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(54) Title: TARGETING MOIETY PEPTIDE EPITOPE COMPLEXES HAVING A PLURALITY OF T-CELL EPITOPES

(57) **Abrégé/Abstract:**

A variety of targeting moiety peptide epitope complexes (TPECs) are described in different embodiments. In each of the embodiments, however, a targeting moiety may be used to deliver the TPEC to an area of unwanted cells, allowing for a therapeutic effect to be delivered locally. The TPEC also contains a plurality of T-cell epitopes. The TPEC further comprises cleavage sites that release the T-cell epitopes from the targeting agent, and in some embodiments from each other, when they are in the microenvironment of the unwanted cells. Although the arrangement and number of T-cell epitopes varies in different embodiments described herein, once cleaved from the targeting agent (and any neighboring T-cell epitopes), the T-cell epitopes function by stimulating an immune response against the unwanted cells.



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(54) Title: TARGETING MOIETY PEPTIDE EPITOPE COMPLEXES HAVING A PLURALITY OF T-CELL EPITOPES

(57) Abstract: A variety of targeting moiety peptide epitope complexes (TPECs) are described in different embodiments. In each of the embodiments, however, a targeting moiety may be used to deliver the TPEC to an area of unwanted cells, allowing for a therapeutic effect to be delivered locally. The TPEC also contains a plurality of T-cell epitopes. The TPEC further comprises cleavage sites that release the T-cell epitopes from the targeting agent, and in some embodiments from each other, when they are in the microenvironment of the unwanted cells. Although the arrangement and number of T-cell epitopes varies in different embodiments described herein, once cleaved from the targeting agent (and any neighboring T-cell epitopes), the T-cell epitopes function by stimulating an immune response against the unwanted cells.

TARGETING MOIETY PEPTIDE EPITOPE COMPLEXES HAVING A PLURALITY OF T-CELL EPITOPES

DESCRIPTION

FIELD

[001] This application relates to immunotherapeutic agents employing a plurality of T-cell epitopes. In particular, it relates to agents that can be used to treat a condition characterized by the presence of unwanted cells, such as cancer or other disease-causing cells.

BACKGROUND

[002] Cancer and other diseases caused by the presence of unwanted cells create significant loss of life, suffering, and economic impact. Immunotherapeutic strategies for targeting cancer have been an active area of translational clinical research.

[003] WO 2012/123755 discusses the concept of re-directed immunotherapy. In this application, an agent for preventing or treating a condition characterized by the presence of unwanted cells includes a targeting moiety that is capable of targeting to the unwanted cells and a T-cell epitope that can be released from the targeting moiety by selective cleavage of a cleavage site in the agent in the vicinity of the unwanted cells.

[004] WO 2014/043523 teaches an agent based on an ScFv directed to cancer cells including from 1-10 immunogenic CD8 T-cell epitopes in one of the following two arrangements: T-c-En-c-Fcn or T-c-Fcn-c-En, where T is the ScFv, En is from 1-10 CD8 T-cell epitopes, c: is a protease cleavage site, and Fcn is from 1-10 Fc portions of an IgG antibody. In this reference, the 1-10 immunogenic CD8 T-cells are released from the ScFv and Fc portions of the agent in a single polypeptide chain, still conjugated to each other.

[005] While some positive test data has been shown with prior approaches, clinically-effective therapeutic strategies must be able to elicit a strong immune response in an individual suffering from a disease such as cancer. Additionally, effective therapies should work well in a wide cross-section of

patients from different racial and ethnic groups. Maximally-effective therapies would also generate an immune response against the unwanted cells without generating an inhibitory immune response against the therapeutic agent itself so that multiple rounds of treatment could be administered over a period of time. Therefore, additional developments in this field of re-directed immunotherapy are required.

SUMMARY

[006] In accordance with the description, a variety of targeting moiety peptide epitope complexes (TPECs) are described in different embodiment of this application. In each of the embodiments, however, a targeting moiety may be used to deliver the TPEC to an area of unwanted cells, allowing for a therapeutic effect to be delivered locally. The TPEC also contains a plurality of T-cell epitopes. The TPEC further comprises cleavage sites that release the T-cell epitopes from the targeting agent, and in some embodiments from each other, when they are in the microenvironment of the unwanted cells. Although the arrangement and number of T-cell epitopes varies in different embodiments described herein, once cleaved from the targeting agent (and any neighboring T-cell epitopes), the T-cell epitopes function by stimulating an immune response against the unwanted cells. In some embodiments, maximal benefits may be achieved by releasing all of the T-cell epitopes from both the targeting agent and from each other in the cleavage process, allowing each T-cell epitope the structural freedom to attract an immune response to the unwanted cell.

[007] Having a plurality of T-cell epitopes, as discussed in detail below, enhances the immune response against the unwanted cells, either by stimulating a stronger immune response in a given patient or by allowing the TPEC to stimulate an immune response across a wide variety of patients in different ethnic and racial groups.

[008] In one embodiment, a composition for retargeting an immune response to unwanted cells comprises a TPEC wherein:

- a. T is a targeting moiety that is capable of targeting unwanted cells;

- b. L is at least one linker capable of chemical linkage to T where L may be a peptide bond, at least one peptide, or a chemical linker;
- c. C is at least one cleavage site
 - i. cleaved by an enzyme expressed by the unwanted cells;
 - ii. cleaved through a pH-sensitive cleavage reaction inside the unwanted cell;
 - iii. cleaved by a complement-dependent cleavage reaction; or
 - iv. cleaved by a protease that is colocalized to the unwanted cell by a targeting moiety that is the same or different from the targeting moiety in the TPEC; and
- d. E is at least one T-cell epitope,

wherein the L, C, and E moieties are arranged in at least one of the following patterns:

- i. a plurality of L-C-E, each attached separately to T,
- ii. at least one of L-(C-E)_n, with each C-E attached to the L in parallel, and/or
- iii. at least one of L-(C-E)_n, with each C-E attached to the L in series.

[009] In one embodiment, a composition for retargeting an immune response to unwanted cells comprises a TPEC having a plurality of T-cell epitopes separately conjugated to a targeting moiety comprising the formula T-(L-C-E)_n or T-(L-C_i-E_j)_n, wherein:

- a. T is a targeting moiety that is capable of targeting unwanted cells;
- b. L is a linker capable of chemical linkage to T;
- c. C is a cleavage site
 - i. cleaved by an enzyme expressed by the unwanted cells;
 - ii. cleaved through a pH-sensitive cleavage reaction inside the unwanted cell;
 - iii. cleaved by a complement-dependent cleavage reaction; or
 - iv. cleaved by a protease that is colocalized to the unwanted cell by a targeting moiety that is the same or different from the targeting moiety in the TPEC; and

- b. E is a T-cell epitope

wherein n is an integer of at least 2 (optionally from about 2 to 50), i is an integer of at least 2 (optionally from about 1 to 50), and j is an integer of at least 2 (optionally from about 1 to 50). The “n” integer refers to the number of L-C-E moieties on the targeting moiety and the “i” and “j” integers refer to how many different types of cleavage sites and epitopes are within the construct.

