cytotoxic activity against HIV-1 infected cells.

[0160] Example 3: A32/CD16 and 7B2/CD16 DARTs

[0161] Dual affinity Re-Targeting molecules A32/CD16 (SEQ ID NOs: 44 and 45) and 7B2/CD16 (SEQ ID NOs: 25 and 27 see Table 2) will be analyzed as described in Example and 6 using Luciferase-based cytotoxicity assay and CD4+ infected target cells along with resting effector cells. For the CD16-DART assay, the effector cells are CD16+ cells, which could be purified by removing CD3+CD20+ cells from whole PBMCs.

[0162] The Luciferase-based killing assay, described in Example 2 will be used to examine and compare the potency and kinetics of CD16-DART-enhanced clearance of productive infection as previously proposed for the CD3-DARTs. The procedure will be the same but the negative selection of the effector cells will provide an enriched population of CD16+ cells.

[0163] Example 4: CH28 and CH44 DARTs

[0164] DART molecules with a HIV-1 arm having the binding specificity of CH28 or CH44, and an effector cell arm targeting CD3 or CD16 will be made and tested in the Luciferase-based killing assay essentially as described in Examples 2 and 3. CH28 or CH44 are HIV-1 CD4 bs neutralizing antibodies. *See* U.S. Provisional Appl. No.: 61/883,220 filed September

27, 2013 and corresponding PCT application. CH28/CD3 comprises SEQ ID NOs: 19 and 19. CH44/CD3 comprises SEQ ID NOs: 21 and 23.

[0165] Example 5: Combinations of CD13- and CD16-DARTs

[0166] The Luciferase-based killing assay will be used to test whether CD13- and CD16-DARTs in a combination formulation provide enhanced benefits. For each DART combination, we will utilize cells expressing the 3 different Fcγ-R IIIa (CD16) phenotypes and the panel of established IMCs to test the ability of DARTs to recruit simultaneously CD3+ and CD16+ effector cells. These assessments will be conducted using leukapheresis samples collected from HIV-1 seronegative donors.

[0167] All documents and other information sources cited herein are hereby incorporated in their entirety by reference.

[0168] Example 6: Dual-Affinity-Re-Targeting (DART) proteins direct T-cells-mediated cytolysis of latently HIV-infected cells

[0169] Enhancement of HIV-specific immunity is likely required to eliminate latent HIV infection. To this aim, a novel immunotherapeutic modality has been developed, Dual Affinity Re-Targeting (DART) proteins that are bispecific antibody-based molecules that can bind two distinct cell surface molecules simultaneously. Described herein are HIVxCD3 DARTs designed with a monovalent HIV-1 envelope (*Env*) binding arm, derived from broadly binding, ADCC-mediating antibodies known to bind to HIV-infected target cells, that is coupled to a monovalent CD3 binding arm designed to engage cytolytic effector T-cells. Thus, DARTs redirect polyclonal T-cells to specifically engage with, and kill Env-expressing cells, including $CD4^+$ T cells infected with different HIV-1 subtypes, thereby obviating the requirement for HIV-specific immunity. Using lymphocytes from patients on suppressive anti-retroviral therapy (ART), DARTs mediated $CD8^+$ T-cell clearance *in vitro* of $CD4^+$ T-cells superinfected with the HIV-1 strain JR-CSF or infected with autologous reservoir viruses isolated from HIV-infected patient resting $CD4^+$ T-cells. Importantly, DARTs also mediated $CD8^+$ T-cell clearance of HIV from resting $CD4^+$ T cell cultures following induction of latent virus expression. Combined with HIV latency reversing agents, HIVxCD3 DARTs have the potential to be effective immunotherapeutic agents to clear latent HIV-1 reservoirs in HIV-infected individuals.

[0170] The inability of antiretroviral therapy (ART) to eradicate HIV was first suggested by the demonstration of latent infection of resting $CD4^+$ T cells (1), and then by the recovery of rare, integrated, replication-competent HIV from the resting $CD4^+$ memory T cells of patients receiving potent ART (2-4). Current ART cannot eradicate HIV infection, because these

long-lived $CD4^+$ T cells remain persistently infected and unrecognized by the immune system, with minimal expression of HIV genes or proteins (1, 5, 6). The persistence of quiescent HIV infection, primarily within central memory T cells, is a major obstacle to eradication of HIV infection (2-4, 7-9).

[0171] Viral persistence is also manifest in a substantial proportion of treated patients by very low levels of detectable viral RNA *(10, 11)* that represents expression of viral particles without effective rounds of new replication and does not appear to lead to drug resistance or failure of therapy *(12, 13)*. However, persistent viremia demonstrates an inability of the immune response to recognize and clear HIV-1 infected cells.

[0172] Chronically infected individuals generally have rapid viral rebound when ART is withdrawn *(14-16)*. This observation has suggested that the immune system in patients cannot control viremia, unless bolstered by a further intervention. Therapeutic immunization, even in individuals who initiated ART when $CD4^+$ and $CD8^+$ cellular immune responses remain relatively preserved, has thus far been unsuccessful in inducing enhanced anti-HIV immunity that can restrict viremia in the absence of ART *(17)*. Therefore, eliminating the latent pool of HIV-infected cells that persist despite ART, and as well, the unknown cells that are the source of low-level viremia found in most patients despite ART, requires new and innovative strategies. One initial step, the disruption of latency and the induction of viral antigen expression in cells that are latently infected, is under intensive investigation *(18, 19)*. However, as early progress is made in the development of latency reversing agents (LRAs), improvements in the ability to clear persistent infection must be sought as well.

[0173] Latently infected cells are very rare, and even if the latent reservoir is as much as 60-times larger than the typical estimates of about 1 infected cell per 10^6 resting central memory $CD4^+$ cells *(20)*, current LRAs might induce proviral transcription in only a fraction of this population, and the quantity of viral antigen presented might be low *(21, 22)*. Therefore, a novel and robust immune response may be necessary to detect and clear both cells producing low-level viremia, and in quiescently infected cells induced to leave the latent state.

[0174] Following the reactivation of latent HIV, viral antigens are presented on the surface of the cell and thus could be targeted by antibodies or antibody-derived molecules. Proof of concept for this approach has been provided by immunotoxins --- bifunctional chimeric proteins consisting of a targeting domain, such as an antibody or a ligand, joined to a toxin effector domain *(23)*. Although initial clinical trials using immunotoxins in HIV-infected individuals failed to have sustained impact on immunological or clinical markers *(24)*,

immunotoxin 3B3-PE38 *(25)* has been reported to reduce levels of HIV-infected cells that persist despite ART in the BLT humanized mouse model *(26)*.

[0175] Several monoclonal antibodies (mAbs) have been reported as capable of recognizing HIV-1 infected cells and engaging Fc-gamma receptor-bearing cells to mediate antibody dependent cellular cytotoxicity (ADCC) *(27),* such as A32 and 7B2, non-neutralizing mAbs that bind to conserved residues in gp120 *(28)* and gp41*(29, 30),* respectively. Based on these properties, two Dual Affinity Re-Targeting (DART) proteins *(31, 32)* were generated in which HIV envelope targeting arms derived from the A32 and 7B2 mAbs were combined with a CD3 effector arm derived from hXR32, a humanized anti-CD3ε mAb, to generate two HIVxCD3 DARTs, A32xCD3 and 7B2xCD3 (**Fig.10**).

[0176] Bispecific molecules that co-engage T cells with antigen-expressing target cells, such as DARTs and Bi-specific T-cell Engager proteins (BiTEs), have been characterized and developed largely for use in oncology *(31-34).* They are dependent on the engagement of both of the binding arms to activate and redirect the cytolytic activity of polyclonal T-cells, in a major histocompatibility complex (MHC) independent manner, against the antigen expressing target cells *(31-34).* This class of bispecific molecules is effective *in vivo* at doses many-fold lower than those typically employed for mAbs *(33, 34),* and has been shown to be clinically potent and efficacious with acceptable safety, as evidenced by the approval of blinatumumab, a CD19xCD3 BiTE, for the treatment of relapsed or refractory B-precursor acute lymphoblastic leukemia (ALL) *(35, 36).* DARTs, which have inter-chain disulfide bonds at their C-termini and are structurally compact, making them well suited for forming stable cell-to-cell contacts between target and effector cells, exhibit greater potency than BiTEs in side-by-side comparisons *(32, 37).*

[0177] Disclosed herein is the ability of HIVxCD3 DARTs to redirect $CD8^+$ T cells against $CD4^+$ cells infected by HIV-1, including ones infected with authentic latent virus isolates emerging from HIV-infected patients' cells in model systems designed to mimic potential clinical HIV eradication strategies. The ability of HIVxCD3 DARTs to recognize conserved HIV-1 antigens on infected cells and simultaneously engage receptors on the membrane of polyclonal effector T-cells, will overcome the need to activate pre-existing HIV-specific cytotoxic effector cells *(38),* thus surmounting a significant hurdle that impedes the effective elimination of the reservoir of infected $CD4^+$ T cells.

[0178] *HIV arm selection for DARTs.* A32 mAb binds to a conformational, CD4-inducible epitope in gp120 C1/C2 (within epitope cluster A) *(28, 39-41)* and 7B2 mAb binds to a linear epitope in gp41 cluster I *(29, 30, 42).* The two mAbs were tested for their ability to mediate

antibody dependent cell-mediated cytotoxicity (ADCC) against a panel of 22 representative HIV-1 infectious molecular clones (IMCs) of subtypes A, AE, B and C (**Figure 18**). The A32 mAb recognized 21 (95%) of the HIV-1 isolates with an average percent specific lysis (%SL) of 43.69% (range 12-86%; **Figure 23**). The 7B2 mAb recognized 20 (91%) of the HIV-1 isolates with an average %SL of 39.58% (range 15-74%; **Figure 23**). In addition to possessing breadth and efficiency in mediating ADCC --- indicating epitope accessibility at the surface of HIV-infected cells, a necessary property for HIVxCD3 DARTs --- the A32 and 7B2 mAbs are attractive sources for *Env* binding domains for DARTs as the residues in Env that influence binding by these mAbs are highly conserved among all HIV-1 subtypes (**Figure 24**). Based on these properties, two HIVxCD3 DARTs were generated in which HIV targeting arms derived from the A32 and 7B2 mAbs were combined with a CD3 effector arm derived from hXR32, a humanized anti-CD3ε mAb (**Figures 10A-10C**). These HIVxCD3 DARTs are named A32xCD3 and 7B2xCD3. Control DARTs with an irrelevant arm – derived from an anti-FITC antibody (4420) or from palivizumab, an antibody to the respiratory syncytial virus (RSV) fusion protein antibody – instead of the HIV arm (4420xCD3, RSVxCD3) or CD3 arm (A32x4420, 7B2x4420) were also generated. Control DARTs with an irrelevant arm derived from an anti-FITC antibody (4420) or palivizumab, an antibody to the respiratory syncytial virus (RSV) fusion protein antibody, instead of the HIV (4420xCD3 and RSVxCD3) or CD3 arm (A32x4420 and 7B2x4420) were also generated.

[0179] *HIV DART binding properties.* A32xCD3 and 7B2xCD3 each exhibited binding to recombinant human CD3 and HIV-1 *Env* proteins, individually and simultaneously, as shown by ELISA (**Figures 11A-11C**). While the binding to CD3 protein was similar for both DARTs, the magnitude of binding to JR-FL gp140 CF was greater for 7B2xCD3 than for A32xCD3, likely due to the fact that the conformational A32 epitope is highly CD4-dependent *(41-44)*. Based on surface plasmon resonance (SPR), the equilibrium dissociation constants (K_D) for CD3 arm binding were 3.6 and 6.1 nM for A32xCD3 and 7B2xCD3, respectively, and K_D for HIV arm binding was 47.7 nM for A32xCD3 using M.ConS gp140 CFI, and 15.1 nM for 7B2xCD3 using JR-FL gp140 CF, respectively (**Figure 19**). Different *Env* proteins were utilized for these two DARTs in the SPR studies, because A32xCD3 binding to JR-FL gp140 CF was inefficient and 7B2xCD3 binding to M.ConS gp140 CFI, due to its lack of the gp41 cluster I sequence, was precluded.

[0180] HIVxCD3 DARTs bind to their cell surface antigens with specificity. DARTs with CD3 effector arms (A32xCD3, 7B2xCD3, 4420xCD3) bind to human $CD3^+$ T cells with similar efficiencies, whereas DARTs with the CD3 arm replaced by an irrelevant arm

(A32x4420, 7B2x4420) or with two irrelevant arms (4420x4420) do not bind (**Figure 11D**). HIVxCD3 DARTs (A32xCD3, 7B2xCD3) bind efficiently to HEK293-D371 cells that express subtype AE CM244 *Env* (**Figure 11E**), and similar binding activity is observed with the A32x4420 and 7B2x4420 control DARTs (**Figure 25**). As expected, the 4420xCD3 control DART does not bind to these cells (**Figure 11E**). A32xCD3 and 7B2xCD3 bind to Jurkat-522 F/Y cells, which express both CD3 and subtype B HXBc2 Env *(45)* and binding via the CD3 arm predominates as shown by the equivalence of 4420xCD3, A32xCD3 and 7B2xCD3 binding. When the CD3 arm is replaced by the irrelevant 4420 arm to ablate CD3 binding, low level binding to the cell surface Env is detected with A32x4420, but not with 7B2x4420 (**Figure 11F**).

[0181] *HIVxCD3 DART redirected T-cell killing of Env-expressing cell lines and concomitant T-cell activation.* Jurkat 522-F/Y is a human $CD4^+$ cell line that expresses *Env* and serves as a model for HIV-infected $CD4^+$ T cells and Jurkat-ΔKS is a control cell line that is identical, except for a deletion/frameshift mutation in the *Env* gene that precludes its expression *(45)*. These cell lines were utilized to evaluate the ability of HIVxCD3 DARTs to mediate redirected T-cell killing of Env^+ target cells. Target cell cytolysis was determined by measuring lactate dehydrogenase (LDH) release with the standard assay and the results were confirmed by luminescence (LUM) assay. As measured by LDH release assays, A32xCD3 and 7B2xCD3 redirected human T cells derived from healthy donors to kill the Jurkat-522 F/Y cells in a concentration dependent manner at an E:T ratio of 10:1, and these two HIVxDARTs exhibited similar potencies after 48h of incubation with fifty percent effective concentrations (EC_{50}) of 160-230 pg/mL **(Figure 12A)**. No DART-mediated redirected T-cell killing of Jurkat-522 F/Y cells occurred with control DARTs (4420xCD3, A32x4420, 7B2x4420) in which the HIV arm or CD3 arm was replaced by an irrelevant one (**Figure 12A**). The A32xCD3 and 7B2xCD3 DARTs did not mediate target cell killing when the effector T-cells were omitted (**Figure 12B**) or when the target cells lacked Env expression **(Figure 12C**). These data demonstrate a strict requirement for Env-expressing target cells and their coengagement with CD3-expressing effector cells for HIVxCD3 DART mediated cytolytic activity.

[0182] As measured by LUM assays, A32xCD3 and 7B2xCD3 exhibited similar potencies for redirected T-cell killing of Jurkat 522-F/Y GF cells with EC_{50} values of 140-170 pg/mL (**Figure 12D**), which were close to those measured with the LDH release assay, indicating consistency across the two different assay modalities. Moreover, with the sensitivity and

specificity of the LUM assay, DART-dependent elimination of the Env^+ target cells was nearly complete (>98%), while the 4420xCD3 control DART mediated no cytotoxicity **(Figure 12D)**. HIVxCD3 DART redirected T cell killing activity was time and E:T ratio dependent. Near complete cytolysis with 7B2xCD3 was reached at 48 hours at E:T ratios of 10:1 and 5:1, whereas high level cytolysis (>80%) at an E:T ratio of 1:1 was delayed until 72 hours (**Figures 12E-12H)**, suggesting that time is the limiting factor for the efficient elimination of target cells at lower E:T cell ratios.

[0183] Concomitant with redirecting T-cell killing activity, the HIVxCD3 DARTs induced T-cell activation (measured by upregulation of the activation marker, CD25) in the presence of the Env^+ target cells with CD25 upregulated in $CD8^+$ T-cells to a greater extent than in $CD4^+$ T-cells (**Figures 26A-26D**). The overall data demonstrate that A32xCD3 and 7B2xCD3 potently activate and redirect T cells, especially $CD8^+$ T-cells, to specifically kill *Env*-expressing target cells. Moreover, the killing data confirm that both DARTs were capable of recognizing and binding to *Env* antigens on the surface of a $CD4^+$ cell line even though the detection of Env binding by FACS analysis was negligible (**Figure 13F**).

[0184] HIVxCD3 DARTs bind to the surface of HIV- infected $CD4^+$T cells and redirect $CD8^+$ T-cells to kill HIV-1 infected $CD4^+$ cells using lymphocytes from HIV-1 seronegative donors. The A32x4420 and 7B2x4420 DARTs were evaluated for their ability to bind and redirect the killing of $CD4^+$ T cell infected with HIV-1 Infectious molecular clones representing the subtype AE CM235, subtype B BaL, and subtype C 1086.C HIV-1 isolates. Each IMC was engineered with a luciferase reporter gene to quantitatively measure the cytolysis of infected target cells. To assess binding to infected cell surface *Env*, A32x4420 and 7B2x4420 DARTs (which lack CD3 effector arms) were compared to the parental A32 and 7B2 mAbs. Similar staining of the $p24^+$ (infected) $CD4^+$ T cells by both HIVxCD3 DARTs independently from the HIV-1 IMC used for the infection (**Figure 27**) was observed. Interestingly, staining with the A32x4420 DART recapitulated closely the staining with the A32 mAbs; in contrast, the 7B2x4420 DART recognized >66% of the HIV-1 infected cells (range 66-78%) compared to the >24% recognized by the 7B2 mAb (range 24-38), suggesting that the DART has a better accessibility to the cluster I gp41 epitope compared to the mAb (**Figure 27**). The secondary conjugated Abs and the Palivizumab mAb utilized as controls recognized less than <5% HIV-1 infected $CD4^+$ T cells.

[0185] The ability of A32xCD3 and 7B2xCD3 to redirect $CD8^+$ T cells from HIV-1 seronegative donors against autologous $CD4^+$ T cells infected with the three HIV-1 IMCs was subsequently investigated. The two HIVxCD3 DARTs redirected autologous $CD8^+$ T effector

cells to kill subtype B BaL (**Figure 13A**), subtype AE CM235 (**Figure 13B**), and subtype C 1086.C (**Figure 13C**) IMC-infected $CD4^+$ target cells in a concentration dependent manner, whereas the control DART (4420xCD3) was inactive. The greater potency exhibited by A32xCD3 ($EC_{50} \leq 1$ ng/mL) compared to 7B2xCD3 (EC_{50} ~10 ng/mL) in these studies with IMC-infected $CD4^+$ cells contrasts with the similar potencies observed in the studies with Env^+ cell lines (**Figures 12A-12C**). DART mediated killing of the IMC-infected $CD4^+$ T cells was dependent on the presence of $CD8^+$ effector cells, and no cytolytic activity was observed in their absence (**Figures 13A-13C**). In time course studies, DART-dependent cytolytic activity was evident at 6 hours with maximal activity (>70% cytolysis) at 48 hours (**Figures 13D-13F**).

[0186] To gain insight into the frequency of effector T cells recruited by the DARTs to kill HIV-1 infected target cells, the ability of DARTs to induce degranulation of the $CD8^+$ T cells obtained from 5 HIV-1 seronegative donors when co-incubated with autologous HIV-1 BaL IMC-infected $CD4^+$ cells under the same conditions used to detect cytolytic activity was assessed. The example of the gating strategy adopted for data analysis is illustrated in **Figures 14A-14G.** The mean frequency of Live/$CD3^+$/$CD8^+$/$CD107^+$ cells (**Figure 14H**) under control conditions (absence of HIVxCD3 DART or presence of control DART) was 0.38% (standard deviation 0.10%; range 0.24-0.51), which increased to an average 3.53% (range 1.5-6.9%) or 18.23% (range 12.30-23.35%) in the presence of 1 ng/mL 7B2xCD3 or A32xCD3, respectively**.** The data demonstrate that HIVxCD3 DARTs can specifically induce degranulation of resting $CD8^+$ T cells in the presence of Env-expressing target cells (autologous HIV-1-infected $CD4^+$ T cells).

[0187] <u>HIVxCD3 DART redirected $CD8^+$ T cell killing activity against JR-CSF infected cells from seronegative donors.</u> A viral clearance assay measuring HIV *gag* p24 antigen production was utilized as an alternative method to assess DART redirected T cell killing activity. $CD4^+$ cells from healthy donors were superinfected with the HIV-1 clade B clone JR-CSF and incubated with autologous $CD8^+$ T cells at an E:T ratio of 1:1 in the absence or presence of 100ng/mL DARTs for 7 days. In experiments with two different donors, addition of the control DART (4420xCD3) did not significantly reduce p24 production compared to incubations performed in the absence of DARTs, whereas addition of A32xCD3 or 7B2xCD3 significantly reduced p24 production to a similar extent (by 72-96% or 87-99% respectively; $p<0.01$ Student T test; **Figures 15A-15B**). The viral clearance assay was also conducted in the presence of integrase and non-nucleoside reverse transcriptase inhibitors once infection was established, at the time of addition of effector cells and DARTs, to block further rounds

of infection. When antiretrovirals (ARVs) were included in the assay, A32xCD3 and 7B2xCD3 still mediated a trend towards reduction in p24 production, although this did not reach statistical significance likely due to low levels of baseline p24 production with the antiretrovirals (**Figure 15C**), suggesting that the DARTs are not acting by inhibition of virus spread but rather through clearance of infected cells.

[0188] HIVxCD3 DARTs redirect $CD8^+$ T-cells to clear JR-CSF-superinfected $CD4^+$ cells using lymphocytes from patients on suppressive ART. Chronic ART is characterized by dysfunctional and exhausted T cell responses *(46, 47)* and thus confirmation of robust DART mediated T-cell redirected clearance activity in patient samples ex vivo is critical. The activity of HIVxCD3 DARTs in viral clearance assays with lymphocytes from 8 HIV-infected individuals on suppressive ART was evaluated. All participants were on ART for at least 6 months at the time of study with virus load <50 copies/mL, but otherwise exhibited diverse clinical backgrounds **(Figure 20)**.

[0189] Because T cells from HIV-1 seropositive subjects could be more susceptible to apoptosis than those from seronegative subjects *(48),* whether HIVxCD3 DARTs, in the absence of target cells, might impact T-cell viability, which could confound the analysis of DART activity with patients' cells, was evaluated. Following 7 days of culture of either $CD4^+$ or $CD8^+$ T cells from HIV-infected, ART-suppressed patients in the presence of 100 ng/mL DART, which mimics the viral clearance assay conditions, no decreases in T cell viability based on Annexin V/7 AAD staining (**Figures 28A-28B**) was observed. Moreover, no changes in activation markers (HLA-DR, CD25) on unstimulated $CD4^+$ or $CD8^+$ T cells were observed after culture with HIVxCD3 or control DARTs (**Figures 28C-28D),** suggesting that engagement of the CD3 arm alone does not activate patients' $CD8^+$ or $CD4^+$ T-cells ex vivo.

[0190] Using the lymphocytes from 8 HIV patients on suppressive ART, viral clearance assays were conducted in which $CD4^+$ cells were superinfected with HIV-1 JR-CSF (target cells) and incubated with autologous $CD8^+$ cells (effectors) at E:T ratios of 0:1, 1:10 or 1:1 in the absence or presence of 100 ng/mL DARTs for 7 days. HIVxCD3 DART activity occurred even in the absence of added $CD8^+$ T cells, indicating, that under these experimental conditions, $CD4^+$ T cells may be recruited as effector cells; compared to control, p24 production was reduced by 0.89 log with 7B2xCD3 ($p<0.05$), by 0.32 log with A32xCD3 (p=NS), and by 0.81 with a 1:1 cocktail of both DARTs ($p<0.05$) (**Figure 16A**). Indeed, the addition of fully active DARTs led to significantly increased degranulation of CD4+ T cells when in the presence of infected target cells (**Figures 16G, 16H**). The addition of $CD8^+$ T

cells as effectors resulted in further reductions in p24 levels; compared to the 0.13 log reduction seen with $CD8^+$ T cells alone at an E:T of 1:10, p24 production was reduced by 1.2 log with 7B2xCD3 ($p<0.05$), by 0.6 log with A32xCD3 (p=NS), and by 1.8 log with a cocktail of the two DARTs ($p<0.05$) (**Figure 16B**). Even more marked reductions were found with the higher E:T ratio of 1:1, where CD8s alone accounted for a 0.7 log reduction, but p24 production was reduced by 2.8 log with 7B2xCD3 ($p<0.05$), by 1.6 log with A32xCD3 (p=NS), and by 2.8 log with a cocktail of the two DARTs ($p<0.05$) (**Figure 16C**). Significant reductions were seen even in the absence of any detectable baseline CD8 T cell antiviral activity, and in three cases no virus was able to be recovered following incubation with DARTs (patient 749 with both fully active DARTs, and patients 720 and 725 with 7B2xCD3). The absolute HIV *gag* p24 antigen values are provided in **Figure 21**.