[0010] In some embodiments, a composition for retargeting an immune response to unwanted cells comprises a TPEC with either a (i) linear and/or bundled polytope or a (ii) branched polytope. Such a TPEC may comprise the formula $T-L-(C_i-E_j)_n$, wherein:

- a. T is a targeting moiety that is capable of targeting unwanted cells;
- b. L is an optional linker capable of chemical or peptide linkage to T;
- c. C is a cleavage site
 - i. cleaved by an enzyme expressed by the unwanted cells;
 - ii. cleaved through a pH-sensitive cleavage reaction inside the unwanted cell;
 - iii. cleaved by a complement-dependent cleavage reaction; or
 - iv. cleaved by a protease that is colocalized to the unwanted cell by a targeting moiety that is the same or different from the targeting moiety in the TPEC; and
- d. E is a T-cell epitope;

wherein n is an integer of at least 2 (optionally from about 2 to 50), i is an integer of at least 2 (optionally from about 1 to 50), and j is an integer of at least 2 (optionally from about 1 to 50). The “n” integer refers to the number of C-E moieties on the targeting moiety and the “i” and “j” integers refer to how many different types of cleavage sites and epitopes are within the construct.

[0011] Further, in some embodiments, a composition for retargeting an immune response to unwanted cells comprises a TPEC having:

- a. a targeting moiety that is capable of targeting unwanted cells;
- b. a plurality of more than 10 T-cell epitopes conjugated to the targeting moiety with at least one cleavage site, wherein the cleavage site is
 - i. cleaved by an enzyme expressed by the unwanted cells;

- ii. cleaved through a pH-sensitive cleavage reaction inside the unwanted cell;
- iii. cleaved by a complement-dependent cleavage reaction; or
- iv. cleaved by a protease that is colocalized to the unwanted cell by a targeting moiety that is the same or different from the targeting moiety in the TPEC.

[0012] Additional objects and advantages will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice. The objects and advantages will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[0013] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the claims.

[0014] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate one (several) embodiment(s) and together with the description, serve to explain the principles described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Figures 1A-B show certain embodiments of TPECs. In Figure 1A, a targeting moiety, optionally an antibody, has a plurality of copies of the same T-cell epitope affixed to it using a chemical linker and a cleavage site. In Figure 1B, a targeting moiety, optionally an antibody, has a plurality of different T-cell epitopes affixed to it using a chemical linker and a cleavage site. The linker-cleavage site-T-cell-epitopes may be affixed to the targeting moiety in random locations and in random amounts, creating a heterogeneous population of TPECs. In these embodiments, the TPEC comprises a plurality of L-C-E, each attached separately to T, where L is linker, C is cleavage site, E is epitope, and T is targeting moiety.

[0016] Figures 2A-C show certain embodiments of TPECs. In Figure 2A, a targeting moiety, optionally an antibody, has a plurality of different T-cell epitopes affixed to it using a chemical or peptide linker, wherein the plurality of T-cell epitopes is separated by cleavage sites and affixed to the targeting moiety in a

bundle. Figure 2B shows the bundle of T-cell epitopes having immunoglobulin framework regions flanking certain domains. Both Figures 2A and 2B include polytopes with a linear configuration. In the embodiments of Figures 2A and 2B, the TPEC comprises at least one of L-(C-E)_n, with each C-E attached to the L in series (in a bundled and/or linear configuration), and wherein n is an integer of at least 2 (optionally from about 2 to 50) and where L is linker, C is cleavage site, E is epitope, and T is targeting moiety. Figure 2C shows one embodiment of a TPEC comprising a polytope with a branched configuration. In another embodiment, not shown, the polytope with a branched configuration may have identical T-cell epitopes. In the embodiments of Figure 2C, the TPEC comprises at least one of L-(C-E)_n, with each C-E attached to the L in parallel (in a branched configuration), and wherein n is an integer of at least 2 (optionally from about 2 to 50) and where L is linker, C is cleavage site, E is epitope, and T is targeting moiety.

[0017] Figures 3A-C show *in vitro* activity of re-directed virus specific T cells (Multiple Peptide TPEC approach). Recognition of a lymphoblastoid lymphoma cell line by CD8⁺ cytomegalovirus-specific cytotoxic T lymphocytes through conjugation of two different viral peptides, RPHERNGFTVL (SEQ ID NO: 2) and NLVPMVATV (SEQ ID NO: 1), to the anti-CD20 antibody Rituximab. Antibody is conjugated using sulfo-SMCC to peptides NLVPMVATVASGV{CIT}GC (SEQ ID NO: 3) and RPHERNGFTVLASGFKGC (SEQ ID NO: 4) at different ratios from 100% NLV:0% RPH through to 0% NLV:100% RPH. Target lymphoma cells labeled with TPEC are recognized by lymphocytes specific towards the NLV peptide (A) at all ratios down to 10% of the conjugated peptide being NLVPMVATVASGV{CIT}GC (SEQ ID NO: 3). Similarly, target lymphoma cells are recognized by lymphocytes specific towards the RPH peptide (B) at all ratios down to 10% of the conjugated peptide being the RPHERNGFTVLASGFKGC (SEQ ID NO: 4). In both (A) and (B), there is no T cell recognition by peptide-specific T cells when the ratio of the conjugated peptide recognized by the T cells is 0%. (C) Overlaid plots of (A) and (B)

demonstrating activity of both NLV and RPH specific T cells using Rituximab TPECs conjugated with the two different peptides.

[0018] Figure 4 illustrates recognition of a lymphoblastoid lymphoma cell line by CD8+ cytomegalovirus-specific cytotoxic T lymphocytes through conjugation of three different viral peptides, VLEETSVML (SEQ ID NO: 5), CRVLCCYVL (SEQ ID NO: 17) and YILEETSVM (SEQ ID NO: 7), to the anti-CD20 antibody Rituximab. Antibody is conjugated using sulfo-SMCC to equal amount of peptides VLEETSVMLASGFKGC (SEQ ID NO: 8), BRVLBBYVLASGFKGC (SEQ ID NO: 9) where B is amino butyric acid, a homolog for cysteine and YILEETSVMASGFKGC (SEQ ID NO: 10). Target lymphoma cells labeled with the TPEC are recognized by T cells specific for each of the three peptides conjugated to the antibody. Untreated target cells are not recognized by any of the peptide-specific T cells (negative control) and free peptide pulsed target cells are strongly recognized by T cells (positive control).

[0019] Figure 5 demonstrates recognition of a lymphoblastoid lymphoma cell line by CD8+ cytomegalovirus-specific cytotoxic T lymphocytes through conjugation of polytope peptides. Antibody is conjugated using sulfo-SMCC to peptides (i) CGVANLVPMVATVAVAVLEETSVML (SEQ ID NO: 11), (ii) CVARPHERNGFTVLVANLVPMVATV (SEQ ID NO: 12) and (iii) CGVANLVPMVATVARPHERNGFTVL (SEQ ID NO: 13). Target lymphoma cells labeled with TPEC are recognized by T cells specific for the NLVPMVATV (SEQ ID NO: 1) peptide. Untreated target cells are not recognized by the peptide-specific T cells (negative control) and free peptide pulsed target cells are strongly recognized by T cells (positive control).

[0020] Figure 6 demonstrates the protein conjugation chemistry of one embodiment of the branched peptide using a stem peptide that contains four azidonorleucine residues and peptides (branches) that contain propargyl glycine at the amino terminus (Figure 6A). Incubation of equimolar concentrations of the branches with the stem peptide in DMSO in the presence of 10mg in 4ml of the CuSO₄·5H₂O catalyst mixed with 10mg in 4 ml ascorbic acid overnight resulted in the formation of the branched peptide used for conjugation. The final concentration of DMSO for the reaction was 50%. The branched peptides were

purified using HPLC and verified using mass spectrometry (Figure 6B). The peptides contain an N-terminal amide group (-nh₂) which is useful in peptide stability compared with the usual carboxyl group (-COOH).

[0021] Figure 7 demonstrates recognition of a lymphoblastoid lymphoma cell line by CD8⁺ cytomegalovirus-specific cytotoxic T lymphocytes through conjugation of polytope peptides in the branched peptide format. Antibody is conjugated using the embodiment in Figure 6B using the following peptides:

[0022] Stem: SEEZSEEZSEEZSEEZ (Z: Azidonorleucine) (SEQ ID NO: 166);

[0023] For Branch 1 TPEC: branch 1-1: BKPAKFFRLTPRVTGGGAM-nh₂ (B: propargyl glycine) (SEQ ID NO: 167);

[0024] Branch 1-2: BKPAKFFRLRPHERNGFTVL-nh₂ (B: propargyl glycine) (SEQ ID NO: 168);

[0025] Branch 1-3: BKPAKFFRLRELRRKMMYM-nh₂ (B: propargyl glycine) (SEQ ID NO: 169); and

[0026] Branch 1-4: BKPAKFFRLNLVPMVATV-nh₂ (B: propargyl glycine) (SEQ ID NO: 170); and

[0027] For Branch 3 TPEC, branch 3-1: BAIPVSLRTPRVTGGGAM-nh₂ (B: propargyl glycine) (SEQ ID NO: 171);

[0028] Branch 3-2: BAIPVSLRRPHERNGFTVL-nh₂ (B: propargyl glycine) (SEQ ID NO: 172);

[0029] Branch 3-3: BAIPVSLRELRRKMMYM-nh₂ (B: propargyl glycine) (SEQ ID NO: 173); and

[0030] Branch 3-4: BAIPVSLVTEHDTLLY-nh₂ (B: propargyl glycine) (SEQ ID NO: 174).

[0031] Target lymphoma cells labeled with branched peptide TPEC are recognized by T cells specific for the RPHERNGFTVL (SEQ ID NO: 2) and TPRVTGGGAM (SEQ ID NO: 49) peptides. Untreated target cells are not recognized by the peptide-specific T cells (negative control) and free peptide pulsed target cells are strongly recognized by T cells (positive control).

[0032] Figure 8 demonstrates recognition of an ovarian carcinoma cell line by CD8⁺ cytomegalovirus-specific cytotoxic T lymphocytes through conjugation

of polytope peptides in the branched peptide format. Antibody is conjugated using the embodiment in Figure 6B using peptides

[0033] Stem: SEEZSEEZSEEZSEEZ (Z: Azidonorleucine) (SEQ ID NO: 166);

[0034] For Branch 1 TPEC, branch 1-1: BKPAKFFRLTPRVTGGGAM-nh2 (B: Propargyl glycine) (SEQ ID NO: 167);

[0035] Branch 1-2: BKPAKFFRLRPHERNGFTVL-nh2 (B: Propargyl glycine) (SEQ ID NO: 168);

[0036] Branch 1-3: BKPAKFFRLRELRRKMMYM-nh2 (B: Propargyl glycine) (SEQ ID NO: 169);

[0037] Branch 1-4: BKPAKFFRLNLVPMVATV-nh2 (B: Propargyl glycine) (SEQ ID NO: 170); and

[0038] For Branch 3 TPEC, branch 3-1: BAIPVSLRTPRVTGGGAM-nh2 (B: Propargyl glycine) (SEQ ID NO: 171);

[0039] Branch 3-2: BAIPVSLRRPHERNGFTVL-nh2 (B: Propargyl glycine) (SEQ ID NO: 172);

[0040] Branch 3-3: BAIPVSLRELRRKMMYM-nh2 (B: Propargyl glycine) (SEQ ID NO: 173); and

[0041] Branch 3-4: BAIPVSLVTEHDTLLY-nh2 (B: Propargyl glycine).

[0042] Target lymphoma cells labeled with branched peptide TPEC are recognized by T cells specific for the RPHERNGFTVL (SEQ ID NO: 2) and TPRVTGGGAM (SEQ ID NO: 49) peptides. Untreated target cells are not recognized by the peptide-specific T cells (negative control) and free peptide pulsed target cells are strongly recognized by T cells (positive control).

[0043] Figures 9A-C provide an embodiment of the bundled domain TPEC. Figure 9A demonstrates the scaffold (Fibronectin type 3 domain from the fibronectin 1 protein) used for the bundled domain TPEC. The bundled domain sequence (Figure 9A) demonstrates the mutations made in the outer loops of the domain and contains viral epitopes separated from the fibronectin sequence on both sides by a protease recognition sequence specific for MMP2. The protease cleavage sites are underlined and the viral epitope are shown in bold and italics. Figure 9B demonstrates the structure of the bundled domain and the “BC”, “DE”

and “FG” loops that contain the viral epitopes. Figure 9C demonstrates recognition of an ovarian carcinoma cell line by CD8⁺ cytomegalovirus specific cytotoxic T lymphocytes. CMV epitopes are contained within an additional protein domain linked to the heavy chain of Cetuximab. The viral epitopes are flanked by an MMP2 protease recognition sequence which will allow release of the viral epitopes in the presence of MMP2, produced by the target cell line. The bundled domain TPEC is produced in Expi293 cells and purified using protein A sepharose beads.

[0044] Target ovarian carcinoma cells labelled with the bundled domain TPEC are recognized by T cells specific for the viral epitope NLVPMVATV by release of IFN- γ . Four different preparations (two TPECs produced on each of two days using different concentrations of DNA) of the bundled domain were used, all of which produced similar amounts of bundled domain TPEC and produced similar recognition of target cells by the peptide-specific T cells. Untreated target cells are not recognized by the peptide-specific T cells (negative control) and free peptide pulsed target cells are strongly recognized by T cells (positive control).

DESCRIPTION OF THE SEQUENCES

[0045] Table 1 provides a listing of certain sequences referenced herein.

Table 1: Description of the Sequences and SEQ ID NOs		
Description	Sequence	#
T-cell epitope from cytomegalovirus (CMV TCE) (pp65, position 495–503, HLA-A2)	NLVPMVATV	1
CMV TCE (pp65, position 265–275, HLA-B7)	RPHERNGFTVL	2
CMV TCE and cleavage site from capthepsin B	NLVPMVATVASGV {Cit} GC cleavage site in bold. {Cit} = citrulline	3
CMV TCE and cleavage site from capthepsin B	RPHERNGFTVL ASGFKGC cleavage site in bold	4
CMV TCE (IE1, position 316–324, HLA-A2)	VLEETSVML	5

CMV TCE (IE1, 309–317, HLA-C7) (Peptidomimetic of SEQ ID NO: 17)	BRVLBBYVL, where B is alpha-aminobutyric acid, a homolog for cysteine	6
CMV TCE (IE1, 315–323, HLA-A2)	YILEETSVM	7
CMV TCE and cleavage site from cathepsin B	VLEETSVML ASGFKGC cleavage site in bold	8
CMV TCE and cleavage site from cathepsin B	BRVLBBYVL ASGFKGC , where B is alpha-aminobutyric acid cleavage site in bold	9
CMV TCE and cleavage site from cathepsin B	YILEETSVM ASGFKGC cleavage site in bold	10
Polytope containing two different CMV-derived epitopes separated by cathepsin B cleavage site	CGVAN LVPMVATVAV AV LEETSV ML cleavage sites in bold	11
Polytope containing two different CMV-derived epitopes separated by cathepsin B cleavage site	CVAR PHERNGFTVL VAN LVPMVA TV cleavage sites in bold	12
Polytope containing two different CMV-derived epitopes separated by cathepsin B cleavage site	CGVAN LVPMVATV AR PHERNGFT VL cleavage sites in bold	13
Polytope containing five different CMV-derived epitopes separated by cathepsin B cleavage sites	GSFR VTEHDTLLY GSFR RPHER NGFTVL GSF RELKRKMIYM GSFR NLVPMVATV cleavage sites in bold	14
CMV TCE (IE1, 32–41, HLA-A68)	ATTFLQTMLR	15
CMV TCE (pp65, 103–114, HLA-B35)	CPSQEPMSIYVY	16
CMV TCE (IE1, 309–317, HLA-C7)	CRVLCCYVL	17
CMV TCE (IE1, 279–287, HLA-A1/B18)	CVETMCNEY	18
CMV TCE (IE1, 198–206, HLA-A1/B18)	DELRRKMMY	19
CMV TCE (pp65, 31–40, HLA-A68)	DTPVLPHETR	20