[0191] HIVxCD3 DARTs redirect $CD8^+$ T-cells to clear autologous reservoir virus (AR)-superinfected $CD4^+$ cells using lymphocytes from patients on suppressive ART. The ability of the DARTs to redirect T-cells against target cells expressing *Env* sequences arising from the latent reservoir through the use of viral clearance assays employing autologous reservoir virus (AR)-infected $CD4^+$ target cells from 5 patients (**Figures 16D-16F**) was evaluated. Patient AR virus isolates were generated from pooled supernatants of limiting dilution cultures of mitogen stimulated resting $CD4^+$ T cells to reflect the diversity of virus that may be encountered in vivo following reactivation of latent virus. Despite the diversity of the AR virus isolates, DART activity mirrored that seen with JR-CSF-infected target cells. Modest activity was observed with AR-infected target cells in the absence of $CD8^+$ effectors (thus attributed to $CD4^+$ T cells; **Figure 16D**), with p24 production reduced by 0.32 log with 7B2xCD3 and by 0.20 log with A32xCD3 (p=N.S. due to higher variance in response to 7B2xCD3) and by 0.51 log with a 1:1 cocktail of both DARTs ($p<0.05$), whereas no activity was observed with the control DARTs (**Fig. 16D**). The addition of HIVxCD3 DARTs to a mixture of AR virus-infected CD4+ target cells and autologous CD8+ effector cells led to significantly enhanced reductions in p24 production. At an E:T ratio of 1:10, p24 production was reduced by 0.51 log with 7B2xCD3 ($p<0.05$), by 0.37 log with A32xCD3 and by 0.79 log with a 1:1 cocktail of the two ($p<0.05$), compared to a reduction of only 0.02 log with $CD8^+$ cells alone **(Figure 16E)**. A trend towards decreased p24 production in the presence of HIVxCD3 DARTs was also seen at the higher E:T ratio of 1:1, although the magnitude of the effect was reduced by the variable baseline $CD8^+$ activity seen in the absence of DARTs **(Figure 16F).** Notably, ex vivo DART activity was observed with lymphocytes from all 5

patients evaluated with at least one of the two HIVxCD3 DARTs, and in all cases with the 1:1 DART cocktail.

[0192] *HIVxCD3 DARTs redirect T cells from HIV-infected individuals on suppressive ART to clear virus from resting CD4$^+$ T cells following induction of latent virus expression.* Ultimately, a reagent used in the "shock and kill" HIV eradication strategy must recognize and clear rare infected cells that are likely to express low levels of antigen as they emerge from latency. A latency clearance assay as previously described *(49)* was employed. This assay seeks to measure the ability of DARTs to redirect autologous CD8$^+$ T cells to reduce viral recovery following induction of resting CD4$^+$ T cells of HIV-infected individuals on suppressive ART. Addition of fully active DARTs or a 1:1 cocktail of A32xCD3 and 7B2xCD3 to a co-culture of CD8$^+$ T cells with PHA-stimulated resting CD4$^+$ T cells at an E:T ratio of 1:10 reduced viral recovery in all 6 out of 6 patients, although the magnitude of reduction varied amongst patients. (**Figures 17A, 22**).

[0193] Reversal of HIV latency using maximal mitogen stimulation in vivo is not clinically practical (50). However, the presentation of viral antigen following the reversal of latency with agents that do not result in global T cell activation, such as vorinostat (VOR), may be less robust than that following maximal mitogen stimulation. To evaluate the HIVxCD3 DARTs in a clinically relevant context, a physiologically relevant exposure to VOR that models that obtained following a single 400 mg *in vivo* dose *(18)* to induce latent viral envelope expression was used. In this setting, addition of CD8$^+$ cells at an E:T ratio of 1:10 plus fully active DARTs led to a reduction in viral recovery following a 24 hour co-culture period when compared to CD8$^+$ cells without or with control DARTs in 4 of 5 patients tested. In the single patient who did not respond to DARTs after a 24 hour co-culture period (patient 795), extending the co-culture period from 24 hours to 96 hours led to complete ablation of viral recovery (**Figures 17B, 22**).

[0194] DISCUSSION

[0195] Significant hurdles in the elimination of the latent HIV-1 reservoir include: 1) the limited ability of the immune system to recognize rare HIV-1 infected cells presenting modest levels of HIV antigen prior to or following induction with latency reversing agents (LRA) *(38, 51)*; 2) the presence of CD8$^+$ cytotoxic T lymphocyte escape mutants in the HIV-1 latent reservoir *(52);* and 3) the low frequency of circulating HIV-specific CD8$^+$ T cells in patients on ART and the necessity to activate them due to inadequate stimuli provided by infected cells *(38)*. Described herein are data that HIVxCD3 DARTs could overcome each of these major obstacles.

[0196] HIVxCD3 DARTs with HIV arms derived from the non-neutralizing mAbs A32 and 7B2 were able to recognize HIV-1 *Env*-expressing cell lines and to elicit redirected T-cell killing activity, even when cell surface *Env* expression appeared low. In addition, HIVxCD3 DARTs were effective *ex vivo* in redirecting $CD8^+$ T cells to clear resting $CD4^+$ T cells obtained from aviremic, ART-treated patients following exposure to VOR.

[0197] HIV-1 isolates represented in the latent reservoir are reported to include escape mutants generated by the $CD8^+$ T cell responses *(52),* which may limit the ability of the MHC class I-restricted $CD8^+$ CTL responses induced by natural infection to clear HIV-1 infected cells. The A32 and 7B2 arms of the HIVxCD3 DARTs are based on broadly reactive non-neutralizing anti-HIV mAbs that interact with highly conserved residues in gp120 and gp41, respectively, and efficiently mediate ADCC activity against cells infected with HIV-1 isolates of various subtypes. Of note, the A32 mAb epitope is the earliest one known to be expressed on the surface of infected cells during the syncytia-formation process *(53)* or following tier 2 virus infection *(54)* and the 7B2 mAb epitope is accessible on gp41 stumps, which are expressed on the surface of infected cells during budding and retained at the membrane surface when gp120 subunits dissociate *(29, 55).* These properties are indicative of the accessibility of the A32 and 7B2 epitopes on the surface of infected cells. Importantly, the existence of CTL escape mutants is not a limitation, because CTL epitopes are irrelevant to DART-mediated redirected killing activity. Further, effector T-cells recruited by bispecific molecules like DARTs are polyclonal and not MHC-restricted *(33).* Consistent with these assertions, A32xCD3 and 7B2xCD3 were effective at redirecting $CD8^+$ T cells from patients to clear $CD4^+$ cells infected by their own autologous reservoir (AR) virus, regardless of the presence of any escape mutations that may have accumulated before initiation of therapy *(52).* Interestingly, upon *in vitro* activation of the $CD4^+$ T cells used as target cells, a specific reduction in virus recovery in absence of $CD8^+$T cells was observed, suggesting that DARTs could also recruit cytotoxic $CD4^+$ T cells under these particular experimental conditions. In line with these, it was found that DARTs induced activation of $CD4^+$ T cells in the presence of Env expressing Jurkat-522 F/Y cells, and were capable of increasing degranulation of $CD4^+$ T cells when co-cultured with infected autologous target cells from HIV positive individuals. Cytotoxic $CD4^+$ T cells have been previously reported in the context of responses to HIV-1 *(56)* and Cytomegalovirus *(57).* Further studies will be necessary to determine whether effective DART recruitment and redirection of cytotoxic $CD4^+$ T cells occurs under in vivo settings.

[0198] The relative potencies of the A32xCD3 and 7B2xCD3 DARTs varied among the different test systems employed in our studies, most likely due to variations in the characteristics of the *Env*-expressing target cells and/or effector T-cells. However, whenever one of the DARTs exhibited greater activity than the other, activity similar to that of the more potent DART when combinations of the two DARTs were utilized in the studies with infected patients' cells (**Figures 16A-16H and 17A-17B**) was consistently observed. Thus, combinations of DARTs targeting different HIV epitopes may be an advantageous strategy to maximize both level and breadth of activity, similar to what has been described for combinations of ADCC-mediating *(58)* or broadly neutralizing anti-HIV-1 mAbs *(59, 60)*.

[0199] Eliminating the pool of latently infected cells by HIV-1-specific $CD8^+$ T cell responses is limited by the low frequency of these cells in infected individuals and the need to activate them from the resting state *(38)*. With resting $CD8^+$ T cells from HIV-1 seronegative individuals lacking any previous exposure to HIV-1 antigens, HIVxCD3 DARTs induced degranulation of up to 23% of these resting $CD8^+$ T cells when incubated with the autologous HIV-1 infected target cells destined to be killed. DARTs were also capable of redirecting $CD8^+$ T cells from HIV-1 seropositive individuals who received antiretroviral therapy in viral clearance assays. Therefore, HIVxCD3 DART proteins can effectively recruit and redirect $CD8^+$ T cytotoxic cells independent of previous exposure to HIV antigens, and regardless of any functional impairment that may remain in chronic HIV-1 infection *(46, 47, 61)*.

[0200] DART redirected T cell activity against HIV-1 Env-expressing targets was dependent on HIVxCD3 DART concentration, effector:target (E:T) cell ratio and incubation time. The monovalent nature of each of the binding arms of the HIVxCD3 DART molecule ensures that target cell killing depends exclusively upon effector/target cell co-engagement, as has been observed with CD19xCD3 and other DARTs *(31, 32, 34)*. No HIVxCD3 DART-mediated T-cell activation or redirected killing activity was observed in the absence of *Env* expression on target cells. Similarly, with T-cells from HIV-infected patients on suppressive ART, no T-cell activation was observed in the absence of virus-infected target cells. Because they should elicit cytotoxic activity from circulating T cells only in the proximity of HIV-1 infected Env-expressing target cells, HIVxCD3 DARTs are not expected to elicit widespread systemic effects, such as inflammatory cytokine release, in HIV-infected patients on ART due to the scarcity of the Env-expressing target cells. The specificity of T-cell redirected responses elicited by HIVxCD3 DARTs will be of critical importance clinically, considering that HIV infection induces nonspecific activation of the immune system in both the acute and chronic

phases of the disease, in HIV-1 specific T-cell subsets as well as in general CD8+ T cell populations *(62-64)*.

[0201] HIV-infected $CD4^+$ T cells expressing cell surface *Env* are the primary in vivo targets for HIVxCD3 DART-redirected T cell killing activity. Because these target cells also express CD3, the DART molecules could mediate synapses between infected and uninfected $CD4^+$ T cells that, rather than or in addition to redirecting the killing of infected cells, conceivably could facilitate the spread of virus to uninfected cells. However, no evidence to suggest that DARTs enhanced the spread of virus was observed, as DARTs reduced p24 production even in the absence of CD8+ T cells (**Figures 16A and 16D**).

[0202] In summary, the experiments described herein demonstrate that HIVxCD3 DARTs, with HIV arms derived from the non-neutralizing A32 and 7B2 mAbs, are specific and potent agents to redirect cytolytic T-cells against target cells consisting of 1) HIV-1 Env-expressing $CD4^+$ cell lines, 2) activated $CD4^+$ cells from seronegative individuals infected with HIV-1 IMCs of different subtypes, 3) activated $CD4^+$ cells from seropositive patients on suppressive ART infected with JR-CSF or autologous reservoir virus, or 4) resting $CD4^+$ cells from HIV-infected patients exposed *ex vivo* to a T-cell mitogen (phytohemagglutinin, PHA) or latency reversing agent (vorinostat, VOR). Importantly, the studies demonstrated that autologous $CD8^+$ T cells from HIV-infected patients on suppressive ART were efficacious as effector cells in the presence of DARTs. The demonstration of HIVxCD3 DART-mediated T cell killing activity in the presence of vorinostat is particularly notable because it provides evidence of activity against authentic latent virus isolates expressed from HIV-infected patients' cells in a model system designed to mimic potential clinical HIV eradication strategies, similar to earlier findings using ex-vivo expanded CTLs *(49)*. Thus, the disclosed data indicate that HIVxCD3 DARTs are suitable agents for testing *in vivo* in combination with LRAs in "shock and kill" HIV eradication strategies.

[0203] Methods

[0204] We have reanalyzed the data using the Dunnett's test for multiple comparisons deemed appropriate due of the relative limited number of samples in our studies. The calculated p values are now indicated in the main text (page 14) and in the legends for figures 5-7. The Methods section for the statistical analyses has also been revised.

[0205] ***Patient Population.*** Leukapheresis samples were obtained from HIV seronegative donors or HIV-infected donors with undetectable plasma viremia (<50 copies/mL) on stable ART for at least 6 months, as indicated. Written informed consent was obtained from each

patient and the study was approved by the Duke and UNC Biomedical Institutional Review Boards.

[0206] ***Infectious Molecular Clones (IMCs).*** HIV-1 IMCs for subtype B BaL, subtype AE CM235 and subtype C 1086.C were generated with the backbone derived from NHL4-3 isolate as previously described *(65, 66)*. All IMCs expressed the *Renilla* luciferase reporter gene and preserved all nine viral open reading frames. The *Renilla* luciferase reporter gene was expressed under the control of the HIV-1 Tat gene. Upon HIV-1 infection of CD4+ T cells, expression of Tat during HIV-1 replication will induce luciferase expression, which allows quantitation of infected cells by measuring relative luminescence units (RLU).

[0207] ***Construction, Expression, and Purification of HIVxCD3 DARTs.*** The DARTs were produced from plasmids that coexpressed two polypeptide chains: one with VL of anti-CD3 linked to VH of anti-HIV; the second with VL of anti-HIV linked to VH of anti-CD3. The carboxy termini of the two polypeptide chains consist of paired oppositely charged E-coil/K-coil dimerization domains, which include an interchain disulfide bond (**Figures 10A-10C**). The HIV arm sequences were derived from the non-neutralizing mAbs, A32 [Genbank accession numbers 3TNM_H and 3TNM_L] and 7B2 [Genbank accession numbers AFQ31502 and AFQ31503], and the CD3 arm sequence was derived from hXR32, a humanized mouse anti-human CD3ε mAb (L. Huang, L. S. Johnson, CD3-binding molecules capable of binding to human and nonhuman CD3, U.S. Patent. 20140099318 (2014)). Control DARTs were similarly constructed by replacing either the HIV or CD3 specificity with an irrelevant specificity from an anti-fluorescein mAb (4420) *(67)* or anti-RSV mAb (palivizumab) *(68)*. DART-encoding sequences were cloned into CET1019AD UCOE vectors (EMD Millipore), transfected into CHO cells and proteins purified as described previously *(31)*. Purified proteins were analyzed by SDS-PAGE (NuPAGE Bis-Tris gel system, Invitrogen) and analytical SEC (TSK GS3000SWxL SE-HPLC, Tosoh Bioscience).

[0208] ***ELISA.*** For monospecific binding assays, a MaxiSorp microtiter plate (Nunc) coated with recombinant proteins (human CD3ε/δ heterodimer, JR-FL gp140ΔCF; *(69)*) in bicarbonate buffer was blocked with 3% BSA and 0.1% Tween-20. DART proteins were applied, followed by sequential addition of biotinylated anti-EK coil antibody and streptavidin-HRP (BD Biosciences). For bispecific binding assays, the plate was coated with JRFL gp140ΔCF and DART application was followed by sequential addition of biotinylated CD3ε/δ and streptavidin-HRP. HRP activity was detected with SuperSignal ELISA Pico chemiluminescent substrate (Thermo Scientific).

[0209] ***SPR Analysis.*** HIVxCD3 DART binding to antigens was analyzed by BIAcore 3000 biosensor (GE, Healthcare) as previously described *(31, 32).* Human CD3ε/δ was immobilized on the CM5 sensor chip according to the manufacturer's procedure. DART binding to immobilized CD3 was analyzed to assess the properties of the CD3 arm and HIV-1 Env protein binding to HIV DART captured on immobilized CD3 was analyzed to assess the properties of the HIV arm. JRFL gp140ΔCF was used to assess 7B2xCD3 binding and M.ConS gp140ΔCFI *(69)* was used to assess A32xCD3 binding. The different Env proteins were utilized because A32xCD3 did not bind efficiently to JR-FL gp140ΔCF and M.ConS gp140ΔCFI lacks the gp41 binding site for 7B2xCD3. Binding experiments were performed in 10 mM HEPES, pH 7.4, 150mM NaCl, 3mM EDTA and 0.005% P20 surfactant. Regeneration of immobilized receptor surfaces was performed by pulse injection of 10mM glycine, pH 1.5. K_D values were determined by a global fit of binding curves to the Langmuir 1:1 binding model (BIA evaluation software v4.1).

[0210] ***Cell Lines***. Jurkat-522 F/Y GF cells, which constitutively express a fusion protein of Copepod Green Fluorescent Protein (copGFP) and Firefly Luciferase (System Biosciences), were generated at Macrogenics from Jurkat-522 F/Y cells by transduction and clone selection. HEK293-D371 cells, which have doxycycline-inducible expression of HIV-1 CM244 (subtype AE) gp140, were obtained from Dr. John Kappes (University of Alabama at Birmingham).

[0211] ***Flow Cytometric Analysis of DART or mAb Binding to Cells.*** DARTs at 4 μg/mL were incubated with 10^5 cells in 200 μL FACS buffer containing 10% human AB serum for 30 minutes at room temperature. After washing, cells were resuspended in 100 μL of 1 μg/mL biotin-conjugated mouse anti-EK antibody (recognizes the E/K heterodimerization region of DART proteins), mixed with 1:500 diluted streptavidin-PE and incubated in the dark for 45 minutes at 2-8°C. Cells were washed, resuspended with FACS buffer, and analyzed with a BD Calibur flow cytometer and FlowJo software (TreeStar, Ashland OR). Binding to IMC-infected $CD4^+$ T cells from normal human donors was conducted as previously described *(54)* for the A32 and 7B2 mAbs, and with biotin-conjugated mouse anti-EK antibody and 1:500 diluted streptavidin-PE for the HIVx4420 DARTs.

[0212] ***Redirected T-Cell Cytotoxicity Assay Against HIV-1 Env-expressing Cell Lines and Assessment of T-Cell Activation.*** *Pan T cells* were isolated from healthy human PBMCs with the Dynabeads® Untouched™ Human T Cells Kit (Invitrogen). HIV-1 *Env* expressing cell lines (1-4 x10^5 cells/mL) were treated with serial dilutions of DARTs, together with human T

cells at an effector:target (E:T) ratio = 10:1, or otherwise at varying E:T ratios as indicated, and incubated at 37°C, 5% CO_2 overnight. Cytotoxicity was measured by lactate dehydrogenase (LDH) release (CytoTox 96® Non-Radioactive Cytotoxicity Assay, Promega) as described previously (32). With the Jurkat-522 F/Y GF cell line, cytotoxicity was also measured by luminescence using Luciferase-Glo substrate (Promega). Specific lysis was calculated from luminiscence counts (RLU): cytotoxicity (%) = 100 x (1-(RLU of Sample ÷ RLU of Control)), where Control = average RLU of target cells incubated with effector cells in the absence of DART. Data were fit to a sigmoidal dose-response function to obtain 50% effective concentration (EC_{50}) and percent maximum specific lysis values. T-cell activation was measured by FACS analysis after cells in the assay plate were labeled with CD8-FITC, CD4-APC, and CD25-PE antibodies (BD Biosciences), followed by cell collection by FACS Calibur flow cytometer equipped with acquisition software CellQuest Pro Version 5.2.1 (BD Biosciences). Data analysis was performed using FlowJo software (Treestar, Inc).

[0213] ***Redirected T-Cell Cytotoxicity Assay Against HIV-1 IMC-Infected CD4$^+$ Cells.*** Cryopreserved resting PBMC from normal healthy HIV-1 seronegative donors were activated for 72 hours with anti-human CD3 (clone OKT3; eBioscience) and anti-human CD28 (clone CD28.2; BD Pharmingen). Subsequently, a $CD4^+$ enriched cell population (purity >92.3%; average±standard deviation 95.73±2.6%) was obtained by depletion of $CD8^+$ T cells using magnetic beads (Miltenyi Biosciences), spinoculated in presence of the luciferase-expressing IMC representing HIV-1 subtype AE (CM235), B (BaL) or C (1086.C) and cultured for 72 hours. $CD4^+$ infected target cells were incubated with resting $CD8^+$ effector cells (isolated by negative selection from autologous PBMC, $CD8^+$ T cell Isolation Kit, Miltenyi Biosciences) at 33:1, 11:1, 3:1, and 0:1 E:T ratios in the absence or presence of DARTs for 6-48 hours at concentration ranging from 1,000 to 0.0001 ng/mL. Uninfected and infected target cells alone were included as additional controls. Each condition was tested in duplicate. After incubation, ViviRen™ Live Cell Substrate (Promega) was added and RLU measured on a luminometer; percentage specific lysis (%SL) of target cells was determined as described previously *(58)*.

[0214] ***T-Cell Degranulation (CD107) Assay.*** As described for the cytotoxicity assay with HIV-1 IMC-infected cells as targets, activated $CD4^+$ cells infected with HIV-1 BaL IMC were plated with resting $CD8^+$ effector cells at a 33:1 E:T ratio in the absence or presence of 1ng/mL DARTs and incubated for 6 hour. For the CD4 T cell degranulation, activated $CD4^+$ T cells were either infected with JR-CSF and labeled with the viability (NFL1) and target

specific (TFL4) markers routinely utilized in our ADCC assay *(70)* or added to targets as effectors at a 10:1 ratio prior to addition of DARTs. Each condition was tested in duplicate. CD107 PE-Cy5 (clone H4A3; eBioscience) was titered and added during the last six hours of the incubation along with Monensin solution (BD GolgiStop) *(71)*. A panel of antibodies consisting of LIVE/DEAD Aqua stain, anti-CD3 APC-H7 (clone SK7; BD Pharmingen), anti-CD4 BV605 (clone OKT4; Biolegend), anti-CD8 BV650 (clone RPA-T8; Biolegend) was used to detect $CD107^+$ $CD8^+$ T cells. After washing and fixation, samples were acquired on a custom made LSRII (BD Bioscience, San Jose, CA) within the next 24 hours. A minimum of 300,000 total viable events was acquired for each test. The analysis of the data was performed using the Flow-Jo software (Treestar, Ashland, OR).

[0215] ***T-Cell Viability and Activation Assays.*** $CD8^+$ T cells and CD8 depleted PBMCs obtained from HIV infected ART suppressed patients were plated at $5x10^4$ cells per well in 96 well plates with 100ng/mL of the indicated DART. Cells were cultured in 0.2mL of cIMDM media supplemented with 10% FBS, 1% Penicillin/Streptomycin and 5U/mL IL-2 for 7 days, and then stained with the following antibodies: HLA-DR-PerCP (clone L243), CD25-PE (clone M-A251), CD8-FITC (clone HIT8a), CD8-PE (clone HIT8a), CD4-FITC (clone RPA-T4), and Annexin V-PE and 7-AAD (all BD biosciences, San Jose, CA).

[0216] ***Redirected T-Cell Viral Clearance Assay.*** $CD8^+$ T-cells were isolated from PBMCs by positive selection (EasySep human $CD8^+$ Selection Kit, Stem Cell). CD8-depleted PBMCs were first activated with 2μg/mL of PHA (Remel, Lenexa, KS) and 60U/mL of IL-2, and then infected by spinoculation at 1200xg for 90 minutes with either JR-CSF or autologous reservoir virus (AR) at an MOI of 0.01 as previously described *(47)*. AR virus was obtained from pooled supernatants of replicate wells from outgrowth assays of resting CD4+ T-cells for each patient performed as previously described *(72)*. Fifty-thousand ($5x10^4$) targets/well were co-cultured with $CD8^+$ T cells in triplicate at the indicated E:T ratio in the absence or presence of 100 ng/mL of DART in 0.2m of cIMDM media supplemented with 10% FBS, 1% Penicillin/Streptomycin and 5 U/mL IL-2. For experiments performed in the presence of antiretrovirals (ARVs), 24 hours after spinoculation cells were washed and 1μM of raltegravir and 4μM of abacavir were added, and then DARTs and $CD8^+$ T-cells were added to cultures. Supernatant was assayed on day 7 by p24 ELISA (ABL, Rockville, MD). Results are calculated as the log (p24 of infected target cells only control divided by p24 of the test condition).

[0217] ***Latency Clearance Assay (LCA).*** The reduction of virus recovery from $CD4^+$ infected cells was assessed by a standard quantitative viral outgrowth assay using the resting $CD4^+$ T

cells of aviremic, ART-treated patients, following the addition of antiviral effector cells and/or molecules, as previously described *(49)*. In this case the LCA was used to model the ability of DARTs to clear virus emerging from the latent reservoir under clinically and pharmacologically relevant conditions. Resting $CD4^+$ T-cells were isolated from a leukapheresis product as previously described *(72)* and exposed to PHA (4μg/mL) and IL-2 (60U/mL) for 24 hours or vorinostat (VOR) (335nM, 6 hours) (Merck Research Laboratories), and plated at 0.5 to 1 x 10^6 cells/well in 12 to 36 replicate wells depending on the size of the reservoir. The VOR was then washed off and CD8s added at an E:T of 1:10 as well as 100 ng/mL of the indicated DART. Cells were co-cultured for 24 hours (unless specified otherwise) following which the DART proteins were washed off and allogeneic CD8-depleted PBMCs from an HIV negative donor were added to amplify residual virus. Supernatant was assayed for the presence of p24 antigen on day 15 for each well. Results are calculated as % viral recovery [(# of positive wells/total number plated)x100], normalized to a control in which no $CD8^+$ T cells are added.

[0218] ***Statistical Analysis.*** Statistical comparisons between groups were analyzed using the Dunnett's test for multiple comparisons using GraphPad Prism Softward (La Jolla, CA); p values <0.05, calculated with Dunnett correction for multiple comparisons, were considered significant. Dunnett's test for multiple comparisons was deemed appropriate due to the relative limited number of samples in the studies.

[0219] References for Example 6

1. Chun T-W et al. In vivo fate of HIV-1-infected T cells: Quantitative analysis of the transition to stable latency. *Nat Med.* 1995;1(12):1284–1290.

2. Chun T-W et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature.* 1997;387(6629):183–188.

3. Finzi D et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science.* 1997;278(5341):1295–1300.

4. Wong JK. Recovery of Replication-Competent HIV Despite Prolonged Suppression of Plasma Viremia. *Science.* 1997;278(5341):1291–1295.

5. Pierson TC et al. Molecular Characterization of Preintegration Latency in Human Immunodeficiency Virus Type 1 Infection. *J Virol.* 2002;76(17):8518–8531.