CMV TCE (IE1, 381–389, HLA-B44)	EEAIVAYTL	21
CMV TCE (pp65, 512–521, HLA-B44)	EFFDANDIY	22
CMV TCE (IE1, 199–207, HLA-B8)	ELKRKMIYM	23
CMV TCE (IE1, 199–207, HLA-B8)	ELRRKMMYM	24
CMV TCE (IE1, 334–342, HLA-A68)	EVISVMKRR	25
CMV TCE (IE2, 381–389, HLA-B41)	FEQPTETPP	26
CMV TCE (IE1, 221–231, HLA-B55)	FPKTTNGCSQA	27
CMV TCE (pp65, 188–195, HLA-B35)	FPTKDVAL	28
CMV TCE (pp65, 369–379, HLA-A24)	FTSQYRIQGKL	29
CMV TCE (pp65, 186–196, HLA-A68)	FVFPTKDVALR	30
CMV TCE (IE2, 242–250, HLA-A2)	IIYTRNHEVK	31
CMV TCE (pp65, 123–131, HLA-B35)	IPSINVHHY	32
CMV TCE (pp150, 101–109, HLA-B7)	KARDHLAVL	33
CMV TCE (IE1, 42–50, HLA-B40)	KEVNSQLSL	34
CMV TCE (IE1, 201–209, HLA-B27)	KRKMIYMCY	35
CMV TCE (pp65, 120–128, HLA-A2)	MLNIPSINV	36
CMV TCE (pp150, 212–220, HLA-B7)	NVRRSWEEL	37
CMV TCE (IE1, 88–96, HLA-B8)	QIKVRVDMV	38
CMV TCE (pp65, 52–61, HLA-B35)	QPSLILVSQY	39
CMV TCE (pp150, 792–802, HLA-A68)	QTVTSTPVQGR	40
CMV TCE (pp65, 341–349, HLA-A24)	QYDVPAALF	41
CMV TCE (pp65, 222–231, HLA-A24)	QYVKVYLESF	42
CMV TCE (pp50, 274–282, HLA-A1)	RGDPFDKNY	43

CMV TCE (pp65, 522–530, HLA-A2)	RIFAELEGV	44
CMV TCE (gB, 4–12, HLA-A2)	RIWCLVVCV	45
CMV TCE (IE1, 341–349, HLA-B27)	RRIEEICMK	46
CMV TCE (IE1, 201–209, HLA-B27)	RRKMMYMCY	47
CMV TCE (pp65, 364–373, HLA-B44)	SEHPTFTSQY	48
CMV TCE (pp65, 417–426, HLA-B7)	TPRVTGGGAM	49
CMV TCE (pp150, 945–955, HLA-A3)	TTVYPPSSTAK	50
CMV TCE (pp50, 52–60, HLA-A3)	TVRSHCVSK	51
CMV TCE (IE1, 81–89, HLA-A2)	VLAELVKQI	52
CMV TCE (pp50, 245–253, HLA-A1)	VTEHDTLLY	53
CMV TCE (pp65, 113–121, HLA-A24)	VYALPLKML	54
CMV TCE (pp65, 363–373, HLA-A1)	YSEHPTFTSQY	55
CMV TCE (pp65, 61–70, HLA-A68)	YTPDSTPCHR	56
CMV TCE (IE1, 316–324, HLA-A2)	YVLEETSVM	57
CMV TCE and cleavage site from ADAM28	CKPAKFFRL NLVPMVATV	58
CMV TCE and cleavage site from ADAM28	CKPAKFFRL RPHERNGFTVL	59
CMV TCE and cleavage site from cathepsin D	CPRSFFRLGK VLEETSVML	60
CMV TCE and cleavage site from ADAM28	CKPAKFFRLE ELKRKMIYM	61
CMV TCE and cleavage site from cathepsin D	CPRSFFRLGK QIKVRVDMV	62
Polytope comprising four different cytomegalovirus epitopes, each separated by either an ADAM28 or cathepsin D cleavage site	CGS CKPAKFFRL YSEHPTFTSQYG S PRSFFRLGK TPRVTGGGAMG S CKPAKFFRL QIKVRVDMVGSPRS FFRLGK ELRRKMMYM	63
T-cell epitope from Epstein Barr Virus (EBV TCE) EBNA1	RPQKRPSCI	64
EBV TCE EBNA1	HPVGEADYF	65
EBV TCE EBNA1	HPVGEADYFEY	66
EBV TCE EBNA1	IPQCRLTPL	67

EBV TCE EBNA1	VLKDAIKDL	68
EBV TCE EBNA2	YHLIVDTDSL	69
EBV TCE EBNA2	DTPLIPLTIF	70
EBV TCE EBNA2	RPTELQPTP	71
EBV TCE EBNA3A	QAKWRLQTL	72
EBV TCE EBNA3A	AYSSWMYSY	73
EBV TCE EBNA3A	RYSIFFDY	74
EBV TCE EBNA3A	FLRGRAYGL	75
EBV TCE EBNA3A	KRPPIFIRRL	76
EBV TCE EBNA3A	RPPIFIRRL	77
EBV TCE EBNA3A	LEKARGSTY	78
EBV TCE EBNA3A	HLAAQGMAY	79
EBV TCE EBNA3A	YPLHEQHGM	80
EBV TCE EBNA3A	VFSDGRVAC	81
EBV TCE EBNA3A	VPAPAGPIV	82
EBV TCE EBNA3A	SVRDRLARL	83
EBV TCE EBNA3A	RLRAEAQVK	84
EBV TCE EBNA3A	VQPPQLTLQV	85
EBV TCE EBNA3B	HRCQAIRKK	86
EBV TCE EBNA3B	TYSAGIVQI	87
EBV TCE EBNA3B	RRARSLSAERY	88
EBV TCE EBNA3B	VSFIEFVGW	88
EBV TCE EBNA3B	AVFDRKSDAK	89
EBV TCE EBNA3B	IVTDFSVIK	90
EBV TCE EBNA3B	AVLLHEESM	
EBV TCE EBNA3B	VEITPYKPTW	91
EBV TCE EBNA3C	EGGVGWRHW	92
EBV TCE EBNA3C	QNGALAINTF	93
EBV TCE EBNA3C	LRGKWQRRYR	94
EBV TCE EBNA3C	RRIYDLIEL	95
EBV TCE EBNA3C	HHIWQNLL	96
EBV TCE EBNA3C	EENLLDFVRF	97
EBV TCE EBNA3C	LLDFVRFMGV	98
EBV TCE EBNA3C	LDFVRFMGV	99
EBV TCE EBNA3C	KEHVIQNAF	100
EBV TCE EBNA3C	FRKAQIQGL	101
EBV TCE EBNA3C	QPRAPIRPI	102
EBV TCE EBNA-LP	SLREWLLRI	103
EBV TCE LMP1	FWLYIVMSD	104
EBV TCE LMP1	FRRDLLCPLGA	105
EBV TCE LMP1	YLLEMLWRL	106
EBV TCE LMP1	YLQQNWWTL	107
EBV TCE LMP1	TLLVDLLWL	108
EBV TCE LMP1	DPHGPVQLSYD	109
EBV TCE LMP2	MGSLEMVPM	110

EBV TCE LMP2	EDPYWGNGDRHSDYQ	111
EBV TCE LMP2	NPVCLPVIVAPYLE	112
EBV TCE LMP2	LPVIVAPYL	113
EBV TCE LMP2	PYLFWLAAI	114
EBV TCE LMP2	ASCFTASVSTVVTA	115
EBV TCE LMP2	FTASVSTVV	116
EBV TCE LMP2	IEDPPFNSL	117
EBV TCE LMP2	RRRWRRRLTV	118
EBV TCE LMP2	RRWRRLTVC	119
EBV TCE LMP2	RRLTVCGGIMF	120
EBV TCE LMP2	TVCGGIMFL	121
EBV TCE LMP2	MFLACVLVLIVDAV	122
EBV TCE LMP2	LIVDAVLQL	123
EBV TCE LMP2	GLGTLGAAI	124
EBV TCE LMP2	LLWTLVLL	125
EBV TCE LMP2	SSCSSCPLSKI	126
EBV TCE LMP2	ILLARLFLY	127
EBV TCE LMP2	FLYALALL	128
EBV TCE LMP2	TYGPVFMCL	129
EBV TCE LMP2	CLGGLTMV	130
EBV TCE LMP2	VMSNTLLSAW	131
EBV TCE LMP2	LTAGFLIFL	132
EBV TCE LMP2	LLSAWILTA	133
EBV TCE BRLF1	LVSDYCNVLNKEFT	134
EBV TCE BRLF1	LVSDYCNVL	135
EBV TCE BRLF1	DYCNVLNKEF	136
EBV TCE BRLF1	AENAGNDAC	137
EBV TCE BRLF1	IACPIVMRYVLDHLI	138
EBV TCE BRLF1	YVLDHLIVV	139
EBV TCE BRLF1	FFIQAPSNRVMIPAT	140
EBV TCE BRLF1	ATIGTAMYK	141
EBV TCE BRLF1	KHSRVRAYTYSKVLG	142
EBV TCE BRLF1	RALIKTLPRASYSSH	143
EBV TCE BRLF1	ERPIFPHPSKPTFLP	144
EBV TCE BRLF1	QKEEAAICGQMDLS	145
EBV TCE BRLF1	EVCQPKRIRPFHPPG	146
EBV TCE BZLF1	LPEPLPQGQLTAY	147
EBV TCE BZLF1	EPLPQGQLTAY	148
EBV TCE BZLF1	APENAYQAY	149
EBV TCE BZLF1	LQHYREVAA	150
EBV TCE BZLF1	DSELEIKRYKNR	151
EBV TCE BZLF1	RKCCRAKFKQLLQHYR	152
EBV TCE BZLF1	RAKFKQLL	153
EBV TCE BZLF1	SENDRLRLL	154

T-cell epitope from Influenza A Virus (IAV TCE) PR8	CTELKLSDY	155
IAV TCE PB1	VSDGGPNLY	156
IAV TCE MP	GILGFVFTL	157
IAV TCE MP	ILGFVFTLTV	158
IAV TCE PR8	ILRGsvAHK	159
IAV TCE MP	SIIPSGPLK	160
IAV TCE MP1	RMVLASTTAK	161
IAV TCE MP2	KSMREEYRK	162
IAV TCE NP	SPIVPSFDM	163
IAV TCE PB1	QPEWFRNVL	164
IAV TCE NP	SRYWAIRTR	165
Stem for branched peptide	SEEZSEEZSEEZSEEZ, wherein Z is Azidonorleucine	166
Branch 1-1 for branched peptide	BKPAKFFRLTPRVTGGGAM- nh ₂ , wherein B is propargyl glycine	167
Branch 1-2 for branched peptide	BKPAKFFRLRPHERNGFTVL- nh ₂ , wherein B is propargyl glycine	168
Branch 1-3 for branched peptide	BKPAKFFRLRELRRKMMYM- nh ₂ , wherein B is propargyl glycine	169
Branch 1-4 for branched peptide	BKPAKFFRLNLVPMVATV-nh ₂ , wherein B is propargyl glycine	170
Branch 3-1 for branched peptide	BAIPVSLRTPRVTGGGAM-nh ₂ , wherein B is propargyl glycine	171
Branch 3-2 for branched peptide	BAIPVSLRRPHERNGFTVL- nh ₂ , wherein B is propargyl glycine	172
Branch 3-3 for branched peptide	BAIPVSLRELRRKMMYM-nh ₂ , wherein B is propargyl glycine	173
Branch 3-4 for branched peptide	BAIPVSLVTEHDTLLY-nh ₂ , wherein B is propargyl glycine	174
C1s cleavage site	YLGRSYKV	175
C1s cleavage site	MQLGRX	176
MASP2 cleavage site	SLGRKIQI	177
C2a cleavage site	GLARSNLDE	178
Cathepsin D cleavage site	PRSFRLGK	179
ADAM28 cleavage site	KPAKFFRL	180
ADAM28 cleavage site	DPAKFFRL	181

ADAM28 cleavage site	KPMKFFRL	182
ADAM28 cleavage site	LPAKFFRL	183
MMP2 cleavage site	AIPVSLR	184
Bundled domain sequence	SASGGGSGGGSVSDVPRDLEVVA ATPTSLLLISWDAPAVGGGGSGGG GSTIPVSLRSTPRVTGGGAMTIP VSLRSGGGGSGGGGSTVRYRIT YGETGGNSPVQEFTVPGSKSTAT ISGLKPGVDYTITVYAVTGGGGG SGGGGSTIPVSLRSNLVPMVATV TIPVSLRSGGGGSGGGGSSPASS KPISINYRTGGGGSGGGGSTIPV SLRSVLEETSVML	185
MMP2 cleavage site	TIPVSLRS	186

DESCRIPTION OF THE EMBODIMENTS

I. Targeting Moiety Peptide Epitope Complexes Having A Plurality of T-Cell Epitopes

[0046] A variety of targeting moiety peptide epitope complexes (TPECs) are described in different embodiments. In each of the embodiments, however, a targeting moiety may be used to deliver the TPEC to an area of unwanted cells, allowing for a therapeutic effect to be delivered locally. The TPEC also contains a plurality of T-cell epitopes. The TPEC further comprises cleavage sites that release the T-cell epitopes from the targeting agent, and in some embodiments from each other, when they are in the microenvironment of the unwanted cells. Although the arrangement and number of T-cell epitopes varies in different embodiments described herein, once cleaved from the targeting agent (and any neighboring T-cell epitopes), the T-cell epitopes function by stimulating an immune response against the unwanted cells. In some embodiments, maximal benefits may be achieved by releasing all of the T-cell epitopes from both the targeting agent and from each other in the cleavage process, allowing each T-cell epitope to attract an immune response to the unwanted cell.

[0047] Having a plurality of T-cell epitopes, as discussed in detail below, enhances the immune response against the unwanted cells, either by stimulating a stronger immune response in a given patient or by allowing the TPEC to

stimulate an immune response across a wide variety of patients in different ethnic and racial groups. In one embodiment, and while not being bound by theory, the cleavage at the cleavage site allows the T-cell epitopes to be trimmed at either or both ends to the appropriate length to produce a peptide that can fit in the peptide-binding groove of HLA class I and be recognized by T-cells, so as to initiate an immune response.