6. Pomerantz RJ. Reservoirs of Human Immunodeficiency Virus Type 1: The Main Obstacles to Viral Eradication. *Clin Infect Dis.* 2002;34(1):91–97.

7. Chomont N et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med.* 2009;15(8):893–900.

8. Bosque A, Famiglietti M, Weyrich AS, Goulston C, Planelles V. Homeostatic Proliferation Fails to Efficiently Reactivate HIV-1 Latently Infected Central Memory CD4+ T Cells. *PLoS Pathog*. 2011;7(10):e1002288.

9. Soriano-Sarabia N et al. Quantitation of Replication-Competent HIV-1 in Populations of Resting CD4+ T Cells. *J Virol*. 2014;88(24):14070–14077.

10. Palmer S et al. New Real-Time Reverse Transcriptase-Initiated PCR Assay with Single-Copy Sensitivity for Human Immunodeficiency Virus Type 1 RNA in Plasma. *J Clin Microbiol*. 2003;41(10):4531–4536.

11. Palmer S et al. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc Natl Acad Sci USA*. 2008;105(10):3879–3884.

12. Dinoso JB et al. Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *Proc Natl Acad Sci USA*. 2009;106(23):9403–9408.

13. Gandhi RT et al. No Evidence for Decay of the Latent Reservoir in HIV-1—Infected Patients Receiving Intensive Enfuvirtide-Containing Antiretroviral Therapy. *J Infect Dis*. 2010;201:293–296.

14. Davey RT et al. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. *Proc Natl Acad Sci USA*. 1999;96(26):15109–15114.

15. Carcelain G et al. Transient Mobilization of Human Immunodeficiency Virus (HIV)-Specific CD4 T-Helper Cells Fails To Control Virus Rebounds during Intermittent Antiretroviral Therapy in Chronic HIV Type 1 Infection. *J Virol*. 2001;75(1):234–241.

16. Rothenberger MK et al. Large number of rebounding/founder HIV variants emerge from multifocal infection in lymphatic tissues after treatment interruption. *Proc Natl Acad Sci USA*. 2015;pii:201414926 [Epub ahead of print].

17. Robb ML, Kim JH. Shot in the HAART: vaccine therapy for HIV. *The Lancet infectious diseases*. 2014;14(4):259–260.

18. Archin NM et al. Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature*. 2012;487(7408):482–485.

19. Denton PW et al. Targeted cytotoxic therapy kills persisting HIV infected cells during ART. *PLoS Pathog*. 2014;10(1):e1003872.

20. Ho Y-C et al. Replication-Competent Noninduced Proviruses in the Latent Reservoir Increase Barrier to HIV-1 Cure. *Cell*. 2013;155(3):540–551.

21. Bullen CK, Laird GM, Durand CM, Siliciano JD, Siliciano RF. New ex vivo approaches distinguish effective and ineffective single agents for reversing HIV-1 latency in vivo. *Nat Med*. 2014;20(4):425–429.

22. Cillo AR et al. Quantification of HIV-1 latency reversal in resting CD4+ T cells from patients on suppressive antiretroviral therapy. *Proc Natl Acad Sci USA*. 2014;111(19):7078–

7083.

23. Pincus SH. Therapeutic potential of anti-HIV immunotoxins. *Antiviral research*. 1996;33(1):1–9.

24. Davey RT et al. Use Of Recombinant Soluble Cd4 Pseudomonas Exotoxin, A Novel Immunotoxin, For Treatment Of Persons Infected With Human Immunodeficiency Virus. *J Infect Dis*. 1994;!170(November):1180–1188.

25. Bera TK, Kennedy PE, Berger EA, Barbas CFI, Pastan I. Specific killing of HIV-infected lymphocytes by a recombinant immunotoxin directed against the HIV-1 envelope glycoprotein. *Molecular Medicine*. 1998;4(6):384.

26. Denton PW et al. Generation of HIV latency in humanized BLT mice. *J Virol*. 2012;86(1):630–634.

27. Pollara J et al. Epitope Specificity of Human Immunodeficiency Virus-1 Antibody Dependent Cellular Cytotoxicity [ADCC] Responses. *Curr HIV Res*. 2013;11(8):378–387.

28. Acharya P et al. Structural Definition of an Antibody-Dependent Cellular Cytotoxicity Response Implicated in Reduced Risk for HIV-1 Infection. *J Virol*. 2014;88(21):12895–12906.

29. Pincus SH et al. In Vivo Efficacy of Anti-Glycoprotein 41, But Not Anti-Glycoprotein 120, Immunotoxins in a Mouse Model of HIV Infection. *J Immunol*. 2003;170:2236–2241.

30. Craig RB, Summa CM, Corti M, Pincus SH. Anti-HIV Double Variable Domain Immunoglobulins Binding Both gp41 and gp120 for Targeted Delivery of Immunoconjugates. *PLoS ONE*. 2012;7(10):e46778.

31. Johnson S et al. Effector Cell Recruitment with Novel Fv-based Dual-affinity Re-targeting Protein Leads to Potent Tumor Cytolysis and in Vivo B-cell Depletion. *J Mol Biol*. 2010;399(3):436–449.

32. Moore PA et al. Application of dual affinity retargeting molecules to achieve optimal redirected T-cell killing of B-cell lymphoma. *Blood*. 2011;117(17):4542–4551.

33. Nagorsen D, Baeuerle PA. Immunomodulatory therapy of cancer with T cell-engaging BiTE antibody blinatumomab. *Experimental Cell Research*. 2011;317(9):1255–1260.

34. Chichili GR et al. A CD3xCD123 bispecific DART for redirecting host T cells to myelogenous leukemia: Preclinical activity and safety in nonhuman primates. *Sci Transl Med*. 2015;7(289):289ra82.

35. Topp MS et al. Phase II Trial of the Anti-CD19 Bispecific T Cell-Engager Blinatumomab Shows Hematologic and Molecular Remissions in Patients With Relapsed or Refractory B-Precursor Acute Lymphoblastic Leukemia. *J Clin Oncol*. 2014;32(36):4134–4140.

36. Topp MS et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. *Lancet Oncol*. 2014;16:57–66.

37. Rader C. DARTs take aim at BiTEs. *Blood*. 2011;117(17):4403–4404.

38. Shan L et al. Stimulation of HIV-1-Specific Cytolytic T Lymphocytes Facilitates Elimination of Latent Viral Reservoir after Virus Reactivation. *Immunity*. 2012;36(3):491–501.

39. Moore JP et al. Exploration of antigenic variation in gp120 from clades A through F of human immunodeficiency virus type 1 by using monoclonal antibodies. *J Virol*. 1994;68(12):8350–8364.

40. Guan Y et al. Diverse specificity and effector function among human antibodies to HIV-1 envelope glycoprotein epitopes exposed by CD4 binding. *Proc Natl Acad Sci USA*. 2013;110(1):E69–78.

41. Veillette M et al. Interaction with Cellular CD4 Exposes HIV-1 Envelope Epitopes Targeted by Antibody-Dependent Cell-Mediated Cytotoxicity. *J Virol*. 2014;88(5):2633–2644.

42. Zhang M-Y et al. Identification and characterization of a broadly cross-reactive HIV-1 human monoclonal antibody that binds to both gp120 and gp41. *PLoS ONE*. 2012;7(9):e44241.

43. Wyatt R et al. Involvement of the V1/V2 variable loop structure in the exposure of human immunodeficiency virus type 1 gp120 epitopes induced by receptor binding. *J Virol*. 1995;69(9):5723–5733.

44. Sanders RW et al. A next-generation cleaved, soluble HIV-1 Env Trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not non-neutralizing antibodies. *PLoS Pathog*. 2013;9(9):e1003618.

45. Cao J, Park IW, Cooper A, Sodroski J. Molecular determinants of acute single-cell lysis by human immunodeficiency virus type 1. *J Virol*. 1996;70(3):1340–1354.

46. Trautmann L, Janbazian L, Chomont N, The E. Upregulation of PD-1 expression on HIV-specific CD8 T cells leads to reversible immune dysfunction. *Nat Med*. 2006;12(10):1198–1202.

47. Blackburn SD et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol*. 2008;10(1):29–37.

48. Groux H. Activation-induced death by apoptosis in CD4+ T cells from human immunodeficiency virus-infected asymptomatic individuals. *J Exp Med*. 1992;175(2):331–340.

49. Sung JA et al. Expanded Cytotoxic T-Cell Lymphocytes Target the Latent Hiv Reservoir. *J Infect Dis*. 2015;:1–15.

50. Van Praag RME et al. OKT3 and IL-2 Treatment for Purging of the Latent HIV-1 Reservoir in Vivo Results in Selective Long-Lasting CD4+ T Cell Depletion - Springer. *J Clin Immunol*. 2001;21(3):218–226.

51. Durand CM, Blankson JN, Siliciano RF. Developing strategies for HIV-1 eradication.

Trends Immunol. 2012;33(11):554–562.

52. Deng K et al. Broad CTL response is required to clear latent HIV-1 due to dominance of escape mutations. *Nature*. 2015;517(7534):381–385.

53. Finnegan CM, Berg W, Lewis GK, DeVico AL. Antigenic properties of the human immunodeficiency virus envelope during cell-cell fusion. *J Virol*. 2001;75(22):11096–11105.

54. Ferrari G et al. An HIV-1 gp120 envelope human monoclonal antibody that recognizes a C1 conformational epitope mediates potent antibody-dependent cellular cytotoxicity (ADCC) activity and defines a common ADCC epitope in human HIV-1 serum. *J Virol*. 2011;85(14):7029–7036.

55. Moore PL et al. Nature of nonfunctional envelope proteins on the surface of human immunodeficiency virus type 1. *J Virol*. 2006;80(5):2515–2528.

56. Johnson S et al. Cooperativity of HIV-Specific Cytolytic CD4 T Cells and CD8 T Cells in Control of HIV Viremia. *J Virol*. 2015;89(15):7494–7505.

57. Casazza JP et al. Acquisition of direct antiviral effector functions by CMV-specific CD4+ T lymphocytes with cellular maturation. *J Exp Med*. 2006;203(13):2865–2877.

58. Pollara J et al. HIV-1 Vaccine-Induced C1 and V2 Env-Specific Antibodies Synergize for Increased Antiviral Activities. *J Virol*. 2014;88(14):7715–7726.

59. Shingai M et al. Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. *Nature*. 2013;503(7475):277–280.

60. Barouch DH et al. Therapeutic efficacy of potent neutralizingHIV-1-specific monoclonal antibodies inSHIV-infected rhesus monkeys. *Nature*. 2013;503(7475):224–228.

61. Yamamoto T et al. Surface expression patterns of negative regulatory molecules identify determinants of virus-specific CD8+ T-cell exhaustion in HIV infection. *Blood*. 2011;117(18):4805–4815.

62. Giorgi JV et al. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. *J Acquir Immune Defic Syndr*. 1993;6(8):904–912.

63. Perfetto SP et al. CD38 expression on cryopreserved CD8+ T cells predicts HIV disease progression. *Cytometry*. 1998;33(2):133–137.

64. Vinikoor MJ et al. Antiretroviral Therapy Initiated During Acute HIV Infection Fails to Prevent Persistent T-Cell Activation. *J Acquir Immune Defic Syndr*. 2013;62(5):505–508.

65. Edmonds TG et al. Replication competent molecular clones of HIV-1 expressing Renilla luciferase facilitate the analysis of antibody inhibition in PBMC. *Virology*. 2010;408(1):1–13.

66. Adachi A et al. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. *J Virol*. 1986;59(2):284–291.

67. Kranz DM, Voss EW. Partial elucidation of an anti-hapten repertoire in BALB/c mice: comparative characterization of several monoclonal anti-fluorescyl antibodies. *Mol Immunol.* 1981;18(10):889–898.

68. Johnson S et al. Development of a Humanized Monoclonal Antibody (MEDI-493) with Potent In Vitro and In Vivo Activity against Respiratory Syncytial Virus. *J Infect Dis.* 1997;176(November):1215–1224.

69. Liao H-X et al. A group M consensus envelope glycoprotein induces antibodies that neutralize subsets of subtype B and C HIV-1 primary viruses. *Virology.* 2006;353:268–282.

70. Pollara J et al. High-throughput quantitative analysis of HIV-1 and SIV-specific ADCC-mediating antibody responses. *Cytometry A.* 2011;79A(8):603–612.

71. Betts MR et al. Sensitive and viable identification of antigen-specific CD8+ T cells by a flow cytometric assay for degranulation. *J Immunol Methods.* 2003;281(1-2):65–78.

72. Archin NM et al. Valproic acid without intensified antiviral therapy has limited impact on persistent HIV infection of resting CD4+ T cells. *AIDS.* 2008;22(10):1131–1135.

[0220] The term 'comprise' and variants of the term such as 'comprises' or 'comprising' are used herein to denote the inclusion of a stated integer or stated integers but not to exclude any other integer or any other integers, unless in the context or usage an exclusive interpretation of the term is required.

[0221] Any reference to publications cited in this specification is not an admission that the disclosures constitute common general knowledge.

ADDITIONAL STATEMENTS OF THE INVENTION

[0222] In certain aspects the invention provides a bispecific molecule comprising a first polypeptide chain, and a second polypeptide chain covalently bonded to one another, wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) comprising the VL CDR3, CDR2 and CDR1 of HIV-1 antibody A32 (SEQ ID NO:78), 7B2 (SEQ ID NO:55), CH28 (SEQ ID NO:68), or CH44 (SEQ ID NO:74);

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) comprising the VH CDR3, CDR2 and CDR1 of an antibody specific for an epitope of CD3 or CD16, wherein domains (A) and (B) are separated from one another by a peptide linker 1; and

(iii) a domain (C) comprising a heterodimer promoting domain including a K coil

or E coil; wherein the heterodimer promoting domain (C) and domain (B) are separated by a peptide linker 2;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) comprising the VL CDR3, CDR2 and CDR1 of an antibody specific for the epitope of CD3 or CD16;

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) comprising the VH CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO:77), 7B2 (SEQ ID NO:56), CH28 (SEQ ID NO:67), or CH44 (SEQ ID NO:73), wherein domains (D) and (E) are separated from one another by a peptide linker 1; and

(iii) a domain (F) comprising a heterodimer promoting domain including a K coil or E coil; wherein the heterodimer promoting domain (F) and domain (E) are separated by a peptide linker 2, and wherein:

the domains (A) and (B) do not associate with one another to form an epitope binding site; the domains (D) and (E) do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like the A32, 7B2, CH28, or CH44 antibody (1);

the domains (B) and (D) associate to form a binding site that binds the epitope of CD3 or CD16;

the K coil comprises residues 240-267 of SEQ ID NO: 19, and the E coil comprises residues 249-276 of SEQ ID NO: 17; and

peptide linker 2 comprises residues 244-248 of SEQ ID NO: 17.

[0223] In certain aspects the invention provides a composition comprising any one of the bispecific molecules of the first embodiment or any combination thereof.

[0224] In certain aspects the invention provides a method to treat or prevent HIV-1 infection in a subject in need thereof comprising administering to the subject a composition comprising any one of the bispecific molecules of the first embodiment or a combination of any one of the bispecific molecules in a therapeutically effective amount.

[0225] In certain aspects the invention provides a vector comprising nucleic acids comprising nucleotides encoding the bispecific molecules of the first embodiment.

[0226] In certain aspects the invention provides a composition comprising a vector comprising a nucleic acid encoding the bispecific molecules of the first embodiment.

[0227] In certain aspects the invention provides the use of a composition comprising any one of the bispecific molecules of the first embodiment or a combination of any one of the bispecific molecules in the manufacture of a medicament to treat or prevent HIV-1 infection in a subject in need thereof.

[0228] In certain aspects the invention provides the use of a latency activating agent, and a composition comprising a bispecific molecule, wherein the bispecific molecule comprises a first polypeptide chain and a second polypeptide chain covalently bonded to one another, wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) comprising the VL CDR3, CDR2 and CDR1 of HIV-1 antibody A32 (SEQ ID NO:78), comprising the VL CDR3, CDR2 and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO:55), comprising the VL CDR3, CDR2 and CDR1 of HIV-1 antibody CH28 (SEQ ID NO:68), or comprising the VL CDR3, CDR2 and CDR1 of HIV-1 antibody CH44 (SEQ ID NO:74);

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) comprising the VH CDR3, CDR2 and CDR1 of an antibody specific for an epitope of CD3 or comprising the VH CDR3, CDR2 and CDR1 of an antibody specific for an epitope of CD16, wherein domains (A) and (B) are separated from one another by a peptide linker 1; and

(iii) a domain (C) comprising a heterodimer promoting domain including a K coil or E coil; wherein the heterodimer promoting domain (C) and domain (B) are separated by a peptide linker 2;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) comprising the VL CDR3, CDR2 and CDR1 of an antibody specific for the epitope of CD3 or comprising the VL CDR3, CDR2 and CDR1 of an antibody specific for the epitope of CD16;

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) comprising the VH CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO:77), comprising the VH CDR3, CDR2, and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO:56), comprising the

VH CDR3, CDR2, and CDR1 of HIV-1 antibody CH28 (SEQ ID NO:67), or comprising the VH CDR3, CDR2, and CDR1 of HIV-1 antibody CH44 (SEQ ID NO:73), wherein domains (D) and (E) are separated from one another by a peptide linker 1; and

(iii) a domain (F) comprising a heterodimer promoting domain including a K coil or E coil; wherein the heterodimer promoting domain (F) and domain (E) are separated by a peptide linker 2;

and wherein:

the domains (A) and (B) do not associate with one another to form an epitope binding site; the domains (D) and (E) do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like A32, 7B2, CH28, or CH44 antibody (1);

the domains (B) and (D) associate to form a binding site that binds an epitope of CD3 or CD16;

the K coil comprises residues 240-267 of SEQ ID NO: 19, and the E coil comprises residues 249-276 of SEQ ID NO: 17; and

peptide linker 2 comprises residues 244-248 of SEQ ID NO: 17;

in the manufacture of a medicament to treat or prevent HIV-1 infection in a subject in need thereof.

What is claimed is:

1. A bispecific molecule comprising a first polypeptide chain, and a second polypeptide chain covalently bonded to one another, wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) comprising the VL CDR3, CDR2 and CDR1 of HIV-1 antibody A32 (SEQ ID NO:78), 7B2 (SEQ ID NO:55), CH28 (SEQ ID NO:68), or CH44 (SEQ ID NO:74);

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) comprising the VH CDR3, CDR2 and CDR1 of an antibody specific for an epitope of CD3 or CD16, wherein domains (A) and (B) are separated from one another by a peptide linker 1; and

(iii) a domain (C) comprising a heterodimer promoting domain including a K coil or E coil; wherein the heterodimer promoting domain (C) and domain (B) are separated by a peptide linker 2;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) comprising the VL CDR3, CDR2 and CDR1 of an antibody specific for the epitope of CD3 or CD16;

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) comprising the VH CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO:77), 7B2 (SEQ ID NO:56), CH28 (SEQ ID NO:67), or CH44 (SEQ ID NO:73), wherein domains (D) and (E) are separated from one another by a peptide linker 1; and

(iii) a domain (F) comprising a heterodimer promoting domain including a K coil or E coil; wherein the heterodimer promoting domain (F) and domain (E) are separated by a peptide linker 2, and wherein:

the domains (A) and (B) do not associate with one another to form an epitope binding site; the domains (D) and (E) do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like the A32, 7B2, CH28, or CH44 antibody (1);

the domains (B) and (D) associate to form a binding site that binds the epitope of CD3 or CD16;

the K coil comprises residues 240-267 of SEQ ID NO: 19, and the E coil comprises residues 249-276 of SEQ ID NO: 17; and

peptide linker 2 comprises residues 244-248 of SEQ ID NO: 17.

2. The bispecific molecule of claim 1, wherein:

(i) the VL2 comprises the VL CDR3, CDR2, and CDR1 of anti-CD3 antibody (SEQ ID NO: 52), and the VH2 comprises the VH CDR3, CDR2, and CDR1 of anti-CD3 antibody (SEQ ID NO: 51); or

(ii) the VL2 comprises the VL CDR3, CDR2, and CDR1 of anti-CD16 antibody (SEQ ID NO: 54), and the VH2 comprises the VH CDR3, CDR2, and CDR1 of anti-CD16 antibody (SEQ ID NO: 53).

3. The bispecific molecule of claim 1, wherein:

(I) (i) the VL1 comprises the VL CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO: 78);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO: 77);

(iii) the VL2 comprises the VL CDR3, CDR2, and CDR1 of anti-CD3 antibody (SEQ ID NO: 52); and

(iv) the VH2 comprises the VH CDR3, CDR2, and CDR1 of anti-CD3 antibody (SEQ ID NO: 51); or wherein

(II) (i) the VL1 comprises VL CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO: 78);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO: 77);

(iii) the VL2 comprises the VL CDR3, CDR2, and CDR1 of anti-CD16 antibody (SEQ ID NO: 54); and

(iv) the VH2 comprises the VH CDR3, CDR2, and CDR1 of anti-CD16 antibody (SEQ ID NO: 53); or wherein

(III) (i) the VL1 comprises the VL CDR3, CDR2, and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO: 55);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO: 56);

(iii) the VL2 comprises the VL CDR3, CDR2, and CDR1 of anti-CD3 antibody (SEQ ID NO: 52); and

(iv) the VH2 comprises the VH CDR3, CDR2, and CDR1 of anti-CD3 antibody (SEQ ID NO: 51); or wherein

(IV) (i) the VL1 comprises the VL CDR3, CDR2, and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO: 55);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO: 56);

(iii) the VL2 comprises the VL CDR3, CDR2, and CDR1 of anti-CD16 antibody (SEQ ID NO: 54); and

(iv) the VH2 comprises the VH CDR3, CDR2, and CDR1 of anti-CD16 antibody (SEQ ID NO: 53); or wherein

(V) (i) the VL1 comprises the VL CDR3, CDR2, and CDR1 of HIV-1 antibody CH28 (SEQ ID NO: 68);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody CH28 (SEQ ID NO: 67);

(iii) the VL2 comprises the VL CDR3, CDR2, and CDR1 of anti-CD3 antibody (SEQ ID NO: 52); and

(iv) the VH2 comprises the VH CDR3, CDR2, and CDR1 of anti-CD3 antibody (SEQ ID NO: 51); or wherein

(VI) (i) the VL1 comprises the CDR1, CDR2 and CDR3 of HIV-1 antibody CH28 (SEQ ID NO: 68);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody CH28 (SEQ ID NO: 67);

(iii) the VL2 comprises the VL CDR3, CDR2, and CDR1 of anti-CD16 antibody (SEQ ID NO: 54); and

(iv) the VH2 comprises the VH CDR3, CDR2, and CDR1 of anti-CD16 antibody (SEQ ID NO: 53); or wherein

(VII) (i) the VL1 comprises the VL CDR3, CDR2, and CDR1 of HIV-1 antibody CH44 (SEQ ID NO: 74);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody CH44 (SEQ ID NO: 73);

(iii) the VL2 comprises the VL CDR3, CDR2, and CDR1 of anti-CD3 antibody (SEQ ID NO: 52); and

(iv) the VH2 comprises the VH CDR3, CDR2, and CDR1 of anti-CD3 antibody (SEQ ID NO: 51); or wherein

(VIII) (i) the VL1 comprises the VL CDR1, CDR2 and CDR3 of HIV-1 antibody CH44 (SEQ ID NO: 74);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody CH44 (SEQ ID NO: 73);

(iii) the VL2 comprises the VL CDR3, CDR2, and CDR1 of anti-CD16 antibody (SEQ ID NO: 54); and

(iv) the VH2 comprises the VH CDR3, CDR2, and CDR1 of anti-CD16 antibody (SEQ ID NO: 53).

4. The bispecific molecule of claim 1 or 2, wherein:
(i) the domain (A) comprises HIV-1 antibody A32 (SEQ ID NO: 78), HIV-1 antibody 7B2 (SEQ ID NO: 55); HIV-1 antibody CH28 (SEQ ID NO: 68); or HIV-1 antibody CH44 (SEQ ID NO: 74);
(ii) the domain (E) comprises HIV-1 antibody A32 (SEQ ID NO: 77 or residues 119-241 of SEQ ID NO: 11), HIV-1 antibody 7B2 (SEQ ID NO: 56); HIV-1 antibody CH28 (SEQ ID NO: 67); or HIV-1 antibody CH44 (SEQ ID NO: 73);
(iii) the domain (B) comprises anti-CD3 antibody (SEQ ID NO: 51) or anti-CD16 antibody (SEQ ID NO: 53); and
(iv) the domain (D) comprises anti-CD3 antibody (SEQ ID NO: 52), or anti-CD16 antibody (SEQ ID NO: 54).

5. The bispecific molecule of claim 1 or 2, wherein:
(i) the domain (A) comprises HIV-1 antibody A32 (SEQ ID NO: 78), the domain (B) comprises anti-CD3 antibody (SEQ ID NO: 51), the domain (D) comprises anti-CD3 antibody (SEQ ID NO: 52), and the domain (E) comprises HIV-1 antibody A32 (SEQ ID NO: 77 or residues 119-241 of SEQ ID NO: 11); or wherein

(ii) the domain (A) comprises HIV-1 antibody 7B2 (SEQ ID NO: 55), the domain (B) comprises anti-CD3 antibody (SEQ ID NO: 51), the domain (D) comprises anti-CD3 antibody (SEQ ID NO: 52), and the domain (E) comprises HIV-1 antibody 7B2 (SEQ ID NO: 56); or wherein

(iii) the domain (A) comprises HIV-1 antibody A32 (SEQ ID NO: 78), the domain (B) comprises anti-CD16 antibody (SEQ ID NO: 53), the domain (D) comprises anti-CD16 antibody (SEQ ID NO: 54), and the domain (E) comprises HIV-1 antibody A32 (SEQ ID NO: 77 or residues 119-241 of SEQ ID NO: 11); or wherein

(iv) the domain (A) comprises HIV-1 antibody 7B2 (SEQ ID NO: 55), the domain (B) comprises anti-CD16 antibody (SEQ ID NO: 53), the domain (D) comprises anti-CD16 antibody (SEQ ID NO: 54), and the domain (E) comprises HIV-1 antibody 7B2 (SEQ ID NO: 56).

6. The bispecific molecule of claim 1, wherein

(i) the VL1 comprises the VL CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO: 78);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO: 77); and

(iii) the domains (B) and (D) associate to form a binding site that binds the epitope of CD3.

7. The bispecific molecule of claim 1, wherein

(i) the VL1 comprises the VL CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO: 78);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO: 77); and

(iii) the domains (B) and (D) associate to form a binding site that binds the epitope of CD16.

8. The bispecific molecule of claim 1, wherein

(i) the VL1 comprises the VL CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO: 78);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO: 77);

(iii) the VL2 comprises the VL CDR3, CDR2, and CDR1 of anti-CD3 antibody (SEQ ID NO: 52); and

(iv) the VH2 comprises the VH CDR3, CDR2, and CDR1 of anti-CD3 antibody (SEQ ID NO: 51).

9. The bispecific molecule of claim 1, wherein

(i) the VL1 comprises the VL CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO: 78);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO: 77);

(iii) the VL2 comprises the VL CDR3, CDR2, and CDR1 of anti-CD16 antibody (SEQ ID NO: 54); and

(iv) the VH2 comprises the VH CDR3, CDR2, and CDR1 of anti-CD16 antibody (SEQ ID NO: 53).

10. The bispecific molecule of claim 1, wherein

the domain (A) comprises VL of HIV-1 antibody A32 (SEQ ID NO: 78);

the domain (E) comprises VH of HIV-1 antibody A32 (residues 119-241 of SEQ ID NO: 11);

the domain (B) comprises VH of anti-CD3 antibody (SEQ ID NO: 51); and

the domain (D) comprises VL of anti-CD3 antibody (SEQ ID NO: 52).

11. The bispecific molecule of claim 1, wherein

the domain (A) comprises VL of HIV-1 antibody A32 (SEQ ID NO: 78);

the domain (E) comprises VH of HIV-1 antibody A32 (residues 119-241 of SEQ ID NO: 11);

the domain (B) comprises VH of anti-CD16 antibody (SEQ ID NO: 53); and

the domain (D) comprises VL of anti-CD16 antibody (SEQ ID NO: 54).

12. The bispecific molecule of claim 1, wherein

(i) the VL1 comprises the VL CDR3, CDR2, and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO: 55);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO: 56); and

(iii) the domains (B) and (D) associate to form a binding site that binds the epitope of CD3.

13. The bispecific molecule of claim 1, wherein

(i) the VL1 comprises the VL CDR3, CDR2, and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO: 55);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO: 56); and

(iii) the domains (B) and (D) associate to form a binding site that binds the epitope of CD16.

14. The bispecific molecule of claim 1, wherein

(i) the VL1 comprises the VL CDR3, CDR2, and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO: 55);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO: 56);

(iii) the VL2 comprises the VL CDR3, CDR2, and CDR1 of anti-CD3 antibody (SEQ ID NO: 52); and

(iv) the VH2 comprises the VH CDR3, CDR2, and CDR1 of anti-CD3 antibody (SEQ ID NO: 51).

15. The bispecific molecule of claim 1, wherein

(i) the VL1 comprises the VL CDR3, CDR2, and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO: 55);

(ii) the VH1 comprises the VH CDR3, CDR2, and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO: 56);

(iii) the VL2 comprises the VL CDR3, CDR2, and CDR1 of anti-CD16 antibody (SEQ ID NO: 54); and

(iv) the VH2 comprises the VH CDR3, CDR2, and CDR1 of anti-CD16 antibody (SEQ ID NO: 53).

16. The bispecific molecule of claim 1, wherein

the domain (A) comprises VL of HIV-1 antibody 7B2 (SEQ ID NO: 55);

the domain (E) comprises VH of HIV-1 antibody 7B2 (SEQ ID NO: 56), and

the domain (B) comprises VH of anti-CD3 antibody (SEQ ID NO: 51); and
the domain (D) comprises VL of anti-CD3 antibody (SEQ ID NO: 52).

17. The bispecific molecule of claim 1, wherein
the domain (A) comprises VL of HIV-1 antibody 7B2 (SEQ ID NO: 55);
the domain (E) comprises VH of HIV-1 antibody 7B2 (SEQ ID NO: 56);
the domain (B) comprises VH of anti-CD16 antibody (SEQ ID NO: 53); and
the domain (D) comprises VL of anti-CD16 antibody (SEQ ID NO: 54).

18. The bispecific molecule of any one of claims 1-17, wherein the bispecific molecule further comprises and Fc-domain.

19. The bispecific molecule of claim 18 wherein:
(i) the first polypeptide chain further comprises a CH2-CH3 domain, wherein the CH2-CH3 domain and domain (C) are separated by a peptide linker 3 or a spacer-linker 3;
(ii) the bispecific molecule further comprises a third polypeptide chain comprises in the N- to C-terminal direction a peptide linker 3 and a CH2-CH3 domain; and
(iii) the CH2-CH3 domains of the first and third polypeptide from the Fc chain.

20. The bispecific molecule of claim 18, wherein:
(i) the CH2-CH3 domain of the first polypeptide chain comprises SEQ ID NO: 42 and the CH2-CH3 domain of the third polypeptide chain comprises SEQ ID NO: 43; or
(ii) the CH2-CH3 domain of the first polypeptide chain comprises SEQ ID NO: 43 and the CH2-CH3 domain of the third polypeptide chain comprises SEQ ID NO: 42.

21. A composition comprising any one of the bispecific molecules of any one of claims 1-20 or any combination thereof.

22. The composition of claim 21, further comprising a second bispecific molecule comprising a first arm with the binding specificity of HIV-1 antibody A32, HIV-1

antibody 7B2, HIV-1 antibody CH28, or HIV-1 antibody CH44 and a second arm targeting CD3 or CD16, wherein the first and second bispecific molecules are different.

23. A method to treat or prevent HIV-1 infection in a subject in need thereof comprising administering to the subject a composition comprising any one of the bispecific molecules of any one of claims 1-20 or a combination of any one of the bispecific molecules in a therapeutically effective amount.

24. The method of claim 23, further comprising administering a latency activating agent.

25. The method of claim 24, wherein the latency activating agent is vorinostat, romidepsin, panobinostat, disulfiram, JQ1, bryostatin, PMA, inonomycin, or any combination thereof.

26. A vector comprising nucleic acids comprising nucleotides encoding the bispecific molecules of any one of claims 1-20.

27. A composition comprising a vector comprising a nucleic acid encoding the bispecific molecules of any one of claims 1-20.

28. Use of a composition comprising any one of the bispecific molecules of any one of claims 1-20 or a combination of any one of the bispecific molecules in the manufacture of a medicament to treat or prevent HIV-1 infection in a subject in need thereof.

29. Use of a latency activating agent, and a composition comprising a bispecific molecule, wherein the bispecific molecule comprises a first polypeptide chain and a second polypeptide chain covalently bonded to one another, wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) comprising the VL CDR3, CDR2 and CDR1 of HIV-1 antibody A32 (SEQ ID NO:78), comprising the VL CDR3, CDR2 and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO:55), comprising the

VL CDR3, CDR2 and CDR1 of HIV-1 antibody CH28 (SEQ ID NO:68), or comprising the VL CDR3, CDR2 and CDR1 of HIV-1 antibody CH44 (SEQ ID NO:74);

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) comprising the VH CDR3, CDR2 and CDR1 of an antibody specific for an epitope of CD3 or comprising the VH CDR3, CDR2 and CDR1 of an antibody specific for an epitope of CD16, wherein domains (A) and (B) are separated from one another by a peptide linker 1; and

(iii) a domain (C) comprising a heterodimer promoting domain including a K coil or E coil; wherein the heterodimer promoting domain (C) and domain (B) are separated by a peptide linker 2;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) comprising the VL CDR3, CDR2 and CDR1 of an antibody specific for the epitope of CD3 or comprising the VL CDR3, CDR2 and CDR1 of an antibody specific for the epitope of CD16;

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) comprising the VH CDR3, CDR2, and CDR1 of HIV-1 antibody A32 (SEQ ID NO:77), comprising the VH CDR3, CDR2, and CDR1 of HIV-1 antibody 7B2 (SEQ ID NO:56), comprising the VH CDR3, CDR2, and CDR1 of HIV-1 antibody CH28 (SEQ ID NO:67), or comprising the VH CDR3, CDR2, and CDR1 of HIV-1 antibody CH44 (SEQ ID NO:73), wherein domains (D) and (E) are separated from one another by a peptide linker 1; and

(iii) a domain (F) comprising a heterodimer promoting domain including a K coil or E coil; wherein the heterodimer promoting domain (F) and domain (E) are separated by a peptide linker 2;

and wherein:

the domains (A) and (B) do not associate with one another to form an epitope binding site; the domains (D) and (E) do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like A32, 7B2, CH28, or CH44 antibody (1);

the domains (B) and (D) associate to form a binding site that binds an epitope of CD3 or CD16;

the K coil comprises residues 240-267 of SEQ ID NO: 19, and the E coil comprises residues 249-276 of SEQ ID NO: 17; and

peptide linker 2 comprises residues 244-248 of SEQ ID NO: 17;

in the manufacture of a medicament to treat or prevent HIV-1 infection in a subject in need thereof.

30. The bispecific molecule of claim 20, wherein:

(i) domain (A) comprises VL of SEQ ID NO: 78;

(ii) domain (B) comprises VH of SEQ ID NO: 51;

(iii) domain (D) comprises VL of SEQ ID NO: 52; and

(iv) domain (E) comprises VH of SEQ ID NO: 77 or residues 119-241 of SEQ ID NO: 11;

wherein the first polypeptide chain further comprises a CH2-CH3 domain, wherein the CH2-CH3 domain and domain (C) are separated by a spacer-linker 3 of SEQ ID NO: 50;

and wherein the CH2-CH3 domain of the first polypeptide chain comprises SEQ ID NO: 42 and the CH2-CH3 domain of the third polypeptide chain comprises SEQ ID NO: 43.

31. The bispecific molecule of claim 1, wherein:

the domain (A) comprises VL of SEQ ID NO: 78;

the domain (E) comprises VH of SEQ ID NO: 77;

the domain (B) comprises VH of SEQ ID NO: 51; and

the domain (D) comprises VL of SEQ ID NO: 52.

32. The bispecific molecule of claim 1, wherein:

the domain (A) comprises VL of SEQ ID NO: 78;

the domain (E) comprises VH of SEQ ID NO: 77;

the domain (B) comprises VH of SEQ ID NO: 53; and

the domain (D) comprises VL of SEQ ID NO: 54.

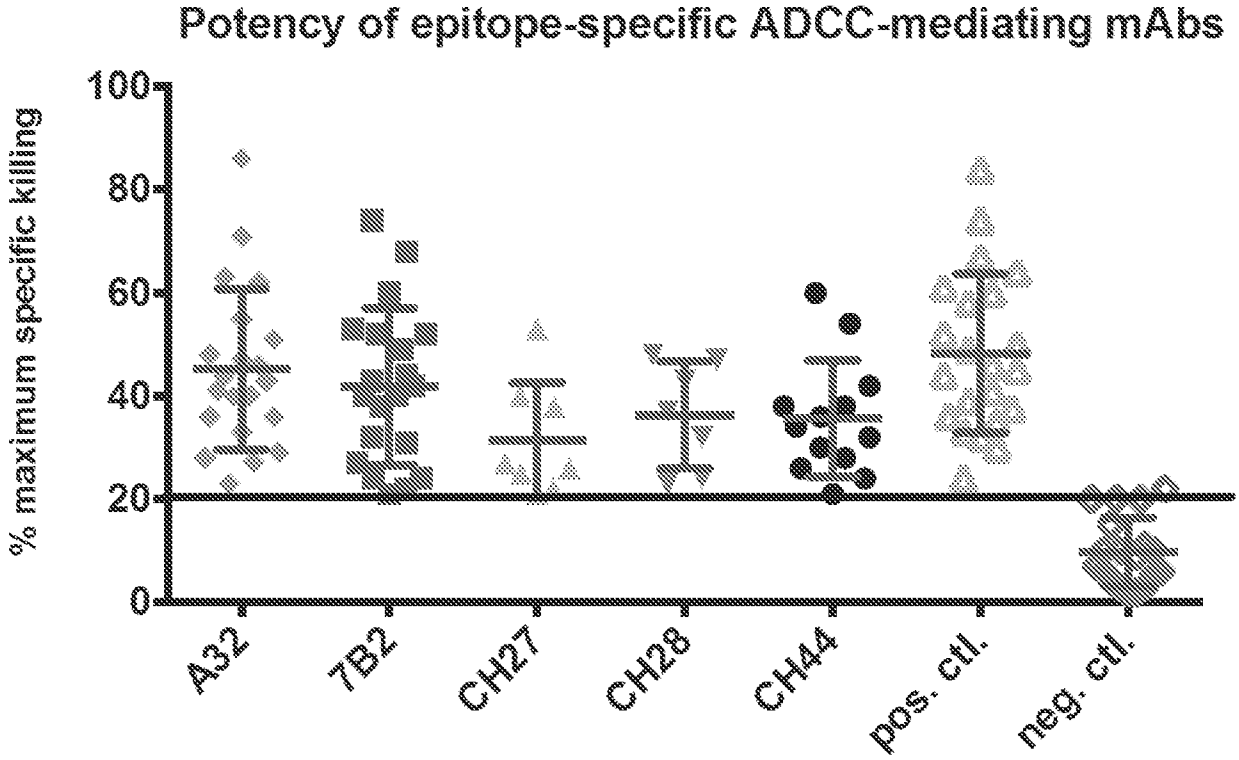

Potency of epitope-specific ADCC-mediating mAbs
% maximum specific killing
100
80
60
40
20
0
A32
7B2
CH27
CH28
CH44
pos. ctl.
neg. ctl.

Figure 1

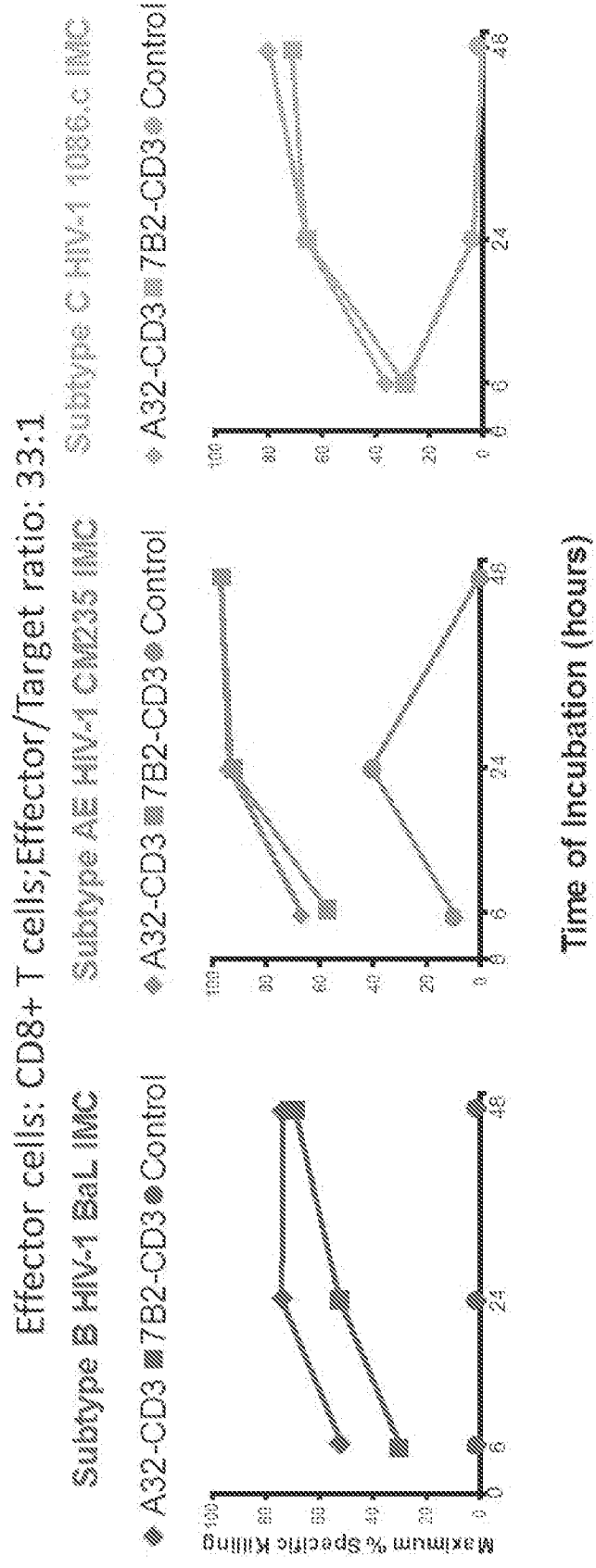

Effector cells: CD8+ T cells;Effector/Target ratio: 33:1
Subtype B HIV-1 BaL IMC
Subtype AE HIV-1 CM235 IMC
Subtype C HIV-1 1086.c IMC
A32-CD3
7B2-CD3
Control
Maximum % Specific Killing
Time of Incubation (hours)

Figure 2

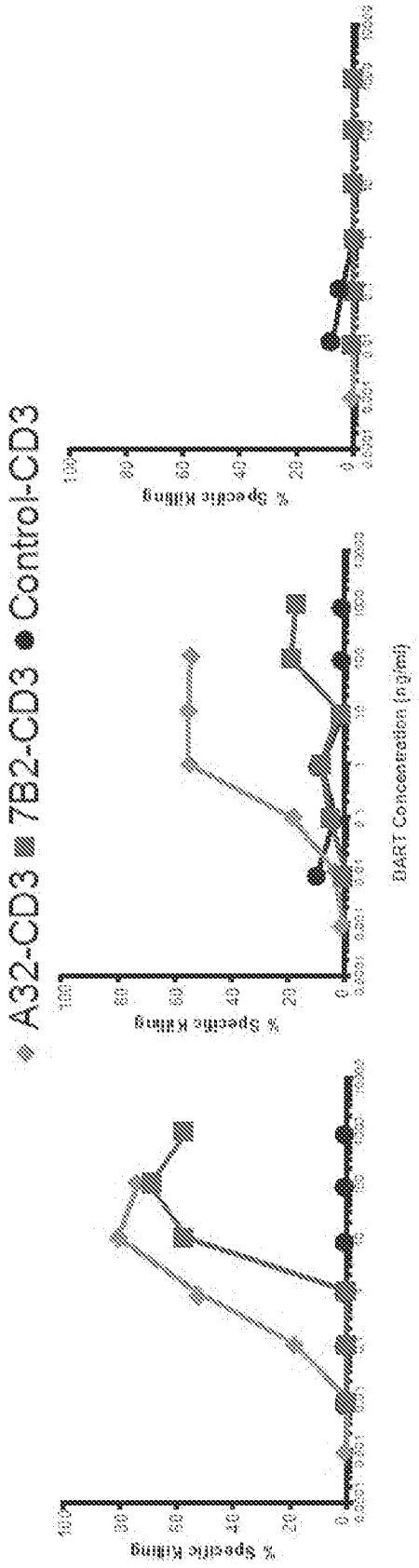

A32-CD3
7B2-CD3
Control-CD3
% Specific Killing
BART Concentration (ng/ml)
Effector/Target ratio = 33:1
11:1
0:1

Figure 3

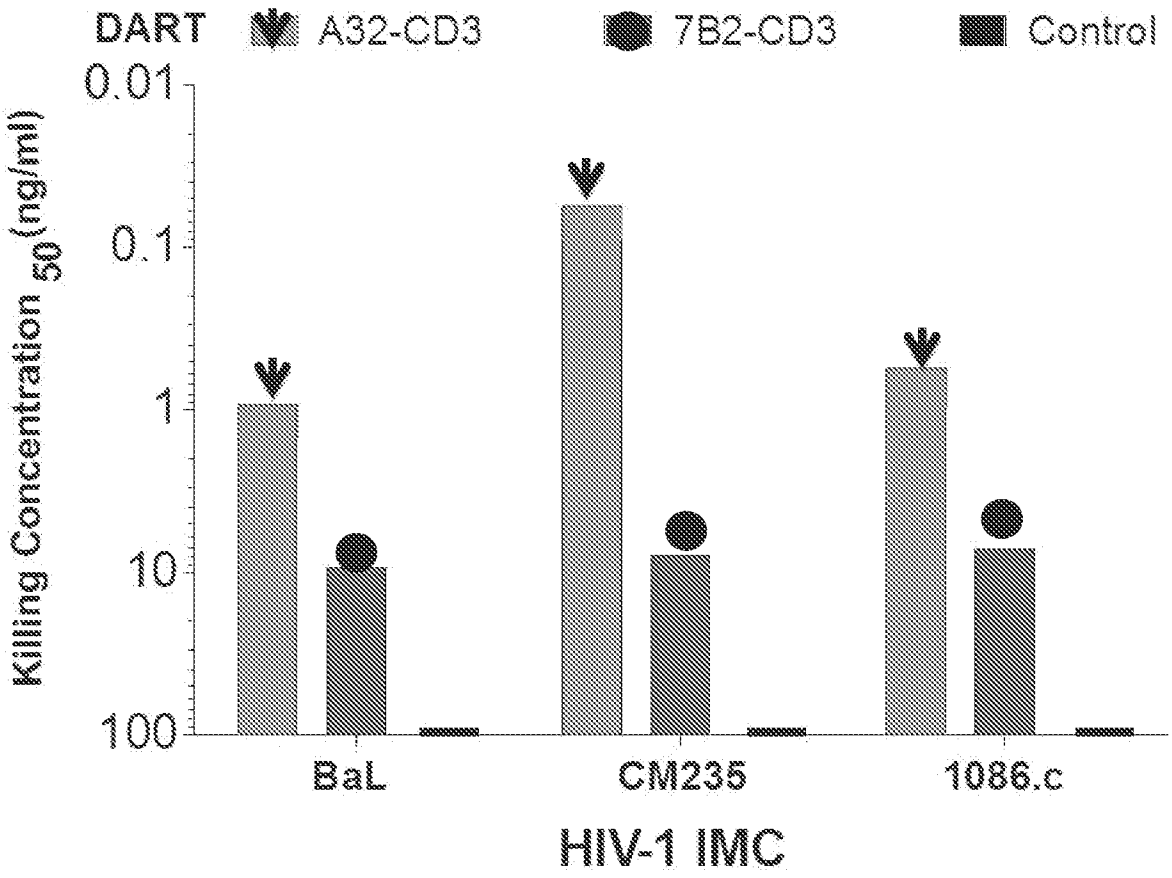

DART
A32-CD3
7B2-CD3
Control
0.01
0.1
1
10
100
Killing Concentration 50 (ng/ml)
BaL
CM235
1086.c
HIV-1 IMC

Figure 4

CDR1 is bold.**CDR2 is** bold green/underlined.**CDR3 is** bold red/italicized.

mAb CH27: original cDNA sequences

>H004021_CH27_HC SEQ ID NO: 57

GAGGTKCAGCTGGTGGAGTCTGGGGGTGGCTTGGTCCAGCCGGGGGGGTCCCTGAGACTCTCTTGTGC
AGCCTCTGGATTCAGCGTCAGCTACGACTATATGGCCTGGGTCCGCCAGGCTCCAGGGAAGGGACTGG
AGTGGGTCTCTATTATTTATGGTGGTGGTAGTCCATATTACGCAGACTCTGTGAAGGGCCGATTCGCC
ATCTCCAGAGACACCTCCAGGAATACACTGGATCTTCAAATGAGCAGCCTGAGACGTGACGACAGCGG
TGTTTACTTCTGTGCGAGGGGACTCGCCTCGCTCTTCGATCTCTGGGGCCGAGGCACCCTGGTCACTG
TCTCGTCAGCATCCCCGACCAGCCCCAAGGTCTTCCCGCTGAGCCTCGACAGCACCAGC

>K003061_CH27_KC SEQ ID NO: 58

GAAATTGTGTTGACRCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGAGAAACAGCCACCCTCTCCTG
CAGGGCCAGTCGGCGTGTTAACGTCAACTACCTAGCCTGGTATCAACACAGACCTGGCCAGAGTCCCA
GGCTCCTCATGTACGGTCCTTACAACAGGCCCACTGGCATCCCGGGCAGGTTCTGGGGCGAGTGGTCT
GGGCCACTCTTCACTCTCAACATCGACAGACTGGAGCCTGTTGATCGAGCAGTCTATTACTGTCTACA
CTTTGACTCTGATACTTCTTCGTGGGCGTTCGGCCGAGGGACCAAGGTGGAGGTCAAACGAACTGTGG
CTGCACCATCTTCTTCACTCTTCCAAAAACATCTGAAGCAGTTTTAATCTCAACTTCTCTCATCAAAC
CCGGGGGGGGAGATCAAGACCGATGGGCCAGCCACGGTTGGTTGGACCGGCACGGGGGCCGGCCCACA
GCGAAAAAAAGGGGGAGACCCAGAGTGTGAGGGCACTAGAGGGGTGGGGACAGACCCTTCTGGGGACT
TGAAGGGGGAGAGTCGCCCCCACATGCCCAACCGGGGGCCCACCACCGGGCGGTTTCACGGACGTTAT
TAACGGGCCGGAATTTTTCCCCCGTGTTTCATACAAGCCCCCCCCTGGAGGGGGGAAACAAACCCCGC
CAAAAAGGGGCCTTTATTCTCAAACCAACAACGCGCGCCCCCAAACCCTCCAAAATTTTTCCCCCCAA
ACCAAACACAAACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
CCCCCCCCCCCCCCCCCCCCCCCCCCCCC