[0048] Without cleavage of these epitopes, the prior art fails to stimulate a sufficient T-cell response because the T-cell epitopes fused together would not be adequately recognized by the patients T-cells and would, thus, not initiate an immune response against the cancer cells.

A. TPECs with a Plurality of T-Cell Epitopes Separately Conjugated

[0049] In one embodiment, the TPEC comprises a plurality of T-cell epitopes that are separately conjugated to the targeting moiety. Because the T-cell epitopes will be separately conjugated to the targeting moiety, with two exemplary embodiments shown in Figures 1A and 1B, this embodiment may be termed the “hairy” embodiment as the T-cell epitopes protrude from the targeting moiety like individual hairs from a person’s skin. In other words, in some embodiments, a composition for retargeting an immune response to unwanted cells may comprise a TPEC comprising the formula $T-(L-C-E)_n$. In such embodiments, a plurality of L-C-E, each attach separately to T, wherein:

[0050] (a) T is a targeting moiety that is capable of targeting unwanted cells;

[0051] (b) L is a linker capable of chemical linkage to T;

[0052] (c) C is a cleavage site that is (i) cleaved by an enzyme expressed by the unwanted cells; (ii) cleaved through a pH-sensitive cleavage reaction inside the unwanted cell; (iii) cleaved by a complement-dependent cleavage reaction; or (iv) cleaved by a protease that is colocalized to the unwanted cell by a targeting moiety that is the same or different from the targeting moiety in the TPEC; and

[0053] (d) E is a T-cell epitope.

[0054] N is an integer of at least 2 (optionally from about 2 to 50). The same cleavage site may optionally be used multiple times in the TPEC and the same epitope may optionally be used multiple times in the TPEC.

[0055] The C and E moieties may be the same in the plurality of L-C-E moieties affixed to a single T or a single T may have different C and E moieties in the plurality of L-C-E moieties. Thus, there may be more than one type of cleavage site or only one type of cleavage site. There may also, independently, be more than one type of T-cell epitope or only one type of T-cell epitope.

[0056] In certain aspects of the embodiments discussed in this section, the L-C-E complexes may be affixed to the targeting moiety in a random fashion. Thus, in a preparation of TPECs with a plurality of T-cell epitopes separately conjugated to the targeting moiety, some TPECs may have greater numbers of T-cell epitopes and some may have fewer numbers. Additionally, in a preparation of TPECs with a plurality of T-cell epitopes separately conjugated to the targeting moiety, the location of TPECs may differ throughout the preparation. In this vein, when these compounds are administered to a patient suffering from a disease such as cancer, the patient's body will have a much more difficult time mounting an inhibitory immune response against the preparation of TPECs. This may allow the patient to receive multiple doses of the TPEC preparation over a significant period of time, as discussed further below in Section III.B below.

B. TPECs with at Least One Polytope

[0057] In another embodiment, the TPEC comprises a plurality of T-cell epitopes that are conjugated to the targeting moiety as a polytope (for poly-epitope), but the conjugation within the polytope and to the targeting moiety occurs through cleavage sites allowing the release of the individual T-cell epitopes in the microenvironment of the unwanted cells. Several embodiments of this arrangement are shown in Figures 2A, 2B, and 2C. Thus, the polytope itself can have a linear and/or bundled configuration (like Figures 2A and 2B) or a branched configuration like a hairbrush with bristles (like Figure 2C).

[0058] In Figures 2A-B, a composition for retargeting an immune response to unwanted cells comprises a TPEC having at least one of L-(C-E)_n with each C-E attached to the L in series and the L attached to the T. It may have one or a

plurality of L-(C-E)_n attached to the T. In the embodiments of Figures 2A-B, the linker may be either a chemical linker, a peptide bond, or at least one peptide.

[0059] In Figure 2C, a composition for retargeting an immune response to unwanted cells comprises a TPEC having at least one of L-(C-E)_n with each C-E attached to the L in parallel and the L attached to the T. It may have one or a plurality of L-(C-E)_n attached to the T. In embodiment 2C, the linker may be a chemical linker.

[0060] In all of the embodiments of Figure 2A-C,

[0061] (a) T is a targeting moiety that is capable of targeting unwanted cells;

[0062] (b) L is a linker capable of chemical or peptide linkage to T (including a peptide bond);

[0063] (c) C is a cleavage site (i) cleaved by an enzyme expressed by the unwanted cells; (ii) cleaved through a pH-sensitive cleavage reaction inside the unwanted cell; (iii) cleaved by a complement-dependent cleavage reaction; or (iv) cleaved by a protease that is colocalized to the unwanted cell by a targeting moiety that is the same or different from the targeting moiety in the TPEC; and

[0064] (d) E is a T-cell epitope;

[0065] wherein n is an integer of at least 2 (optionally from about 2 to 50).

[0066] The C and E moieties may be the same in the plurality of C-E moieties affixed through L to a single T or a single T may have different C and E moieties in the plurality of C-E moieties. Thus, there may be more than one type of cleavage site or only one type of cleavage site. There may also, independently, be more than one type of T-cell epitope or only one type of T-cell epitope.

[0067] In some embodiments, the plurality of the T-cell epitopes being conjugated in a polytope allow for a uniform product to be produced that contains a standard group, number, and arrangement of T-cell epitopes, while still allowing for the release of all of the T-cell epitopes in the microenvironment of the unwanted cells. Microenvironment means the specific set of physical, chemical, and biological conditions in the vicinity of cells within a distance where these conditions can have an effect on or be sensed by the cells.

[0068] In some embodiments, more than one polytope are conjugated to the targeting moiety. In some embodiments from about 1 to 30, 1 to 20, or 1 to 10 polytopes are conjugated to the targeting moiety. In some embodiments, about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 polytopes are conjugated to the targeting moiety, each group by one cleavage site. In these embodiments, the polytopes may be the same or different.

[0069] In some embodiments, the TPEC may have both at least one separately conjugated T-cell epitope and at least one polytope, according to sections I.A and I.B, respectively.

C. TPECs with More Than 10 T-Cell Epitopes

[0070] In another embodiment, the TPEC comprises a plurality of T-cell epitopes that, irrespective of how they are arranged on the targeting moiety, comprise more than 10 T-cell epitopes. In these embodiments, the T-cell epitopes may be separately conjugated or conjugated in a polytope. In other words, a composition for retargeting an immune response to unwanted cells may comprise a TPEC having:

[0071] (a) a targeting moiety that is capable of targeting unwanted cells;

[0072] (b) a plurality of more than 10 T-cell epitopes conjugated to the targeting moiety with at least one cleavage site, wherein the cleavage site is (i) cleaved by an enzyme expressed by the unwanted cells; (ii) cleaved through a pH-sensitive cleavage reaction inside the unwanted cell; (iii) cleaved by a complement-dependent cleavage reaction; or (iv) cleaved by a protease that is colocalized to the unwanted cell by a targeting moiety that is the same or different from the targeting moiety in the TPEC.

[0073] In some embodiments, including TPECs with more than 10 T-cell epitopes, the immune response against the unwanted cells is notably stronger. In some embodiments, including TPECs with more than 10 T-cell epitopes, the TPEC can be used to treat a greater proportion of patients suffering from the condition characterized by unwanted cells.

[0074] In some compositions with TPECs having more than 10 T-cell epitopes, the T-cell epitopes may be separately conjugated to the targeting moiety, each T-cell epitope by a cleavage site. In some embodiments, with TPECs having

more than 10 T-cell epitopes, the T-cell epitopes may be conjugated to the targeting moiety as at least one polytope, each polytope conjugated to the targeting moiety by a cleavage site. In some embodiments, the T-cell epitopes within a polytope have cleavage sites between them.