Figure 5

mAb CH27: cleaned DNA sequences

>H004021_CH27_HC_clean SEQ ID NO: 59

GAGGTKCAGCTGGTGGAGTCTGGGGGTGGCTTGGTCCAGCCGGGGGGGTCCCTGAGACTCTCTTGTGC
AGCCTCTGGATTCAGCGTC**AGCTACGACTATATGGCC**TGGGTCCGCCAGGCTCCAGGGAAGGGACTGG
AGTGGGTCTCTATTATTTATGGTGGTGGTAGTCCATATTACGCAGACTCTGTGAAGGGCCGATTCGCC
ATCTCCAGAGACACCTCCAGGAATACACTGGATCTTCAAATGAGCAGCCTGAGACGTGACGACAGCGG
TGTTTACTTCTGTGCGAGGGGACTCGCCTCGCTCTTCGATCTCTGGGGCCGAGGCACCCTGGTCACTG
TCTCGTCAGCA

>K003061_CH27_KC_clean SEQ ID NO: 60

GAAATTGTGTTGACRCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGAGAAACAGCCACCCTCTCCTG
CAGGGCCAGTCGGCGTGTT**AACGTCAACTACCTAGCC**TGGTATCAACACAGACCTGGCCAGAGTCCCA
GGCTCCTCATGTACGGTCCTTACAACAGGCCCACTGGCATCCCGGGCAGGTTCTGGGGCGAGTGGTCT
GGGCCACTCTTCACTCTCAACATCGACAGACTGGAGCCTGTTGATCGAGCAGTCTATTACTGTCTACA
CTTTGACTCTGGATACTTCTTCGTGGGCGTTCGGCCGAGGGACCAAGGTGGAGGTCAAACGA

mAb CH27: amino acid sequences

>H004021_CH27_HC_AA SEQ ID NO: 61

EVQLVESGGGLVQPGGSLRLSCAASGFSV**SYDYMA**WVRQAPGKGLEWVSIIYGGGSPYYADSVKGRFA
ISRDTSRNTLDLQMSSLRRDDSGVYFCARGLASLFDIWGRGTLVTVSSA

>K003061_CH27_KC_AA SEQ ID NO: 62

EIVLTQSPGTLSLSPGETATLSCRASRRV**NVNYLA**WYQHRPGQSPRLLMYGPYNRPTGIPGRFWGEWS
GPLFTLNIDRLEPVDRAVYYCLNFDSDTSSWAFGRGTKVEVKR

Figure 5 cont.

mAb CH28: original cDNA sequences

>H004367_CH28_HC SEQ ID NO: 63

CAGGTGCAGCTGGTRCAGTCTGGGACTTAATCCTCTACACACACCCATATCTCCTACGCTCCATAACG
GACCTACTTTGCACTAATCCTTAGGACACGCCGACTCCTCCTCGGGGTAAACTCTATAAAGATGACAA
AAGATAGTAACCTAGGAATAGAACAATGCAATATACTAAAGAACTGATCCTTGACACTGCTACCGCCT
AGGTATTCGCAATATAACAAATATCATCATCCGACCCAAGTATATGCTGGATTTGTTAAAATAGTCAG
AGAACTATATCTATCTGCAATGGCTTACAGCTAAGCCAGAATCTACAATAAAAACAACAAGCGGGAAC
GCTTCCAAAGAAAAAAACCATATACTCAGGTCTGTTCACCATCTCAATCACCGACTTTCACACATTTT
TTCCCAGGCTTACAAAGATATTCAGGGTTTTTTTTTCCTTTTCCATTCCAGAGGAACCACAGTGCAGC
GTGTCAGTAGGGGGGTAAGGAAAATAAGCCTGGTTCCTCAGTGTGTCTCTCTGGCAAGTCTTGGGGCG
CCTCCTTTAGAAATAATCTCACCCCAGCCCCCCGCCACCTCCAGGGAATGGACGAGTAGTCGAGACAA
ATCTTCCCCCTTATGTTAGAAGAAACAACCAACCCAGATAAAATGCCGCGGAAGCGTCACATTTCTCC
CAAACTCAACCTATATCGCATCTAACAGGGGATAAAGCAGCTCAAAAAAAGACACGGCGGCCTGTATG
TGGGAGAGATCGGTTTCTCTCCTTACAGAACGGAGACCCCCGCGAATTAAAAATGGGGCCAAGAGAAA
AGAGTTAATTTTTCTTCTGGGTATGTGATGGGGGGGGAGAATAATCTGCGCACCAGACCACAGGGTAG
CTGCGTACGTCTCGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCAGCGTCAGGAACGACC
AGATGGCCTGGGTCCGCCAGGCTCCAGGGAAGCGACTGGAGTGGGTCT

>K003331_CH28_KC SEQ ID NO: 64

GAAATTGTGTTGACRCAGTCTCCAGGCACCCTGTCCTTGTCTCCAGGGGAGAGAGCCACCCTCTCCTG
CAGGGCCAATCGGCGGATTGACATGAACGCCTTGGCCTGGTACCAGCACCGATCTGGCCAGGCTCCCA
GGCTCCTCACCCATGGTGTCTATAACAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCTATTGGTCT
GGGCCAGAGTTCACCCTCGTCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTCTATTACTGTGTACA
CTTTCTCTACGAAAATCCAGCGTGGGCGTTCGGCCGAGGGACCAAGATAGAGGTCAAGCGAACTGTGG
CTGCACCATCTGCTCACATCTTCCAACCATATGATGAGCACTTGAAATCTGAAACTGCCTCATCACCC
CTGGCGGGGAAAAACAAGAACGGTGGGGGCGCCACAGGTGCCAAAAACTTTATGTCGCTCCGGGCAAA
AGCCCCCGTGGAATTTCATGAAAAAGGGGTACCCATTAGTAGACCGCAAAATGCCGGGCCAAATCTCC
TAAATAACAGAGGTAGAATTAACCAAACAGATGTCCAAAGAACTTTGCTGGAACACCGTAAGCGGTAT
CAAAGAGGAGGGGGGAGAGAACCGGGGGGCCCCTGCCGGATATATATCTGTAAACAGCCGGGCCAATG
GATTTCCTCCCCCCTGTGGGCCCTAAAAAAAGGGGGTGTTCTTAAACCCACAAAAAAAGGGAAAACGG
TGTCCCGGGAAACACACCTTTCTCCCAATATTATTTCGCGCGGGGAAAAAAAGAAAAACAAAAAAAAA
AACCCC

Figure 5 cont.

mAb CH28: cleaned DNA sequences

>H004367_CH28_HC_clean SEQ ID NO: 65

GAGGTGCAGCTGGTGGAGTCTGGGGGTAGCTGCGTACGTCTCGGGGGGTCCCTGAGACTCTCCTGTGC
AGCCTCTGGATTCAGCGTC**AGGAACGACCAGATGGCC**TGGGTCCGCCAGGCTCCAGGGAAGCGACTGG
AGTGGGTCTCTATTATTAACGATGGTGCTAGTCCATACTACGCAGACTCTGTGAAGGGCCGCTTCGCC
ATGTCCAGAGACACCTCCAAGAATACAGTGTTTCTTCAGATGAACAGCCTGAGACGTGACGACACAGC
TGTTTATTTCTGTGCGAGGGGGATCGCCTCATTCTTCGATGTCTGGGGCCGTGGCACGCTGGTCGCTG
TCTCGTCAGCA

>K003331_CH28_KC_clean SEQ ID NO: 66

GAAATTGTGTTGACRCAGTCTCCAGGCACCCTGTCCTTGTCTCCAGGGGAGAGAGCCACCCTCTCCTG
CAGGGCCAATCGGCGGATT**GACATGAACGCCTTGGCC**TGGTACCAGCACCGATCTGGCCAGGCTCCCA
GGCTCCTCACCCATGGTGTCTATAACAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCTATTGGTCT
GGGCCAGAGTTCACCCTCGTCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTCTATTACTGTGTACA
CTTTCTCTACGAAAATCCAGCGTGGGCGTTCGGCCGAGGGACCAAGATAGAGGTCAAGCGA

mAb CH28: amino acid sequences

>H004367_CH28_HC_AA SEQ ID NO: 67

EVQLVESGGSCVRLGGSLRLSCAASGFSV**RNDQMA**WVRQAPGKRLEWVSIINDGASPYYADSVKGRFA
MSRDTSKNTVFLQMNSLRRDDTAVYFCARGIASFFDVWGRGTLVAVSSA

>K003331_CH28_KC_AA SEQ ID NO: 68

EIVLTQSPGTLSLSPGERATLSCRANRRI**DMNALA**WYQHRSGQAPRLLTNGVYNRATGIPDRFSGYWS
GPEFTLVISRLEPEDFAVYYCVHFLYENPANAFGRGTKIEVKR

Figure 5 cont.

mAb CH44: original cDNA sequences

>H004016_CH44_HC SEQ ID NO: 69

GAGGTKCAGCTGGTGGAGTCTGGGGGTGGCGTGGTCCACCCTGGGGGGTCCCTGAGACTCTCCTGTGC
AGCCTCTGGATTCAGCGTCAGTCACGACTTCATGGCCTGGATCCGCCAGGCTCCAGGAAAGGGACTGG
AGTGGATCTCTATCATATATAACACTGGTTCTCGATACTACTACGCAGACTCTGTGAAGGGCCGCTTC
GCCCTCTCCAGAGATACGTCCAACAACACACTGATTCTTCACATGAGCGGCCTGAGACGTGACGACAC
GGCTATTTATTTCTGTGCCAGGGGAGTCGCCTCGTTCTTCGAATTGTGGGGCCGTGGCACCCTGGTCA
CTGTCTCGTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGC

>K003056_CH44_KC SEQ ID NO: 70

GAAACGACACTCACGCAGTCTCCAGCCATCCTGTCTGTGTCTCCAGGGGAAACCGCCACCCTCTCCTG
CAGGGCCAGTCGCCGTGTTGACATGAACGGCCTCGCCTGGTACCAACACAGGCCTGGCCAGGCTCCCA
GGCTCCTCATGCATGGTGTTTATAATAGGGCCGCCGGCATCTCAGGCAGGTTCACTGGCAGTTGGTCT
GGGCCAGTCTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGGAGTCTATTACTGTCAACA
CTTTTACTATGAGACTTCAGCGTGGGCGTTCGGCCGAGGGACCAGGGTGGAGGGCAAACGAACTGTGG
CTGCACCATCTTCTCCTATCTTCCAAAAAAACAACCTCCCAATGACAGCTGTAAATGTAACATAACTG
TGGCACAATCCATCAGCATCCGCCGCGGAAGGGCGAGCAAAGGGCCCCCCGCCGCCCGCCCGGGGGGG
GGGGGGGGGGGGGTGGGGGGGGCCCCCCTGGGCGGGGGGCCCCACCCCCCCCGGCCCCCCCCCGGGCCC
GGCCCCCCCCCCCCCCCCGGCCCCCCCCCCCCGGGCGGGGCCCCCACAGGGCCCCGGGGGGGCCCCCTCC
CCCCCCCCCCCCAAACCCCCCCCCCCCCCCCCCCCCCCCCCCCGCCGCCCCCCCCCCCCCCCCCCCCCC
CCCCCCCCCCAAACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
CCCCCCCCCCCCCCCCCCCC

Figure 5 cont.

mAb CH44: cleaned DNA sequences

>H004016_CH44_HC_clean SEQ ID NO: 71

GAGGTKCAGCTGGTGGAGTCTGGGGGTGGCGTGGTCCACCCTGGGGGGTCCCTGAGACTCTCCTGTGC
AGCCTCTGGATTCAGCGTC**AGTCACGACTTCATGGCC**TGGATCCGCCAGGCTCCAGGAAAGGGACTGG
AGTGGATCTCTATCATATATAACACTGGTTCTCGATACTACTACGCAGACTCTGTGAAGGGCCGCTTC
GCCCTCTCCAGAGATACGTCCAACAACACACTGATTCTTCACATGAGCGGCCTGAGACGTGACGACAC
GGCTATTTATTTCTGTGCCAGGGGAGTCGCCTCGTTCTTCGAATTGTGGGGCCGTGGCACCCTGGTCA
CTGTCTCGTCAGCC

>K003056_CH44_KC_clean SEQ ID NO: 72

GAAACGACACTCACGCAGTCTCCAGCCATCCTGTCTGTGTCTCCAGGGGAAACCGCCACCCTCTCCTG
CAGGGCCAGTCGCCGTGTT**GACATGAACGGCCTCGCC**TGGTACCAACACAGGCCTGGCCAGGCTCCCA
GGCTCCTCATGCATGGTGTTTATAATAGGGCCGCCGGCATCTCAGGCAGGTTCACTGGCAGTTGGTCT
GGGCCAGTCTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGGAGTCTATTACTGTCAACA
CTTTTACTATGACACTTCAGCGTGGGCGTTCGGCCGAGGGACCAGGGTGGAGGGCAAACGA

mAb CH44: amino acid sequences

>H004016_CH44_HC_AA SEQ ID NO: 73

EVQLVESGGGVVHPGGSLRLSCAASGFSV**SHDFMA**WIRQAPGKGLEWISIIYNTGSRYYYADSVKGRF
ALSRDTSNNTLILHMSGLRRDDTAIYFCARGVASFFELWGRGTLVTVSSA

>K003056_CH44_KC_AA SEQ ID NO: 74

ETTLTQSPAILSVSPGETATLSCRASRRV**DMNGLA**WYQHRPGQAPRLLMNGVYNRAAGISGRFTGSWS
GPVFTLTISRLEPEDFGVYYCQHFYYETSAWAFGRGTRVEGKR

Figure 5 cont.

>A32VH
CAGGTGCAGCTGTGCGGAGTCGGGCCAGGACTGGTGAAGCCTTCACAGACCTTGTCCCTCACTTGCACTGTC
TCTGGTGGCTCCAGCAGTAGTGGTGCTCACTACTGGAGTTGGATCCGCCAGTACCCAGGGAAGGGCCTGGAG
TGGATTGGTTACATCCATTACAGTGGGAACACTTACTACAACCCGTCCCTCAAGAGTCGAATTACCATATCA
CAACACACGTCTGAGAACCAGTTCTCCCTGAAGCTCAACTCTGTGACTGTTGCAGACACGGCCGTCTATTAC
TGTGCGAGAGGGACCCCGTCTCCGGACACTACGGAATGCTTTTGATATTTGGGGCCAGGGGACAAGGGTCACC
GTCTCTTCA (SEQ ID NO:75)
>A32VL
CAGTCTGCCCTGACTCAGCCTCCCTCCGCGTCCGGGTCTCCTGGACAGTCAGTCACCATCTCCTGCACTGGA
ACCAGCAGTGACGTTGGTGGTTATAACTATGTTTCCTGGTACCAACACCACCCAGGCAAAGCCCCCAAACTC
ATAATTTCTGAGGTCAATAACCGGCCCTCAGGGGTCCCTGATCGTTTCTCTGGCTCCAAGTCTGGCAACACG
GCCTCCCTGACCGTCTCTGGGCTCCAGGCTGAGGATGAGGCTGAATATTACTGCAGCTCATACACAGACATC
CACAATTTCGTCTTCGGCGGAGGGACCAAGCTGACCGTCCTA (SEQ ID NO:76)

>A32VH
QVQLCGVGPGLVKPSQTLSLSCTVSGGSSSSGAHYWSWIRQYPGKGLEWIGYIHYSGNTYYNPSLKSRITIS
QHTSENQFSLKLNSVTVADTAVYYCARGTRLETLRNAFDIWGQGTRVTVSS (SEQ ID NO:77)
>A32VL
QSALTQPPSASGSPGQSVTISCTGTSSDVGGYNYVSWYQHHPGKAPKLIISEVNNEPSGVPDRFSGSKSGNT
ASLTVSGLQAEDEAEYYCSSYTDIHNFVFGGGTELTVL (SEQ ID NO:78)

Figure 6

>7B2VH
CAGVTGCAGCTGGTGCAGTCTGGGGGAGGCGTTTTCAAGCCTGGAGGGTCCCTGAGACTCTCCTG
TGAAGCCTCTGGATTCACATTTACTGAATATTACATGACTTGGGTCCGCCAGGCTCCTGGGAAGG
GGCTGGAGTGGCTTGCGTATATTAGTAAGAATYGTGAATATTCAAAATATTCACCGTCCTCAAAC
GGCCGGTTCACCATCTCCAGAGACAACGCCAAGAACTCAGTGTTTCTGCAATTGGACAGACTGAG
CGCCGACGACACGGCCGTCTATTACTGTGCGAGAGCGGACGGATTAACATACTTCTCTGAATTAC
TCCAATACATTTTTGACCTCTGGGGCCAGGGAGCCCGGGTCACCGTCTCCTCG(SEQ ID NO:79)

>7B2VH
METDTLLLWVLLLWVPGSTGDQVQLVQSGGGVFKPGGSLRLSCEASGFTFTEYYMTWVRQAPGKG
LEWLAYISKNGEYSKYSPSSNGRFTISRDNAKNSVFLQLDRLSADDTAVYYCARADGLTYFSELL
QYIFDLWGQGARVTVSS(SEQ ID NO:80)

>7B2VK
METDTLLLWVLLLWVPGSTGDETTLTQSPDSLAVSPGERATIHCKSSQTLLYSSNNRHSIAWYQQ
RPGQPPKLLLYWASMRLSGVPDRFSGSGSGTDFTLTINNLQAEDVAIYYCHQYSSHPPTFGHGTE
VELR (SEQ ID NO:81)
>7B2VK
GAAACGACACTCACGCAGTCTCCAGACTCCCTGGCTGTGTCTCCGGGCGAGAGGGCCACCATCCA
CTGCAAGTCCAGCCAGACTCTTTTGTACAGCTCCAACAATAGACACTCCATTGCTTGGTACCAAC
AGAGACCAGGACAGCCTCCTAAATTACTCCTTTATTGGGCATCTATCCGGCTTTCCGGGGTCCCT
GACCGATTCAGTGGCAGCGGGTCTGGGACAGATTTCACTCTCACCATCAACAACCTGCAGGCTGA
GGATGTGGCCATCTATTACTGTCACCAATATTCCAGTCATCCCCCGACGTTCGGCCACGGGACCA
GGGTGGAGCTCAGA(SEQ ID NO:82)

Figure 7

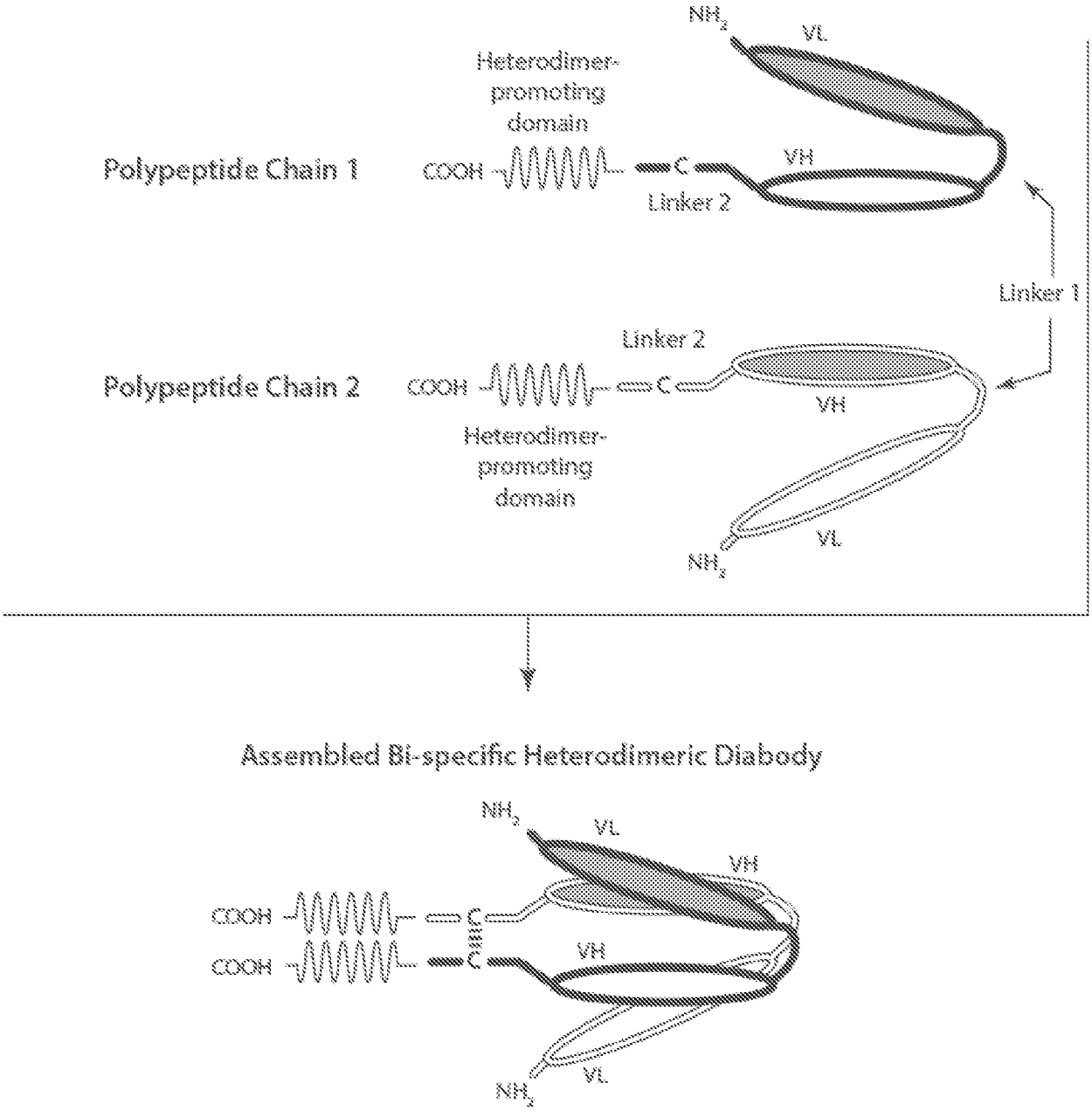

Polypeptide Chain 1
Heterodimer-promoting domain
COOH
C
Linker 2
VH
VL
NH2
Linker 1
Polypeptide Chain 2
Linker 2
COOH
C
VH
Heterodimer-promoting domain
VL
NH2
Assembled Bi-specific Heterodimeric Diabody
NH2
VL
VH
COOH
C
COOH
C
VH
VL
NH2

Figure 8A

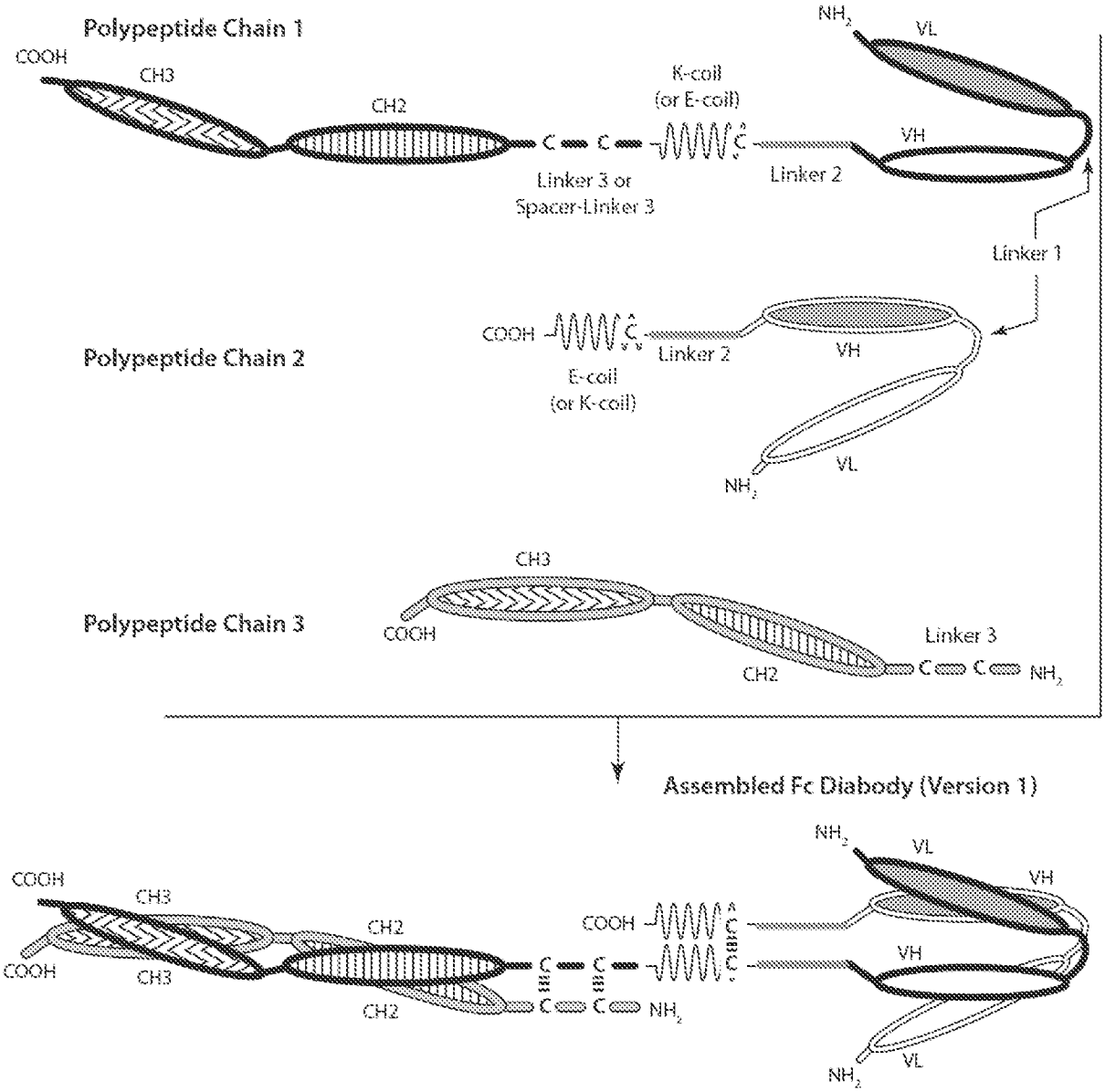

Polypeptide Chain 1
COOH
CH3
CH2
K-coil
(or E-coil)
Linker 3 or
Spacer-Linker 3
Linker 2
VH
VL
NH2
Linker 1
Polypeptide Chain 2
COOH
E-coil
(or K-coil)
Linker 2
VH
VL
NH2
Polypeptide Chain 3
CH3
COOH
CH2
Linker 3
NH2
Assembled Fc Diabody (Version 1)
COOH
CH3
CH3
COOH
CH2
CH2
NH2
VL
VH
VH
VL
NH2

Figure 8B

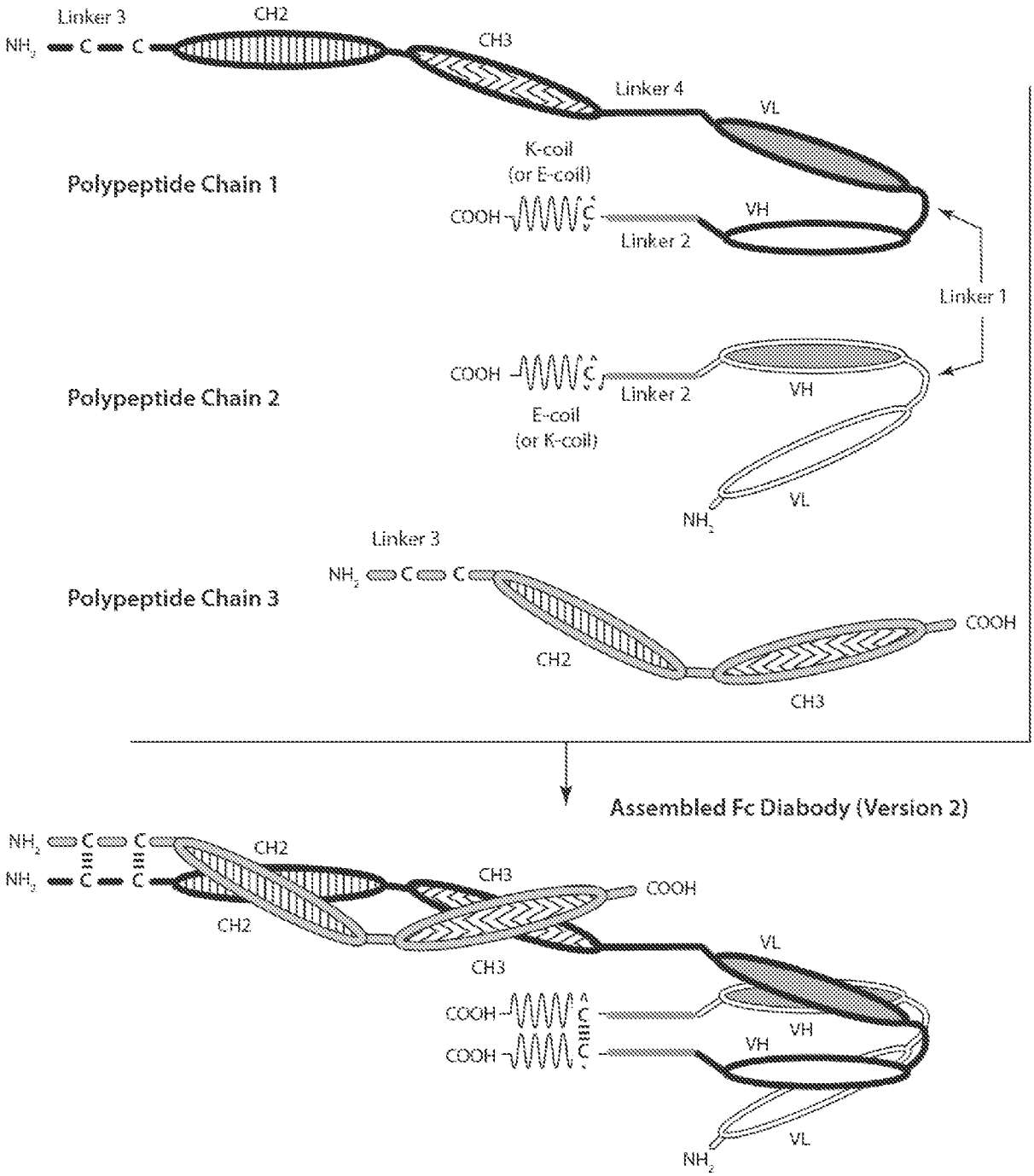

Linker 3
CH2
CH3
Linker 4
VL
K-coil
(or E-coil)
Polypeptide Chain 1
COOH
VH
Linker 2
Linker 1
Polypeptide Chain 2
COOH
Linker 2
VH
E-coil
(or K-coil)
VL
Linker 3
Polypeptide Chain 3
CH2
COOH
CH3
Assembled Fc Diabody (Version 2)
CH2
CH3
COOH
CH2
VL
CH3
VH
VH
VL

Figure 8C

Sequences

SEQ ID NO: 1
GGGSGGGG

SEQ ID NO: 2
GGCGGG

SEQ ID NO: 3
GVEPKSC

SEQ ID NO: 4
VEPKSC

SEQ ID NO: 5
GFNRGEC

SEQ ID NO: 6
FNRGEC

SEQ ID NO: 7
EVAALEKEVA ALEKEVAALE KEVAALEK

SEQ ID NO: 8
KVAALKEKVA ALKEKVAALK EKVAALKE

Figure 9

SEQ ID NO: 9

QSALTQPPSA SGSPGQSVTI SCTGTSSDVG GYNYVSWYQH HPGKAPKLII
SEVNNRPSGV PDRFSGSKSG NTASLTVSGL QAEDEAEYYC SSYTDIHNFV
FGGGTKLTVL GGGSGGGGEV QLVESGGGLV QPGGSLRLSC AASGFTFSTY
AMNWVRQAPG KGLEWVGRIR SKYNNYATYY ADSVKGRFTI SRDDSKNSLY
LQMNSLKTED TAVYYCVRHG NFGNSYVSWF AYWGQGTLVT VSSGGCGGGE
VAALEKEVAA LEKEVAALEK EVAALEK

SEQ ID NO: 10

cagagcgcac tgactcagcc cccttccgcc tccgggtctc ctggacagag cgtgacaatc
tcatgcactg ggacttcaag cgatgtgggc gggtacaact atgtgagttg gtaccagcac
catcccggga aggcacctaa actgatcatt agcgaagtga acaatcgacc aagcggcgtc
cccgaccggt tcagcggcag caagtctggc aataccgcca gtctgacagt ctcaggcctg
caggccgagg atgaagctga gtactattgc tcatcataca ctgacatcca taacttcgtc
ttcggcggcg gaactaaact gaccgtgctg ggtggcggat ccggcggcgg aggcgaggtg
cagctggtgg agtctggggg aggcttggtc cagcctggag ggtccctgag actctcctgt
gcagcctctg gattcacctt cagcacatac gctatgaatt gggtccgcca ggctccaggg
aaggggctgg agtgggttgg aaggatcagg tccaagtaca acaattatgc aacctactat
gccgactctg tgaagggtag attcaccatc tcaagagatg attcaaagaa ctcactgtat
ctgcaaatga acagcctgaa aaccgaggac acggccgtgt attactgtgt gagacacggt
aacttcggca attcttacgt gtcttggttt gcttattggg gacaggggac actggtgact
gtgtcttccg gaggatgtgg cggtggagaa gtggccgcac tggagaaaga ggttgctgct
ttggagaagg aggtcgctgc acttgaaaag gaggtcgcag ccctggagaa a

SEQ ID NO: 11

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI GGTNKRAPWT
PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF GGGTKLTVLG GGGSGGGGQV
QLQESGPGLV KPSQTLSLSC TVSGGSSSSG AHYWSWIRQY PGKGLEWIGY IHYSGNTYYN
PSLKSRITIS QHTSENQFSL KLNSVTVADT AVYYCARGTR LRTLRNAFDI WGQGTLVTVS
SGGCGGGKVA ALKEKVAALK EKVAALKEKV AALKE

Figure 9 cont.

SEQ ID NO: 12

caggctgtgg tgactcagga gccttcactg accgtgtccc caggcggaac tgtgaccctg
acatgcagat ccagcacagg cgcagtgacc acatctaact acgccaattg ggtgcagcag
aagccaggac aggcaccaag gggcctgatc gggggtacaa acaaaagggc tccctggacc
cctgcacggt ttctggaag tctgctgggc ggaaaggccg ctctgactat taccggggca
caggccgagg acgaagccga ttactattgt gctctgtggt atagcaatct gtgggtgttc
ggggtggca caaaactgac tgtgctggga ggggtggat ccggcggcgg aggccaggtg
cagctgcagg agtccggccc cggactggtc aaaccctctc agactctgtc tctgtcatgt
accgtgtcag gcggctcttc cagctccggg gcacactact ggagctggat caggcagtat
cccggcaagg ggctggagtg gatcggatac attcattata gcggcaacac atactataat
ccttctctga agagtcggat cactatttca cagcacacca gcgaaaacca gttcagcctg
aagctgaaca gcgtgaccgt cgccgacaca gccgtgtact attgcgcccg gggcaccaga
ctgagaactc tgagaaacgc atttgacatc tggggacagg ggacactggt gacagtgagc
tccggaggat gtggcggtgg aaaagtggcc gcactgaagg agaaagttgc tgctttgaaa
gagaaggtcg ccgcacttaa ggaaaaggtc gcagccctga aagag

SEQ ID NO: 13

DIVMTQSPDS LAVSPGERAT IHCKSSQTLL YSSNNRHSIA WYQQRPGQPP KLLLYWASMR
LSGVPDRFSG SGSGTDFTLT INNLQAEDVA IYYCHQYSSH PPTFGHGTRV EIKGGGSGGG
GEVQLVESGG GLVQPGGSLR LSCAASGFTF STYAMNWVRQ APGKGLEWVG RIRSKYNNYA
TYYADSVKGR FTISRDDSKN SLYLQMNSLK TEDTAVYYCV RHGNFGNSYV SWFAYWGQGT
LVTVSSGGCG GGEVAALEKE VAALEKEVAA LEKEVAALEK

SEQ ID NO: 14

gacatcgtga tgacccagtc tccagactcc ctggctgtgt ctccgggcga gagggccacc
atccactgca agtccagcca gactcttttg tacagctcca acaatagaca ctccattgct
tggtaccaac agagaccagg acagcctcct aaattactcc tttattgggc atctatgcgg
ctttccgggg tccctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc
atcaacaacc tgcaggctga ggatgtggcc atctattact gtcaccaata ttccagtcat
ccccgacgt tcggccacgg gaccagggtg gagatcaaag gtggaggatc cggcggcgga
ggcgaggtgc agctggtgga gtctgggga ggcttggtcc agcctggagg gtccctgaga
ctctcctgtg cagcctctgg attcaccttc agcacatacg ctatgaattg ggtccgccag

Figure 9 cont.

gctccaggga aggggctgga gtgggttgga aggatcaggt ccaagtacaa caattatgca
acctactatg ccgactctgt gaagggtaga ttcaccatct caagagatga ttcaaagaac
tcactgtatc tgcaaatgaa cagcctgaaa accgaggaca cggccgtgta ttactgtgtg
agacacggta acttcggcaa ttcttacgtg tcttggtttg cttattgggg acaggggaca
ctggtgactg tgtcttccgg aggatgtggc ggtggagaag tggccgcact ggagaaagag
gttgctgctt tggagaagga ggtcgctgca cttgaaaagg aggtcgcagc cctggagaaa

SEQ ID NO: 15
QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI GGTNKRAPWT
PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF GGGTKLTVLG GGGSGGGGQV
QLVQSGGGVF KPGGSLRLSC EASGFTFTEY YMTWVRQAPG KGLEWLAYIS KNGEYSKYSP
SSNGRFTISR DNAKNSVFLQ LDRLSADDTA VYYCARADGL TYFSELLQYI FDLWGQGARV
TVSSGGCGGG KVAALKEKVA ALKEKVAALK EKVAALKE

SEQ ID NO: 16
caggctgtgg tgactcagga gccttcactg accgtgtccc caggcggaac tgtgaccctg
acatgcagat ccagcacagg cgcagtgacc acatctaact acgccaattg ggtgcagcag
aagccaggac aggcaccaag gggcctgatc gggggtacaa acaaaagggc tccctggacc
cctgcacggt tttctggaag tctgctgggc ggaaaggccg ctctgactat taccggggca
caggccgagg acgaagccga ttactattgt gctctgtggt atagcaatct gtgggtgttc
ggggggtggca caaaactgac tgtgctggga gggggtggat ccggcggagg tggacaggtg
cagctggtgc agtctggggg aggcgttttc aagcctggag ggtccctgag actctcctgt
gaagcctctg gattcacatt tactgaatat tacatgactt gggtccgcca ggctcctggg
aaggggctgg agtggcttgc gtatattagt aagaatggtg aatattcaaa atattcaccg
tcctcaaacg gccggttcac catctccaga gacaacgcca agaactcagt gtttctgcaa
ttggacagac tgagcgccga cgacacggcc gtctattact gtgcgagagc ggacggatta
acatacttct ctgaattact ccaatacatt tttgacctct ggggccaggg agcccgggtc
accgtctcct ccggaggatg tggcggtgga aaagtggccg cactgaagga gaaagttgct
gctttgaaag agaaggtcgc cgcacttaag gaaaaggtcg cagccctgaa agag

Figure 9 cont.

SEQ ID NO: 17

EIVLTQSPGT LSLSPGERAT LSCRANRRID MNALAWYQHR SGQAPRLLTH GVYNRATGIP
DRFSGYWSGP EFTLVISRLE PEDFAVYYCV HFLYENPAWA FGQGTKLEIK GGGSGGGGEV
QLVESGGGLV QPGGSLRLSC AASGFTFSTY AMNWVRQAPG KGLEWVGRIR SKYNNYATYY
ADSVKGRFTI SRDDSKNSLY LQMNSLKTED TAVYYCVRHG NFGNSYVSWF AYWGQGTLVT
VSSASTKGEV AACEKEVAAL EKEVAALEKE VAALEK

SEQ ID NO: 18

gaaattgtgt tgacgcagtc tccaggcacc ctgtccttgt ctccagggga gagagccacc
ctctcctgca gggccaatcg gcggattgac atgaacgcct tggcctggta ccagcaccga
tctggccagg ctcccaggct cctcacccat ggtgtctata acagggccac tggcatccca
gacaggttca gtggctattg gtctgggcca gagttcaccc tcgtcatcag cagactggag
cctgaagatt ttgcagtcta ttactgtgta cactttctct acgaaaatcc agcgtgggcg
ttcggccagg ggaccaagct ggagatcaag ggtggaggat ccggcggcgg aggcgaggtg
cagctggtgg agtctggggg aggcttggtc cagcctggag ggtccctgag actctcctgt
gcagcctctg gattcacctt cagcacatac gctatgaatt gggtccgcca ggctccaggg
aaggggctgg agtgggttgg aaggatcagg tccaagtaca acaattatgc aacctactat
gccgactctg tgaagggtag attcaccatc tcaagagatg attcaaagaa ctcactgtat
ctgcaaatga acagcctgaa aaccgaggac acggccgtgt attactgtgt gagacacggt
aacttcggca attcttacgt gtcttggttt gcttattggg gacaggggac actggtgact
gtgtcttccg cctccaccaa gggcgaagtg gccgcatgtg agaaagaggt tgctgctttg
gagaaggagg tcgctgcact tgaaaaggag gtcgcagccc tggagaaa

SEQ ID NO: 19

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI GGTNKRAPWT
PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF GGGTKLTVLG GGGSGGGGEV
QLVESGGGVV HPGGSLRLSC AASGFSVRND QMAWVRQAPG KRLEWVSIIN DGASPYYADS
VKGRFAMSRD TSKNTVFLQM NSLRRDDTAV YFCARGIASF FDVWGRGTLV TVSSASTKGK
VAACKEKVAA LKEKVAALKE KVAALKE

Figure 9 cont.

SEQ ID NO: 20

caggctgtgg tgactcagga gccttcactg accgtgtccc caggcggaac tgtgaccctg
acatgcagat ccagcacagg cgcagtgacc acatctaact acgccaattg ggtgcagcag
aagccaggac aggcaccaag gggcctgatc gggggtacaa acaaaagggc tccctggacc
cctgcacggt ttctggaag tctgctgggc ggaaaggccg ctctgactat taccggggca
caggccgagg acgaagccga ttactattgt gctctgtggt atagcaatct gtgggtgttc
gggggtggca caaaactgac tgtgctggga gggggtggat ccggcggagg tggagaggtg
cagctggtgg agtctggagg tggcgtggtc caccctggag gaagcctgag actctcctgt
gcagcctctg gattcagcgt caggaacgac cagatggcct gggtccgcca ggctccaggg
aagcgactgg agtgggtctc tattattaac gatggtgcta gtccatacta cgcagactct
gtgaagggcc gcttcgccat gtccagagac acctccaaga atacagtgtt tcttcagatg
aacagcctga gacgtgacga cacagctgtt tatttctgtg cgaggggat cgcctcattc
ttcgatgtct ggggccgtgg cacgctggtc actgtctcgt cagcctccac caagggcaaa
gtggccgcat gtaaggagaa agttgctgct ttgaaagaga aggtcgccgc acttaaggaa
aaggtcgcag ccctgaaaga
g

SEQ ID NO: 21

EIVLTQSPAI LSVSPGETAT LSCRASRRVD MNGLAWYQHR PGQAPRLLMH GVYNRAAGIS
GRFTGSWSGP VFTLTISRLE PEDFGVYYCQ HFYYETSAWA FGQGTRLEIK GGGSGGGGEV
QLVESGGGLV QPGGSLRLSC AASGFTFSTY AMNWVRQAPG KGLEWVGRIR SKYNNYATYY
ADSVKGRFTI SRDDSKNSLY LQMNSLKTED TAVYYCVRHG NFGNSYVSWF AYWGQGTLVT
VSSASTKGEV AACEKEVAAL EKEVAALEKE VAALEK

SEQ ID NO: 22

gaaattgtgt tgacgcagtc tccagccatc ctgtctgtgt ctccagggga aaccgccacc
ctctcctgca gggccagtcg ccgtgttgac atgaacggcc tcgcctggta ccaacacagg
cctggccagg ctcccaggct cctcatgcat ggtgtttata atagggccgc cggcatctca
ggcaggttca ctggcagttg gtctgggcca gtcttcactc tcaccatcag cagactggag
cctgaagatt ttggagtcta ttactgtcaa cacttttact atgagacttc agcgtgggcg
ttcggccagg ggaccaggct ggagatcaaa ggtggaggat ccggcggcgg aggcgaggtg
cagctggtgg agtctggggg aggcttggtc cagcctggag ggtccctgag actctcctgt

Figure 9 cont.

gcagcctctg gattcacctt cagcacatac gctatgaatt gggtccgcca ggctccaggg
aaggggctgg agtgggttgg aaggatcagg tccaagtaca acaattatgc aacctactat
gccgactctg tgaagggtag attcaccatc tcaagagatg attcaaagaa ctcactgtat
ctgcaaatga acagcctgaa aaccgaggac acggccgtgt attactgtgt gagacacggt
aacttcggca attcttacgt gtcttggttt gcttattggg gacaggggac actggtgact
gtgtcttccg cctccaccaa gggcgaagtg gccgcatgtg agaaagaggt tgctgctttg
gagaaggagg tcgctgcact tgaaaaggag gtcgcagccc tggagaaa

SEQ ID NO: 23
QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI GGTNKRAPWT
PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF GGGTKLTVLG GGGSGGGGEV
QLVESGGGVV HPGGSLRLSC AASGFSVSHD FMAWIRQAPG KGLEWISIIY NTGSRYYYAD
SVKGRFALSR DTSNNTLILH MSGLRRDDTA IYFCARGVAS FFELWGRGTL VTVSSASTKG
KVAACKEKVA ALKEKVAALK EKVAALKE

SEQ ID NO: 24
caggctgtgg tgactcagga gccttcactg accgtgtccc caggcggaac tgtgaccctg
acatgcagat ccagcacagg cgcagtgacc acatctaact acgccaattg ggtgcagcag
aagccaggac aggcaccaag gggcctgatc gggggtacaa acaaaagggc tccctggacc
cctgcacggt ttttctggaag tctgctgggc ggaaaggccg ctctgactat taccggggca
caggccgagg acgaagccga ttactattgt gctctgtggt atagcaatct gtgggtgttc
gggggtggca caaaactgac tgtgctggga gggggtggat ccggcggagg tggagaggtg
cagctggtgg agtctggagg tggcgtggtc caccctggag gaagcctgag actctcctgt
gcagcctctg gattcagcgt cagtcacgac ttcatggcct ggatcaggca ggctccagga
aagggactgg agtggatctc tatcatatat aacactggtt ctcgatacta ctacgcagac
tctgtgaagg gccgcttcgc cctctccaga gatacgtcca acaacacact gatttcttcac
atgagcggcc tgagacgtga cgacacggct atttatttct gtgccagggg agtcgcctcg
ttctttgaat tgtggggccg tggcaccctg gtcactgtct cgtcagcctc caccaagggc
aaagtggccg catgtaagga gaaagttgct gctttgaaag agaaggtcgc cgcacttaag
gaaaaggtcg cagccctgaa
agag

Figure 9 cont.

SEQ ID NO: 25

DIVMTQSPDS LAVSPGERAT IHCKSSQTLL YSSNNRHSIA WYQQRPGQPP KLLLYWASMR
LSGVPDRFSG SGSGTDFTLT INNLQAEDVA IYYCHQYSSH PPTFGHGTRV EIKGGGSGGG
GQVTLRESGP ALVKPTQTLT LTCTFSGFSL STSGMGVGWI RQPPGKALEW LAHIWWDDDK
RYNPALKSRL TISKDTSKNQ VVLTMTNMDP VDTATYYCAQ INPAWFAYWG QGTLVTVSSG
GCGGGEVAAL EKEVAALEKE VAALEKEVAA LEK

SEQ ID NO: 26

gacatcgtga tgacccagtc tccagactcc ctggctgtgt ctccgggcga gagggccacc
atccactgca agtccagcca gactcttttg tacagctcca acaatagaca ctccattgct
tggtaccaac agagaccagg acagcctcct aaattactcc tttattgggc atctatgcgg
ctttccgggg tccctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc
atcaacaacc tgcaggctga ggatgtggcc atctattact gtcaccaata ttccagtcat
ccccgacgt tcggccacgg gaccagggtg gagatcaaag gtggaggatc cggcggcgga
ggccaggtta ccctgagaga gtctggccct gcgctggtga agcccacaca gaccctcaca
ctgacttgta ccttctctgg gttttcactg agcacttctg gtatgggtgt aggctggatt
cgtcagcctc ccgggaaggc tctagagtgg ctggcacaca tttggtggga tgatgacaag
cgctataatc cagccctgaa gagccgactg acaatctcca aggatacctc caaaaaccag
gtagtcctca caatgaccaa catggaccct gtggatactg ccacatacta ctgtgctcaa
ataaaccccg cctggtttgc ttactggggc caagggactc tggtcactgt gagctccgga
ggatgtggcg gtggagaagt ggccgcactg gagaaagagg ttgctgcttt ggagaaggag
gtcgctgcac ttgaaaagga ggtcgcagcc ctggagaaa

SEQ ID NO: 27

DIVMTQSPDS LAVSLGERAT INCKASQSVD FDGDSFMNWY QQKPGQPPKL LIYTTSNLES
GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQSNEDPY TFGQGTKLEI KGGGSGGGGQ
VQLVQSGGGV FKPGGSLRLS CEASGFTFTE YYMTWVRQAP GKGLEWLAYI SKNGEYSKYS
PSSNGRFTIS RDNAKNSVFL QLDRLSADDT AVYYCARADG LTYFSELLQY IFDLWGQGAR
VTVSSGGCGG GKVAALKEKV AALKEKVAAL KEKVAALKE

Figure 9 cont.

SEQ ID NO: 28

gacatcgtga tgacccaatc tccagactct ttggctgtgt ctctagggga gagggccacc
atcaactgca aggccagcca aagtgttgat tttgatggtg atagttttat gaactggtac
caacagaaac caggacagcc acccaaactc ctcatctata ctacatccaa tctagaatct
ggggtcccag acaggtttag tggcagtggg tctgggacag acttcaccct caccatcagc
agcctgcagg ctgaggatgt ggcagtttat tactgtcagc aaagtaatga agatccgtac
acgttcggac aggggaccaa gcttgagatc aaaggaggcg gatccggcgg aggtggacag
gtgcagctgg tgcagtctgg gggaggcgtt ttcaagcctg gagggtccct gagactctcc
tgtgaagcct ctggattcac atttactgaa tattacatga cttgggtccg ccaggctcct
gggaaggggc tggagtggct tgcgtatatt agtaagaatg gtgaatattc aaaatattca
ccgtcctcaa acggccggtt caccatctcc agagacaacg ccaagaactc agtgtttctg
caattggaca gactgagcgc cgacgacacg gccgtctatt actgtgcgag agcggacgga
ttaacatact tctctgaatt actccaatac atttttgacc tctggggcca gggagccccgg
gtcaccgtct cctccggagg atgtggcggt ggaaaagtgg ccgcactgaa ggagaaagtt
gctgctttga aagagaaggt cgccgcactt aaggaaaagg tcgcagccct gaaaagag

SEQ ID NO: 29

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI GGTNKRAPWT
PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF GGGTKLTVLG GGGSGGGGEV
KLDETGGGLV QPGRPMKLSC VASGFTFSDY WMNWVRQSPE KGLEWVAQIR NKPYNYETYY
SDSVKGRFTI SRDDSKSSVY LQMNNLRVED MGIYYCTGSY YGMDYWGQGT SVTVSSGGCG
GGEVAALEKE VAALEKEVAA LEKEVAALEK

SEQ ID NO: 30

DVVMTQTPFS LPVSLGDQAS ISCRSSQSLV HSNGNTYLRW YLQKPGQSPK VLIYKVSNRF
SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP WTFGGGTKLE IKGGGSGGGG
QVQLQESGPG LVKPSQTLSL SCTVSGGSSS SGAHYWSWIR QYPGKGLEWI GYIHYSGNTY
YNPSLKSRIT ISQHTSENQF SLKLNSVTVA DTAVYYCARG TRLRTLRNAF DIWGQGTLVT
VSSGGCGGGK VAALKEKVAA LKEKVAALKE KVAALKE

Figure 9 cont.