D. Compositions Comprising TPECs

[0075] A composition may comprise a plurality of TPECs. In some embodiments, all of the TPECs in the composition are the same. In some embodiments, at least some of the TPECs in the composition are not identical.

[0076] In some embodiments, each TPEC is conjugated to a plurality of identical T-cell epitopes.

[0077] In some embodiments, at least some of the TPECs in the composition are conjugated to a plurality of T-cell epitopes that are not identical.

[0078] In some embodiments, a composition may comprise at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or more different TPECs.

E. T-Cell Epitopes

[0079] T-cell epitopes function in the TPECs to attract the patient's own immune response to attack the unwanted cell by labeling it with antigenic T-cell epitopes from infectious agents.

[0080] Depending on desired aspects of the therapy, a variety of optional factors may be considered when choosing T-cell epitopes. Every person has six HLA class-I molecules that can bind peptides to present to CD8 T cells with some HLA types more prevalent in the population than others, such as HLA-A201 which is found in ~45% of the Caucasian population. In situations where the epitopes chosen for conjugation are able to bind to a limited set of HLA molecules that are only found in a proportion of the population, the TPEC may not have any or as much effectiveness in other segments of the population. Using HLA-A201 as an example, if the targeting moiety is conjugated with a T-cell epitope that binds to HLA-A201 then only patients that express HLA-A201 would bind the epitope and present it to T cells and initiate an immune response. However, in patients who are HLA-A201 negative the epitope may not be able to bind to their HLA molecules and there would be no or a lesser immune response. In situations where the therapy may be provided to a wide segment of the

population having different HLA molecules, using different epitopes that bind to more HLA molecules could enhance the effectiveness of the TPEC across the population.

[0081] As an additional factor to consider, in some embodiments, T-cell epitopes may be chosen from those that a particular person has been exposed to, a wide variety of people have been exposed to, or for which vaccines have or can be administered. T cells generally recognize the epitope in complex with an HLA molecule when the patient has been previously infected with the virus from which the epitope is derived (or in instances where the patient has previously received a vaccine containing those epitopes). Vaccines may have been administered for a prior purpose (such as childhood vaccines) or may be administered preceding TPEC treatment with the same epitope.

[0082] In certain instances, T-cell epitopes are chosen from cytomegalovirus (CMV), influenza, Epstein Barr virus (EBV), varicella zoster, mumps, measles, rubella, adenovirus, polio, vaccinia, RSV, rotavirus, tetanus, vaccinia, and yellow fever T-cell epitopes. Epitopes may be chosen from infectious agents that are prevalent across the population in question or for which there are vaccines that are regularly administered or could be administered as part of a combination therapy approach.

[0083] In certain embodiments, the T-cell epitopes are chosen from at least 2, 3, 4, or 5 different infectious agents. In certain embodiments, at least some of the T-cell epitopes are CMV epitopes.

[0084] In any of the various TPECs described herein, in some embodiments, the plurality of T-cell epitopes are not all identical. In some embodiments, the plurality of T-cell epitopes are the same. In certain embodiments, the plurality of T-cell epitopes comprise some that are the same and some that are different.

[0085] The T-cell epitopes may be all MHC Class I restricted peptides, all MHC class II restricted peptides, or a combination of both Class I and Class II.

[0086] In some embodiments, the plurality of T-cell epitopes are from about 7 to 14 amino acids in length, from about 8 to 13, from about 9 to 12, about 9, or about 10 amino acids.

[0087] In some embodiments, at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 different T-cell epitopes are used in the TPECs, whether the TPECs are the same or different. In some embodiments, using a plurality of different T-cell epitopes allows the agent to stimulate a T-cell response in at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90% of the human population.

[0088] In some embodiments, the T-cell epitopes are chosen from HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, CD1d, and MR1. In certain embodiments, the T-cell epitopes are chosen from HLA-A*01, HLA-A*02, HLA-A*03, HLA-A*11, HLA-A*24, HLA-B*44, HLA-B*07, HLA-B*08, HLA-B*15, HLA-B*35, HLA-B*40, HLA-C*07, HLA-C*03, HLA-C*05, HLA-C*04, HLA-C*06, and HLA-E*0101 restricted antigens.

[0089] In certain embodiments, the composition comprises at least the following T-cell epitopes HLA-A*02, HLA-A*01, and HLA-A*03.

[0090] When desired, more immunodominant T-cell epitopes may be selected. By immunodominant, we mean those epitopes that elicit the strongest immune response in a given patient and/or those that are known to be immunogenic across a wide cross-section of people.

[0091] In some embodiments, the T-cell epitopes comprise at least one of the epitopes provided in Table 2. In some embodiments, the T-cell epitopes are chosen from the epitopes provided in Table 2.

Table 2: Exemplary T-Cell Epitopes			
Peptide from	Sequence	Position	HLA Type
Cytomegalovirus Epitopes			
IE1	ATTFLLQTMLR (SEQ ID NO: 15)	32–41	HLA-A68
IE1	BRVLBBYVL (SEQ ID NO: 6) (variant of SEQ ID NO: 17, where the C residues are changed to B residues, wherein B is alpha- aminobutyric acid)	309–317	HLA-C7
pp65	CPSQEPMSIYVY (SEQ ID NO: 16)	103–114	HLA-B35
IE1	CRVLCCYVL (SEQ ID NO: 17)	309–317	HLA-C7
IE1	CVETMCNEY (SEQ ID NO: 18)	279–287	HLA-A1/B18

IE1	DELRRKMMY (SEQ ID NO: 19)	198–206	HLA-A1/B18
pp65	DTPVLPHETR (SEQ ID NO: 20)	31–40	HLA-A68
IE1	EEAIVAYTL (SEQ ID NO: 21)	381–389	HLA-B44
pp65	EFFDANDIY (SEQ ID NO: 22)	512–521	HLA-B44
IE1	ELKRKMIYM (SEQ ID NO: 23)	199–207	HLA-B8
IE1	ELRRKMMYM (SEQ ID NO: 24)	199–207	HLA-B8
IE1	EVISVMKRR (SEQ ID NO: 25)	334–342	HLA-A68
IE2	FEQPTETPP (SEQ ID NO: 26)	381–389	HLA-B41
IE1	FPKTTNGCSQA (SEQ ID NO: 27)	221–231	HLA-B55
pp65	FPTKDVAL (SEQ ID NO: 28)	188–195	HLA-B35
pp65	FTSQYRIQGKL (SEQ ID NO: 29)	369–379	HLA-A24
pp65	FVFPTKDVALR (SEQ ID NO: 30)	186–196	HLA-A68
IE2	IIYTRNHEVK (SEQ ID NO: 31)	242–250	HLA-A2
pp65	IPSINVHHY (SEQ ID NO: 32)	123–131	HLA-B35
pp150	KARDHLAVL (SEQ ID NO: 33)	101–109	HLA-B7
IE1	KEVNSQLSL (SEQ ID NO: 34)	42–50	HLA-B40
IE1	KRKMIYMCY (SEQ ID NO: 35)	201–209	HLA-B27
pp65	MLNIPSINV (SEQ ID NO: 36)	120–128	HLA-A2
pp65	NLVPMVATV (SEQ ID NO: 1)	495–503	HLA-A2
pp150	NVRRSWEEL (SEQ ID NO: 37)	212–220	HLA-B7
IE1	QIKVRVDMV (SEQ ID NO: 38)	88–96	HLA-B8
pp65	QPSLILVSQY (SEQ ID NO: 39)	52–61	HLA-B35
pp150	QTVTSTPVQGR (SEQ ID NO: 40)	792–802	HLA-A68
pp65	QYDVPAALF (SEQ ID NO: 41)	341–349	HLA-A24
pp65	QYVKVYLESF (SEQ ID NO: 42)	222–231	HLA-A24
pp50	RGDPFDKNY (SEQ ID NO: 43)	274–282	HLA-A1
pp65	RIFAELEGV (SEQ ID NO: 44)	522–530	HLA-A2
gB	RIWCLVVCV (SEQ ID NO: 45)	4–12	HLA-A2
pp65	RPHERNGFTVL (SEQ ID NO: 2)	265–275	HLA-B7
IE1	RRIEEICMK (SEQ ID NO: 46)	341–349	HLA-B27
IE1	RRKMMYMCY (SEQ ID NO: 47)	201–209	HLA-B27
pp65	SEHPTFTSQY (SEQ ID NO: 48)	364–373	HLA-B44
pp65	TPRVTGGGAM (SEQ ID NO: 49)	417–426	HLA-B7
pp150	TTVYPPSSTAK (SEQ ID NO: 50)	945–955	HLA-A3
pp50	TVRSHCVSK (SEQ ID NO: 51)	52–60	HLA-A3
IE1	VLAELVKQI (SEQ ID NO: 52)	81–89	HLA-A2
IE1	VLEETSVML (SEQ ID NO: 5)	316–324	HLA-A2
pp50	VTEHDTLLY (SEQ ID NO: 53)	245–253	HLA-A1
pp65	VYALPLKML (SEQ ID NO: 54)	113–121	HLA-A24
IE1	YILEETSVM (SEQ ID NO: 7)	315–323	HLA-A2
pp65	YSEHPTFTSQY (SEQ ID NO: 55)	363–373	HLA-A1
pp65	YTPDSTPCHR (SEQ ID NO: 56)	61–70	HLA-A68
IE1	YVLEETSVM (SEQ ID NO: 57)	316–324	HLA-A2
Epstein Barr Virus			