SEQ ID NO: 31
DVVMTQTPFS LPVSLGDQAS ISCRSSQSLV HSNGNTYLRW YLQKPGQSPK VLIYKVSNRF
SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP WTFGGGTKLE IKGGGSGGGG
QVTLRESGPA LVKPTQTLTL TCTFSGFSLS TSGMGVGWIR QPPGKALEWL AHIWWDDDKR
YNPALKSRLT ISKDTSKNQV VLTMTNMDPV DTATYYCAQI NPAWFAYWGQ GTLVTVSSGG
CGGGEVAALE KEVAALEKEV AALEKEVAAL EK

SEQ ID NO: 32
DIVMTQSPDS LAVSLGERAT INCKASQSVD FDGDSFMNWY QQKPGQPPKL LIYTTSNLES
GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQSNEDPY TFGQGTKLEI KGGGSGGGGE
VKLDETGGGL VQPGRPMKLS CVASGFTFSD YWMNWVRQSP EKGLEWVAQI RNKPYNYETY
YSDSVKGRFT ISRDDSKSSV YLQMNNLRVE DMGIYYCTGS YYGMDYWGQG TSVTVSSGGC
GGGKVAALKE KVAALKEKVA ALKEKVAALK E

SEQ ID NO: 33
DIVMTQSPDS LAVSPGERAT IHCKSSQTLL YSSNNRHSIA WYQQRPGQPP KLLLYWASMR
LSGVPDRFSG SGSGTDFTLT INNLQAEDVA IYYCHQYSSH PPTFGHGTRV EIKGGGSGGG
GEVKLDETGG GLVQPGRPMK LSCVASGFTF SDYWMNWVRQ SPEKGLEWVA QIRNKPYNYE
TYYSDSVKGR FTISRDDSKS SVYLQMNNLR VEDMGIYYCT GSYYGMDYWG QGTSVTVSSG
GCGGGEVAAL EKEVAALEKE VAALEKEVAA LEK

SEQ ID NO: 34
DVVMTQTPFS LPVSLGDQAS ISCRSSQSLV HSNGNTYLRW YLQKPGQSPK VLIYKVSNRF
SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP WTFGGGTKLE IKGGGSGGGG
QVQLVQSGGG VFKPGGSLRL SCEASGFTFT EYYMTWVRQA PGKGLEWLAY ISKNGEYSKY
SPSSNGRFTI SRDNAKNSVF LQLDRLSADD TAVYYCARAD GLTYFSELLQ YIFDLWGQGA
RVTVSSGGCG GGKVAALKEK VAALKEKVAA LKEKVAALKE

SEQ ID NO: 35
QSALTQPPSA SGSPGQSVTI SCTGTSSDVG GYNYVSWYQH HPGKAPKLII SEVNNRPSGV
PDRFSGSKSG NTASLTVSGL QAEDEAEYYC SSYTDIHNFV FGGGTKLTVL GGGSGGGGEV
KLDETGGGLV QPGRPMKLSC VASGFTFSDY WMNWVRQSPE KGLEWVAQIR NKPYNYETYY
SDSVKGRFTI SRDDSKSSVY LQMNNLRVED MGIYYCTGSY YGMDYWGQGT SVTVSSGGCG
GGEVAALEKE VAALEKEVAA LEKEVAALEK

SEQ ID NO: 36
DVVMTQTPFS LPVSLGDQAS ISCRSSQSLV HSNGNTYLRW YLQKPGQSPK VLIYKVSNRF
SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP WTFGGGTKLE IKGGGSGGGG
QVQLQESGPG LVKPSQTLSL SCTVSGGSSS SGAHYWSWIR QYPGKGLEWI GYIHYSGNTY
YNPSLKSRIT ISQHTSENQF SLKLNSVTVA DTAVYYCARG TRLRTLRNAF DIWGQGTLVT
VSSGGCGGGK VAALKEKVAA LKEKVAALKE KVAALKE

Figure 9 cont.

SEQ ID NO: 37
QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI GGTNKRAPWT
PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF GGGTKLTVLG GGGSGGGGQV
TLRESGPALV KPTQTLTLTC TFSGFSLSTS GMSVGWIRQP PGKALEWLAD IWWDDKKDYN
PSLKSRLTIS KDTSKNQVVL KVTNMDPADT ATYYCARSMI TNWYFDVWGA GTTVTVSSGG
CGGGEVAALE KEVAALEKEV AALEKEVAAL EK

SEQ ID NO 38
DIQMTQSPST LSASVGDRVT ITCRASQSVG YMHWYQQKPG KAPKLLIYDT SKLASGVPSR
FSGSGSGTEF TLTISSLQPD DFATYYCFQG SGYPFTFGGG TKLEIKGGGS GGGGEVQLVE
SGGGLVQPGG SLRLSCAASG FTFSTYAMNW VRQAPGKGLE WVGRIRSKYN NYATYYADSV
KGRFTISRDD SKNSLYLQMN SLKTEDTAVY YCVRHGNFGN SYVSWFAYWG QGTLVTVSSG
GCGGGKVAAL KEKVAALKEK VAALKEKVAA LKE

SEQ ID NO: 39
APSSS

SEQ ID NO: 40
APSSSPME

SEQ ID NO: 41
APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL
TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK

SEQ ID NO:42
APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
LPPSREEMTK NQVSLWCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL
TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK

SEQ ID NO: 43
APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
LPPSREEMTK NQVSLSCAVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLVSKL
TVDKSRWQQG NVFSCSVMHE ALHNRYTQKS LSLSPGK

Figure 9 cont.

SEQ ID NO: 44
SQSALTQPPS ASGSPGQSVT ISCTGTSSDV GGYNYVSWYQ HHPGKAPKLI ISEVNNRPSG
VPDRFSGSKS GNTASLTVSG LQAEDEAEYY CSSYTDIHNF VFGGGTKLTV LGGGSGGGGQ
VTLRESGPAL VKPTQTLTLT CTFSGFSLST SGMGVGWIRQ PPGKALEWLA HIWWDDDKRY
NPALKSRLTI SKDTSKNQVV LTMTNMDPVD TATYYCAQIN PAWFAYWGQG TLVTVSSGGC
GGGEVAALEK EVAALEKEVA ALEKEVAALE K

SEQ ID NO: 45
DIVMTQSPDS LAVSLGERAT INCKASQSVD FDGDSFMNWY QQKPGQPPKL LIYTTSNLES
GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQSNEDPY TFGQGTKLEI KGGGSGGGGQ
VQLQESGPGL VKPSQTLSLS CTVSGGSSSS GAHYWSWIRQ YPGKGLEWIG YIHYSGNTYY
NPSLKSRITI SQHTSENQFS LKLNSVTVAD TAVYYCARGT RLRTLRNAFD IWGQGTLVTV
SSGGCGGGKV AALKEKVAAL KEKVAALKEK VAALKE

SEQ ID NO: 46
DIVMTQSPDS LAVSPGERAT IHCKSSQTLL YSSNNRHSIA WYQQRPGQPP KLLLYWASMR
LSGVPDRFSG SGSGTDFTLT INNLQAEDVA IYYCHQYSSH PPTFGHGTRV EIKGGGSGGG
GEVQLVESGG GLVQPGGSLR LSCAASGFTF STYAMNWVRQ APGKGLEWVG RIRSKYNNYA
TYYADSVKGR FTISRDDSKN SLYLQMNSLK TEDTAVYYCV RHGNFGNSYV SWFAYWGQGT
LVTVSSGGCG GGEVAALEKE VAALEKEVAA LEKEVAALEK GGGDKTHTCP PCPAPEAAGG
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE
MTKNQVSLWC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW
QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

SEQ ID NO: 47

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI GGTNKRAPWT
PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF GGGTKLTVLG GGGSGGGGQV
QLVQSGGGVF KPGGSLRLSC EASGFTFTEY YMTWVRQAPG KGLEWLAYIS KNGEYSKYSP
SSNGRFTISR DNAKNSVFLQ LDRLSADDTA VYYCARADGL TYFSELLQYI FDLWGQGARV
TVSSGGCGGG KVAALKEKVA ALKEKVAALK EKVAALKE

SEQ ID NO: 48
APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
LPPSREEMTK NQVSLSCAVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLVSKL
TVDKSRWQQG NVFSCSVMHE ALHNRYTQKS LSLSPGK

Figure 9 cont.

SEQ ID NO: 49 (Linker 3)

DKTHTCPPCP

SEQ ID NO: 50 (Spacer Linker 3)

GGGDKTHTCPPCP

SEQ ID NO: 51 (VH CD3)

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR IRSKYNNYAT YYADSVKGRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR HGNFGNSYVS WFAYWGQGTL VTVSS

SEQ ID NO: 52 (VL CD3)

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF GGGTKLTVLG

SEQ ID NO: 53 (VH CD16)

QVTLRESGP ALVKPTQTLT LTCTFSGFSL STSGMGVGWI RQPPGKALEW LAHIWWDDDK RYNPALKSRL TISKDTSKNQ VVLTMTNMDP VDTATYYCAQ INPAWFAYWG QGTLVTVSS

SEQ ID NO: 54 (VL CD16)

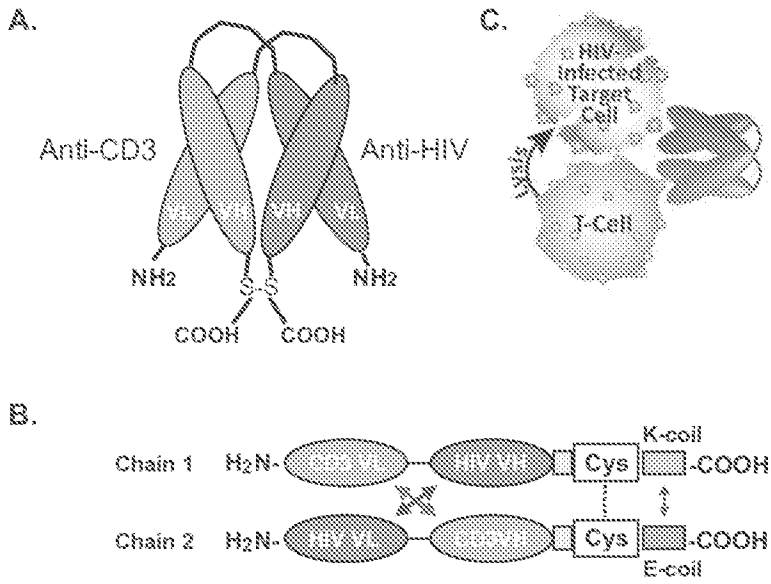
DIVMTQSPDS LAVSLGERAT INCKASQSVD FDGDSFMNWY QQKPGQPPKL LIYTTSNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQSNEDPY TFGQGTKLEI K

SEQ ID NO: 55 (VL 7B2 GenBank: AFQ31503.1DIVMTQSPDS LAVSPGERAT IHCKSSQTLL YSSNNRHSIA WYQQRPGQPP KLLLYWASMR LSGVPDRFSG SGSGTDFTLT INNLQAEDVA IYYCHQYSSH PPTFGHGTRV

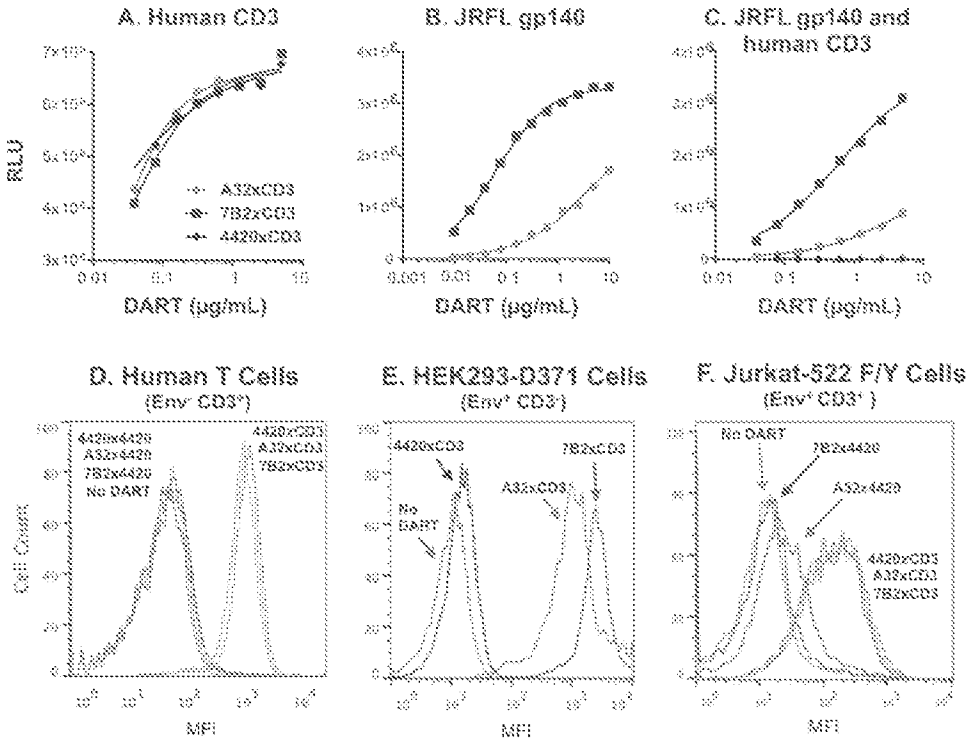
SEQ ID NO: 56 (VH 7B2 GenBank: AFQ31502.1)

QVQLVQSGGG VFKPGGSLRL SCEASGFTFT EYYMTWVRQA PGKGLEWLAY ISKNGEYSKY SPSSNGRFTI SRDNAKNSVF LQLDRLSADD TAVYYCARAD GLTYFSELLQ YIFDLWGQGA RVTVSS

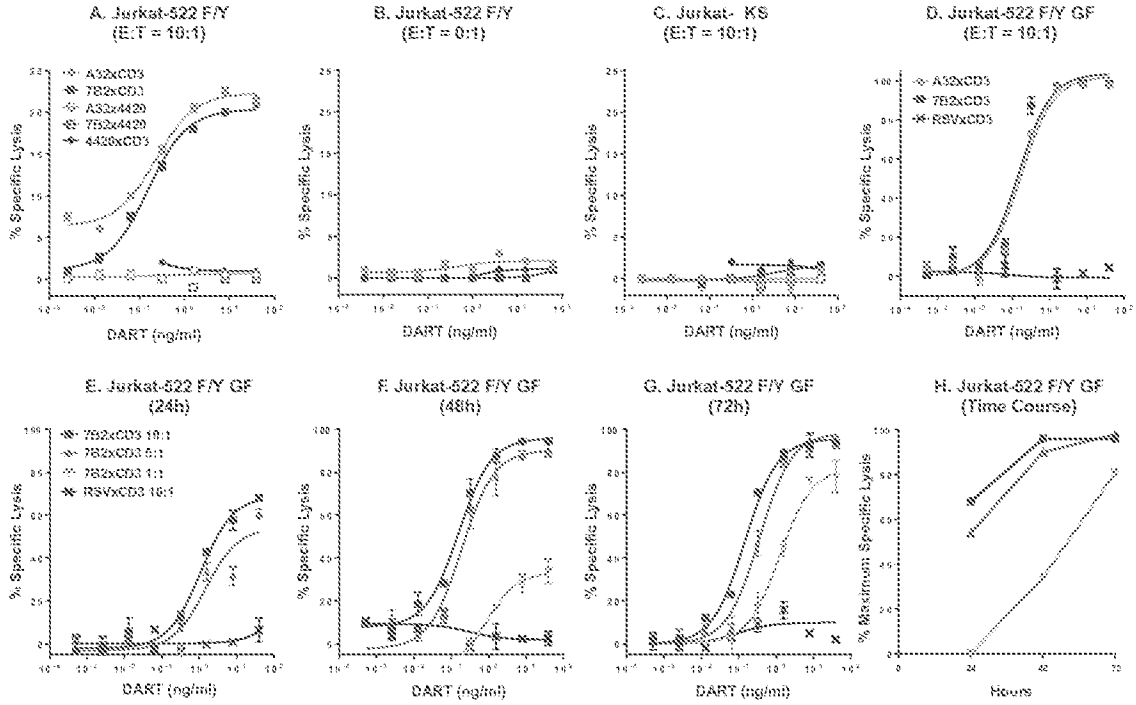
Figure 9 cont.



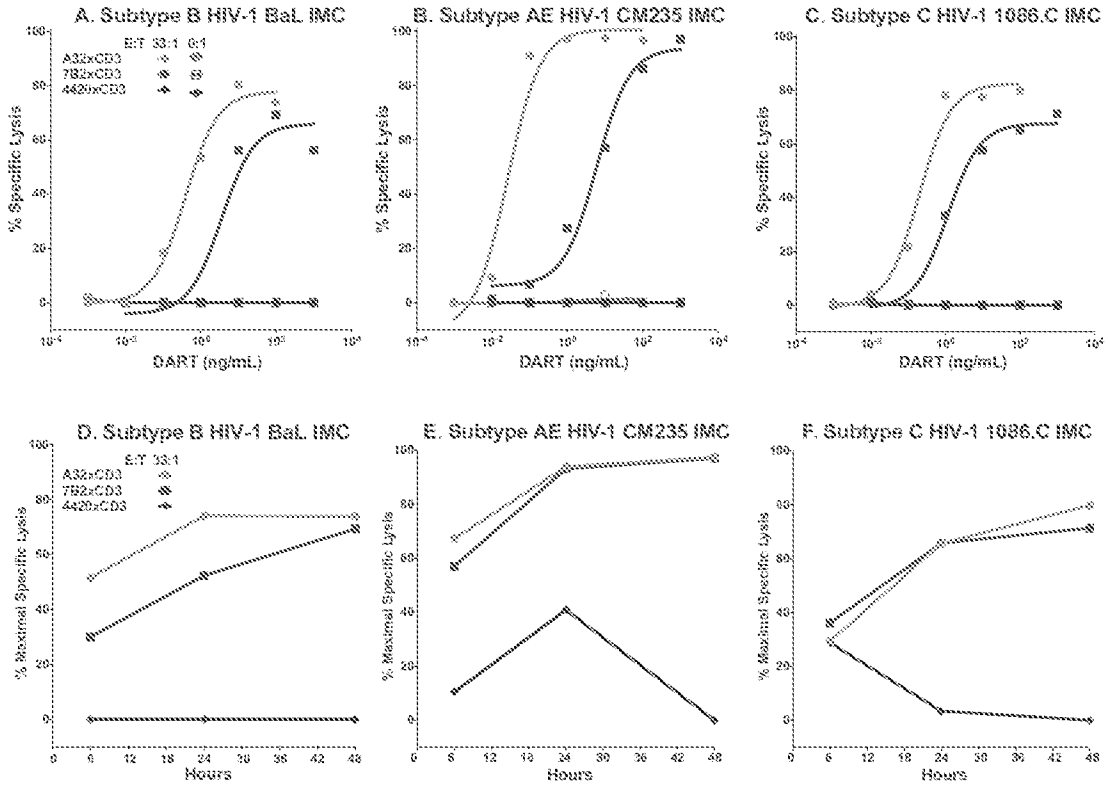
Figures 10A-10C



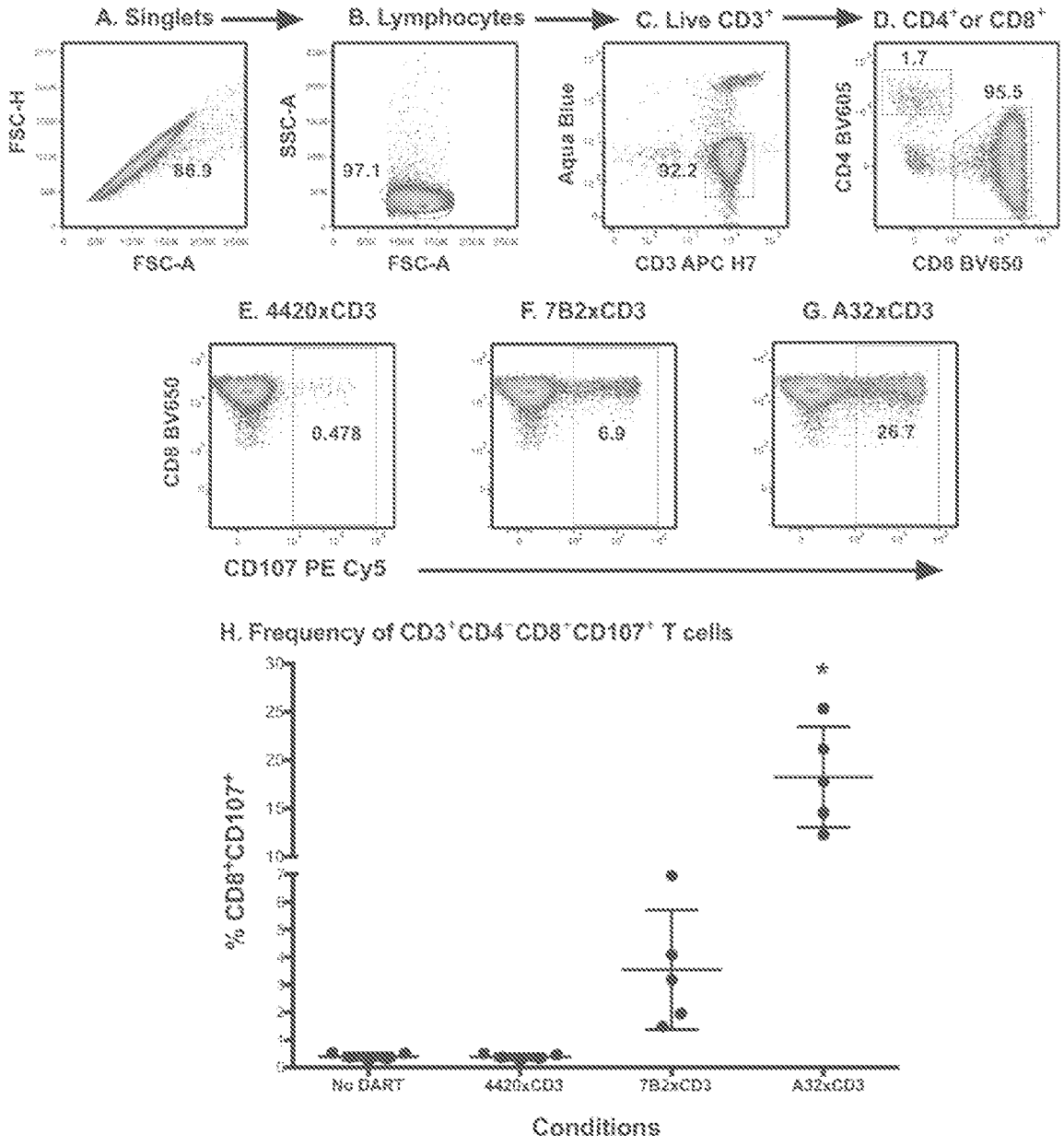
Figures 11A-11F



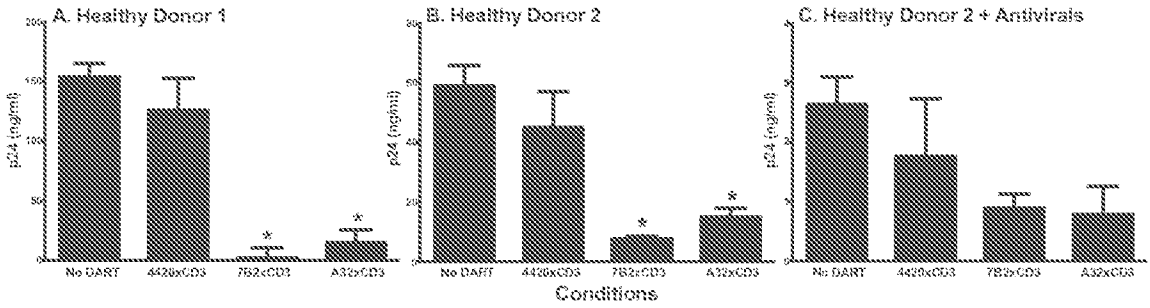
Figures 12A-12H



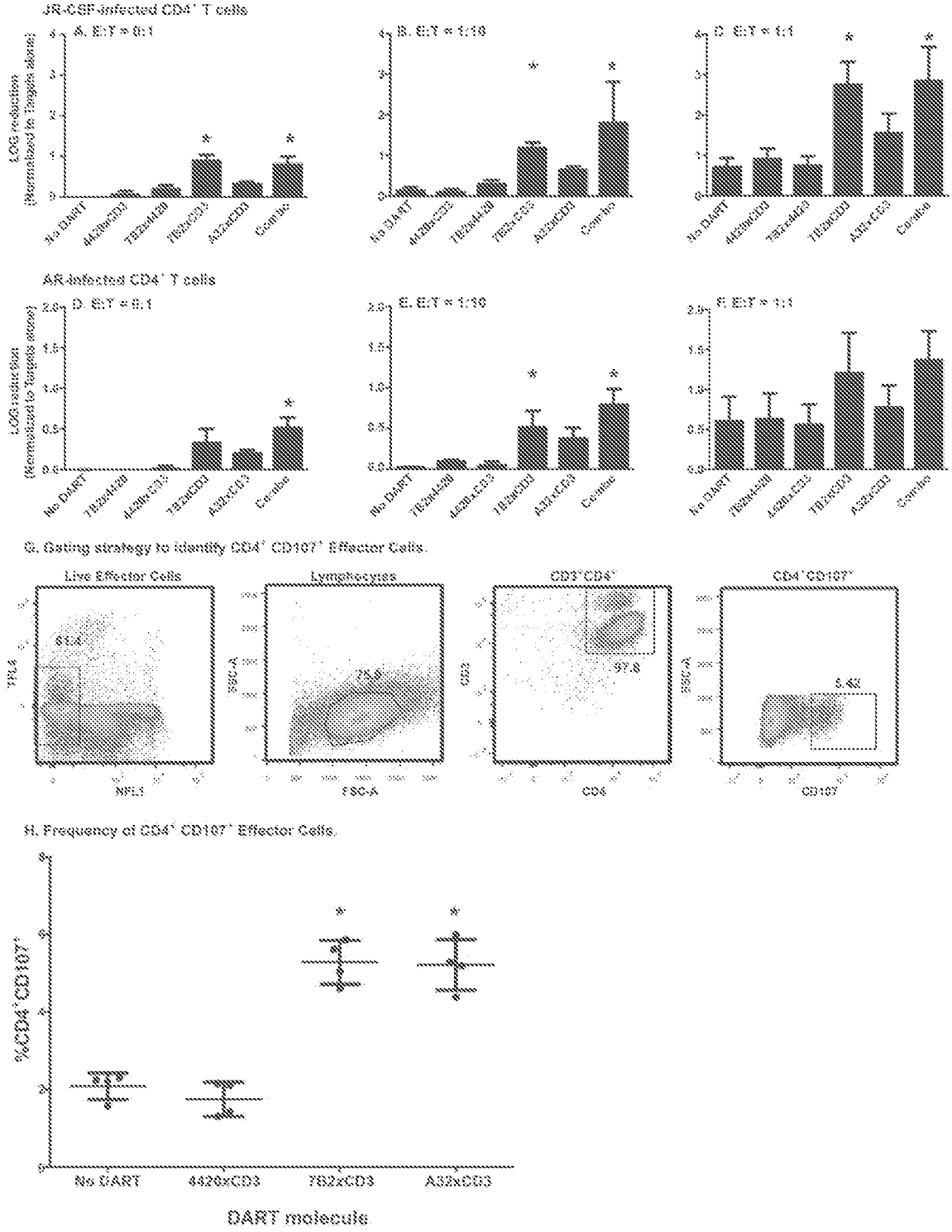
Figures 13A-13F



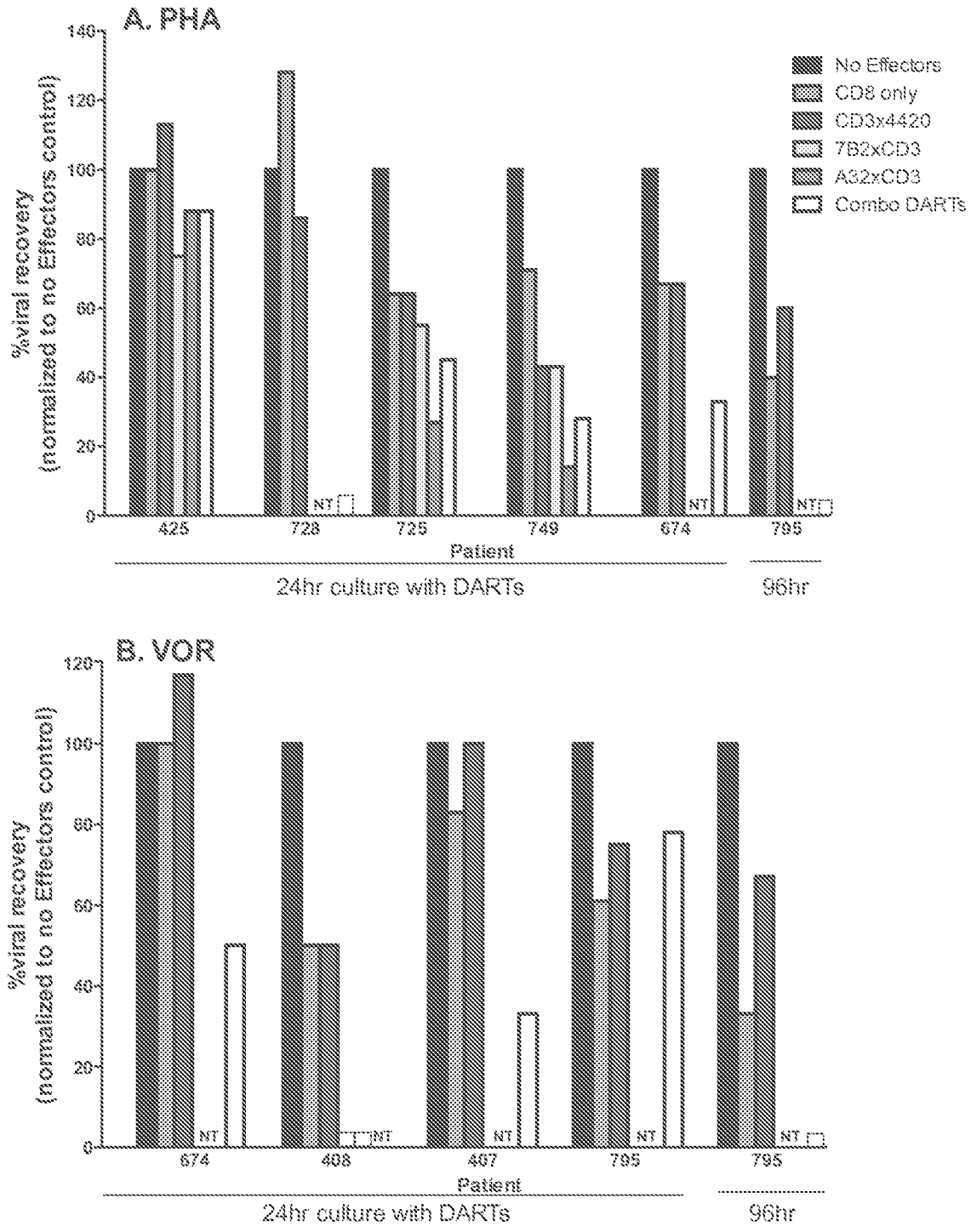
Figures 14A-14H



Figures 15A-15C



Figures 16A-16H



Figures 17A-17B

	Subtype A	Subtype AE	Subtype B	Subtype C
Tier 1	Q32.17	92TH023	BaL SF162	MW96.5
Tier 2		CM235 CM244 C1080.C03 427299 816763	CH040 CH058 SUMA WITO YU2	CAP45 CH505 DU151 DU422 TV1 1086.C 246-F3.C10

Figure 18

Antigen	DART	k_a (±SD), $M^{-1}s^{-1}$	k_d (±SD), s^{-1}	K_D (±SD), nM
Human CD3ε/δ	A32xCD3	6.8 (± 0.1) x10^5	2.4 (± 0.0) x10^{-3}	3.6 (± 0.1)
	7B2xCD3	4.1 (± 0.0) x10^4	2.5 (± 0.0) x10^{-4}	6.1 (± 0.0)
M.ConS gp140	A32xCD3	1.7 (± 0.0) x10^4	8.1 (± 1.8) x10^{-4}	47.7 (± 10.8)
JR-FL gp140	7B2xCD3	2.0 (± 0.1) x10^4	3.1 (± 0.9) x10^{-4}	15.1 (± 3.5)

Figure 19

Patient	Virus used for infection in VCA*	Pre-ART Viral Load (copies/ml)	Nadir CD4	Current CD4 count	Duration of suppression (years)
493	JR-CSF	78,115	257	637	2.5
532	JR-CSF	unknown	130	623	14
673	JR-CSF	Unknown	unknown	707	7
527	JR-CSF, AR	184,781	600	718	4.5
728	JR-CSF, AR	45,000	354	456	4
749	JR-CSF, AR	unknown	404	402	1.5
725	JR-CSF, AR	234,048	475	789	3
720	JR-CSF, AR	586,930	166	446	3
425	Autologous	750,001	604	783	8
407	Autologous	1,042,734 (AHI)	499	706	2
408	Autologous	6095 (AHI)	830	1168	2
795	Autologous	175,718	526	850	2.5
674	Autologous	185,042	338	1045	4.5

* Virus Clearance Assay
AR = autologous reservoir virus
Autologous indicates patient cells used in latency clearance assay, no superinfection used.
AHI = Acute HIV Infection

Figure 20

Patient	E:T Ratio	HIV-1 gag p24 concentration (ng/ml) (SEM)					
		No DART	CD3x4420	7B2x4420	7B2xCD3	A32xCD3	Combo
493	No CD8s	10.6(2)	12.6(4)	NT	1.44(1.0)	10.8 (2.2)	NT
	1:10	4.7(1.0)	7.35 (2.1)	NT	1.05 (0.6)	3.5 (1.3)	NT
	1:1	.92 (0.2)	1.29 (.03)	NT	.04 (.02)	.343 (.01)	NT
532	No CD8s	12.9 (2.7)	12.6 (3.9)	NT	1.44(1.0)	10.8(4.1)	NT
	1:10	8.9(1.7)	7.35(2.1)	NT	1.05(.629)	3.5(1.3)	NT
	1:1	0.92(0.2)	1.5(0.16)	NT	0.04(0.02)	0.16 (0.03)	NT
673	No CD8s	345(6.4)	194(25)	253(17)	213(28)	139(4.6)	NT
	1:10	329(1.2)	137(5.2)	184(12)	73(4.6)	57(5.2)	NT
	1:1	94(6.9)	8.8(0.2)	13.5(0.5)	4.4(0.5)	11.4(1.0)	NT
527	No CD8s	154(5.2)	185(22)	106(9.4)	27.3(12.3)	54.2(29.3)	62.9(3.4)
	1:10	164(16)	210(11)	78(6.6)	12.1(2.1)	21(2.8)	68(5.9)
	1:1	116(53)	139(10.8)	80(21)	13(6)	8.4(0.9)	34.9(5.6)
728	No CD8s	103(25)	111(10)	92(24)	19(10)	122(51)	30(11)
	1:10	114(18)	117(13)	90(2.2)	3.7(2.6)	54(11)	15.9(5.4)
	1:1	178(14)	121(32)	60(23)	1.9(1.1)	44(9.5)	0.9(0.4)
749	No CD8s	27.9(11)	7.3(6.8)	13.8(2.9)	0.9(0.5)	19.3(7.7)	16.5(12.2)
	1:1	11.4(0.5)	6.8(3.3)	5.5(3)	ND	ND	ND
725	No CD8s	191(3.7)	177(37)	189(24)	29(21)	134(16)	45(13)
	1:10	234(53)	187(14)	163(22)	6.5(5.5)	122(17)	ND
	1:1	60(22)	71(5)	53(17)	ND	4.5(3.3)	ND
720	No CD8s	15(4.8)	26.3(4.8)	22(2.3)	0.7(0.2)	5.9(2.6)	0.6(0.2)
	1:10	5.7(2.5)	17(2.3)	16(4.6)	0.4(0.1)	2.5(0.5)	1.0(0.6)
	1:1	1.1(0.1)	0.4(0.3)	1.1(0.3)	ND	1.3(0.4)	0.2(0.02)

SEM=standard error of the mean of 3 replicates

ND = not detected

NT = not tested due to cell availability

Figure 21

LRA	Patient	#wells plated	#positive wells after addition of DART MOLECULE					
			No Effectors	No DART	4420 xCD3	7B2 xCD3	A32 xCD3	Combo
PHA	425	12	8	8	9	6	7	7
	728	12	7	9	6	NT	NT	0
	725	12	11	7	7	6	3	5
	749	12	7	5	3	3	1	2
	674	12	6	4	4	NT	NT	2
	795*	12	10	4	6	NT	NT	0
VOR	674	12	6	6	7	NT	NT	3
	408	12	2	1	1	0	0	NT
	407	24	6	5	6	NT	NT	2
	795	36	28	17	21	NT	NT	22
	795*	12	3	1	2	NT	NT	0

LRA=latency reversing agent

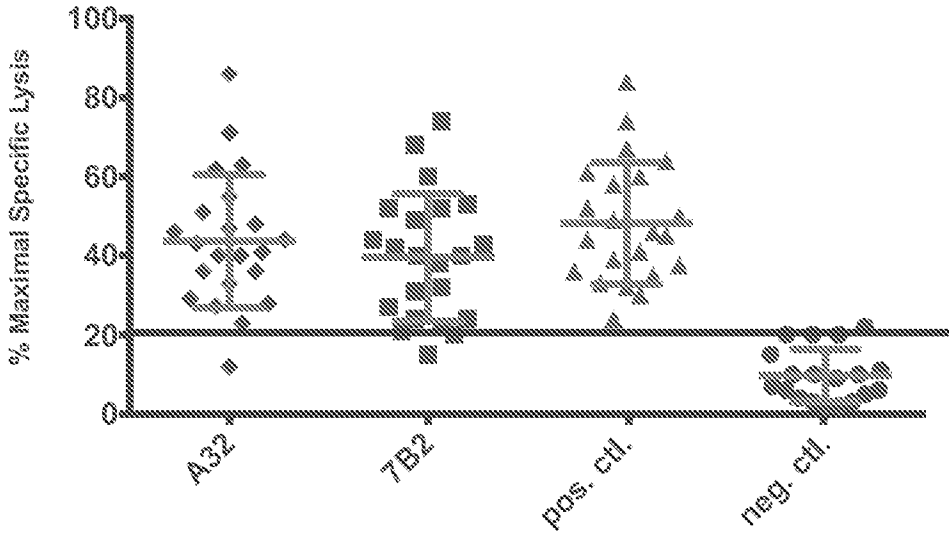
NT=not tested due to cell availability

combo= Addition of 50ng/ml of 7B2xCD3 DART and 50ng/ml of A32xCD3 DART

* evaluated using 96 hours co-culture

VOR=vorinostat

Figure 22



IMC Recognized	21	20	22	1
Percentage	95	91	100	5
Mean Max %SL	43.69	39.58	48.25	9.73
Range %SL	12-86	15-74	24-84	2-22

Figure 23

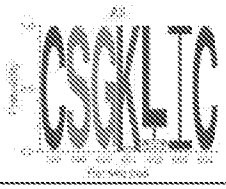
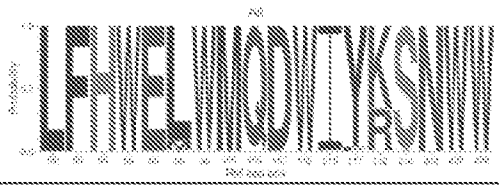
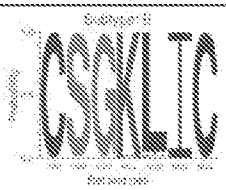
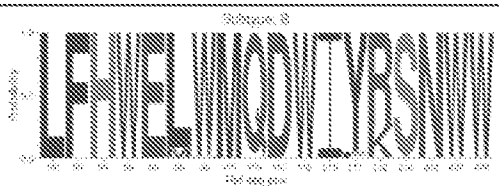
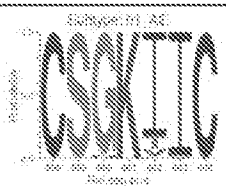
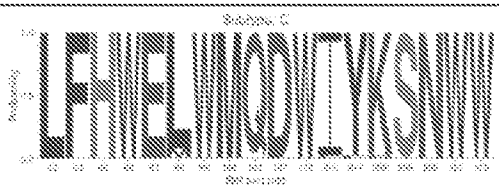
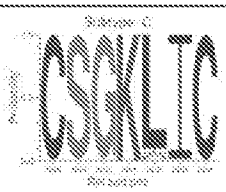
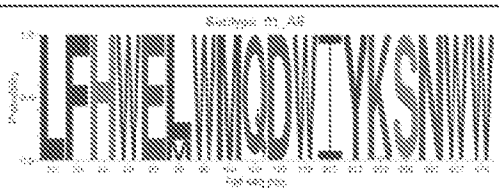
HIV-1 *Env* (# isolates)	Residues Influencing 7B2 Binding	Residues Influencing A32 Binding
All subtypes (4556)		
Subtype B (1529)		
Subtype AE (426)		
Subtype C (1191)		

Figure 24

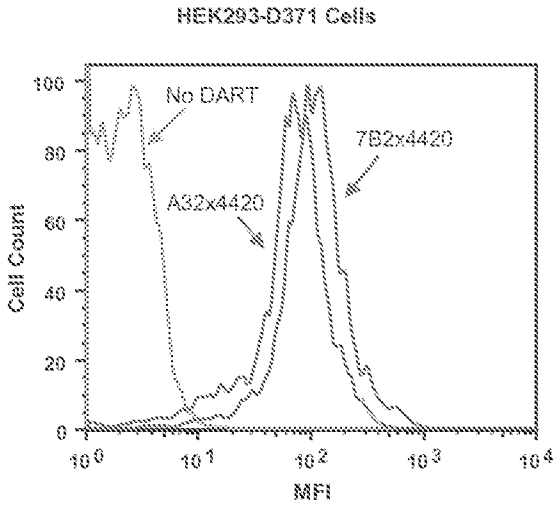
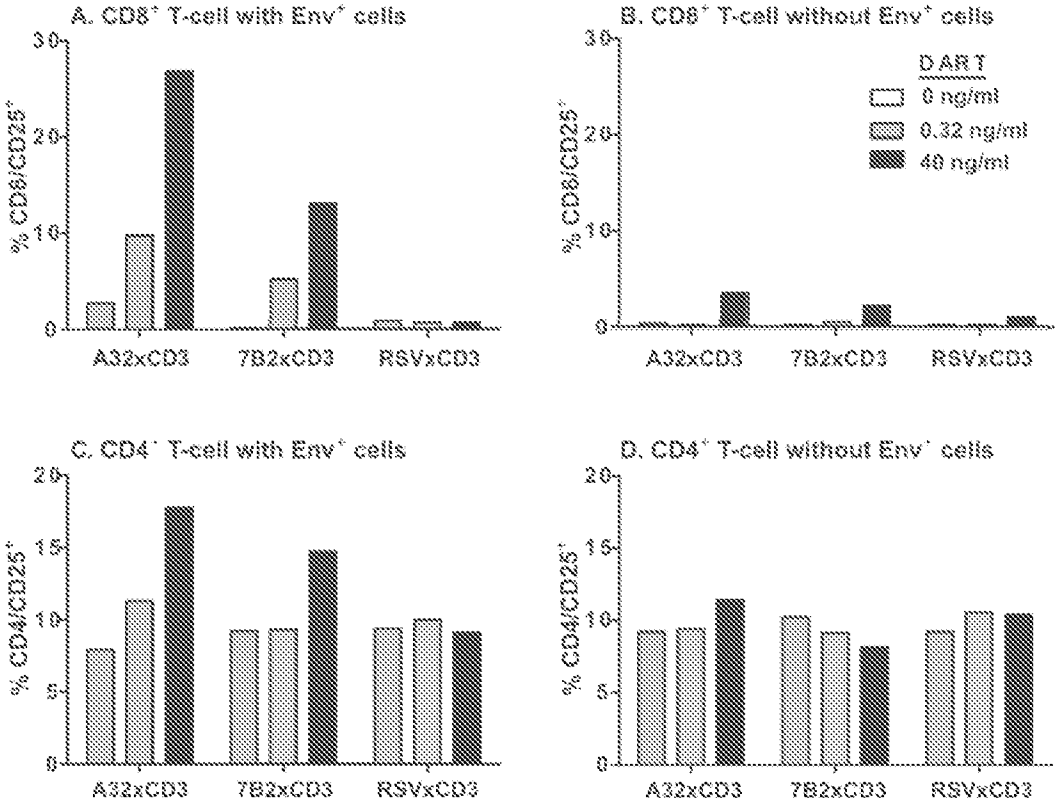

HEK293-D371 Cells
No DART
7B2x4420
A32x4420
Cell Count
MFI
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4

Figure 25



Figures 26A-26D

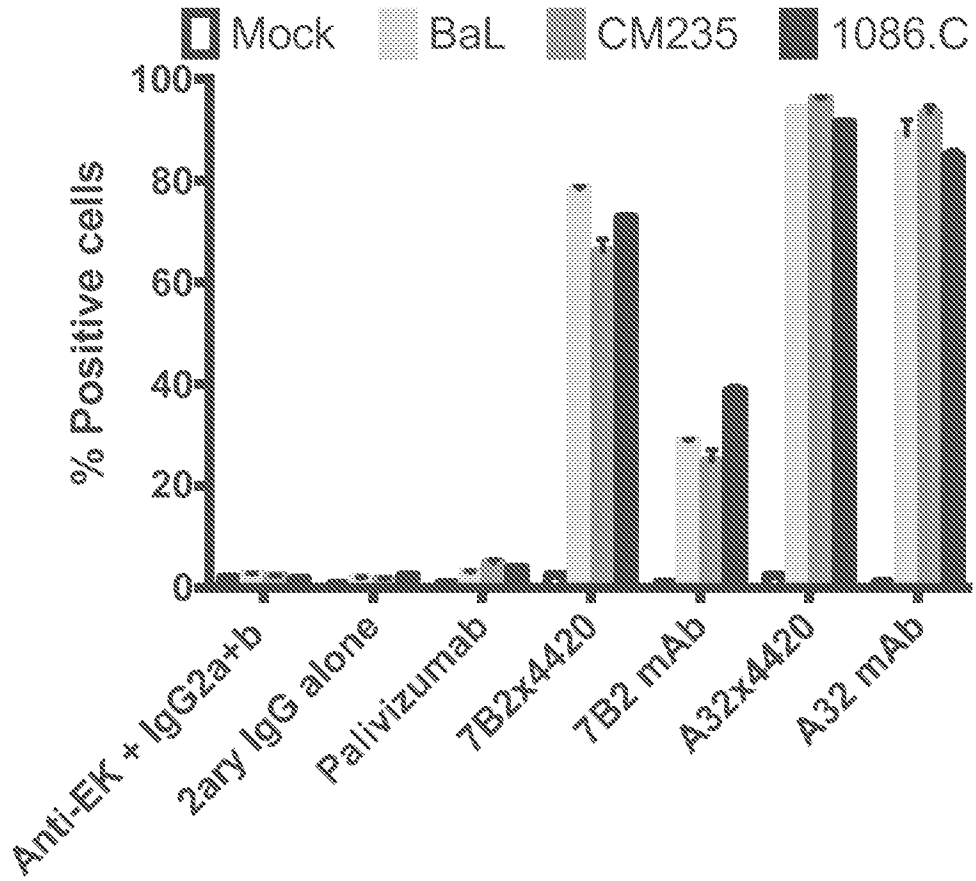
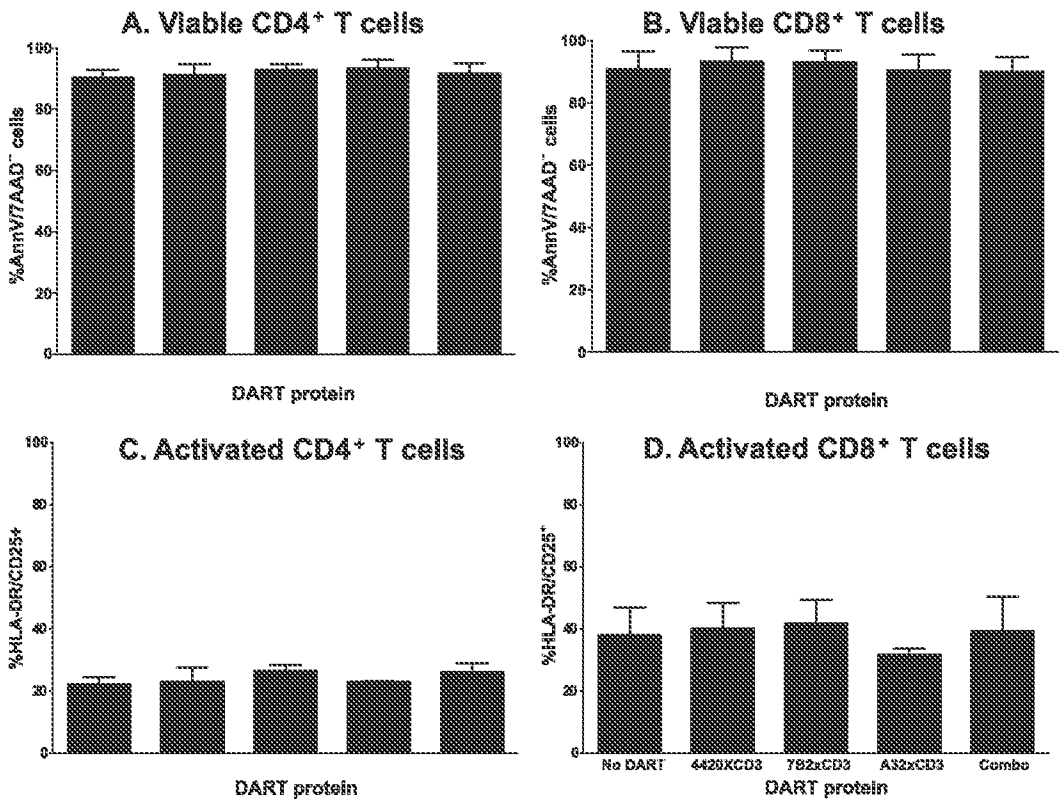

Mock
BaL
CM235
1086.C
% Positive cells
100
80
60
40
20
0
Anti-EK + IgG2a+b
2ary IgG alone
Palivizumab
7B2x4420
7B2 mAb
A32x4420
A32 mAb

Figure 27



Figures 28A-28D