EBNA1	RPQKRPSCI (SEQ ID NO: 64)	72-80	HLA-B7
EBNA1	HPVGEADYF (SEQ ID NO: 65)	407-415	HLA-B53
EBNA1	HPVGEADYFEY (SEQ ID NO: 66)	407-417	HLA-B35
EBNA1	IPQCRLTPL (SEQ ID NO: 67)	528-536	HLA-B7
EBNA1	VLKDAIKDL (SEQ ID NO: 68)	574-582	HLA-A2
EBNA2	YHLIVDTDSL (SEQ ID NO: 69)	14-23	HLA-B38
EBNA2	DTPLIPLTIF (SEQ ID NO: 70)	42-51	HLA-A2/B51
EBNA2	RPTTELQPTP (SEQ ID NO: 71)	234-242	HLA-B55
EBNA3A	QAKWRLQTL (SEQ ID NO: 72)	158-166	HLA-B8
EBNA3A	AYSSWMYSY (SEQ ID NO: 73)	176-184	HLA-A30
EBNA3A	RYSIFFDY (SEQ ID NO: 74)	246-253	HLA-A24
EBNA3A	FLRGRAYGL (SEQ ID NO: 75)	325-333	HLA-B8
EBNA3A	KRPPIFIRRL (SEQ ID NO: 76)	378-387	HLA-B27
EBNA3A	RPPIFIRRL (SEQ ID NO: 77)	379-387	HLA-B7
EBNA3A	LEKARGSTY (SEQ ID NO: 78)	406-414	HLA-B62
EBNA3A	HLAAQGMAY (SEQ ID NO: 79)	450-458	HLA-A1
EBNA3A	YPLHEQHGM (SEQ ID NO: 80)	458-466	HLA-B35
EBNA3A	VFSDGRVAC (SEQ ID NO: 81)	491-499	HLA-A29
EBNA3A	VPAPAGPIV (SEQ ID NO: 82)	502-510	HLA-B7
EBNA3A	SVRDRLARL (SEQ ID NO: 83)	596-604	HLA-A2
EBNA3A	RLRAEAQVK (SEQ ID NO: 84)	603-611	HLA-A3
EBNA3A	VQPPQLTLQV (SEQ ID NO: 85)	617-625	HLA-B46
EBNA3B	HRCQAIRKK (SEQ ID NO: 86)	149-157	HLA-B27
EBNA3B	TYSAGIVQI (SEQ ID NO: 87)	217-225	HLA-A24
EBNA3B	RRARSLSAERY (SEQ ID NO: 88)	244-254	HLA-B27
EBNA3B	VSFIEFVGW (SEQ ID NO: 89)	279-287	HLA-B58
EBNA3B	AVFDRKSDAK (SEQ ID NO: 90)	399-408	HLA-A11
EBNA3B	IVTDFSVIK (SEQ ID NO: 91)	416-424	HLA-A11
EBNA3B	AVLLHEESM (SEQ ID NO: 92)	488-496	HLA-B35.01
EBNA3B	VEITPYKPTW (SEQ ID NO: 93)	657-666	HLA-B44
EBNA3C	EGGVGWRHW (SEQ ID NO: 94)	163-171	HLA-B44
EBNA3C	QNGALAINTF (SEQ ID NO: 95)	213-222	HLA-B62
EBNA3C	LRGKWQRRYR (SEQ ID NO: 96)	249-258	HLA-B27
EBNA3C	RRIYDLIEL (SEQ ID NO: 97)	258-266	HLA-B27
EBNA3C	HHIWQNLL (SEQ ID NO: 98)	271-278	HLA-B39
EBNA3C	EENLLDFVRF (SEQ ID NO: 99)	281-290	HLA-B44
EBNA3C	LLDFVRFMGV (SEQ ID NO: 100)	284-293	HLA-A2
EBNA3C	LDFVRFMGV (SEQ ID NO: 101)	285-293	HLA-B37
EBNA3C	KEHVIQNAF (SEQ ID NO: 102)	335-343	HLA-B44
EBNA3C	FRKAQIQGL (SEQ ID NO: 103)	343-351	HLA-B27
EBNA3C	QPRAPIRPI (SEQ ID NO: 104)	881-889	HLA-B7
EBNA-LP	SLREWLLRI (SEQ ID NO: 105)	284-292	HLA-A2 (A*0203)
LMP1	FWLYIVMSD (SEQ ID NO: 106)	38-46	MHC Class I

LMP1	FRRDLLCPLGA (SEQ ID NO: 107)	72-82	HLA-B40
LMP1	YLLEMLWRL (SEQ ID NO: 108)	125-133	HLA-A2
LMP1	YLQQNWWTL (SEQ ID NO: 109)	159-167	HLA-A2
LMP1	TLLVDLLWL (SEQ ID NO: 110)	166-174	HLA-A2
LMP1	DPHGVPQLSYD (SEQ ID NO: 111)	375-386	HLA-B51
LMP2	MGSLEMVPM (SEQ ID NO: 112)	1-9	HLA-B35
LMP2	EDPYWGNGDRHSDYQ (SEQ ID NO: 113)	61-75	MHC Class I
LMP2	NPVCLPVIVAPYLF (SEQ ID NO: 114)	121-134	MHC Class I
LMP2	LPVIVAPYL (SEQ ID NO: 115)	125-133	HLA-B53
LMP2	PYLFWLAAI (SEQ ID NO: 116)	131-139	HLA-A23
LMP2	ASCFTASVSTVVTA (SEQ ID NO: 117)	141-154	MHC Class I
LMP2	FTASVSTVV (SEQ ID NO: 118)	144-152	HLA-A68
LMP2	IEDPPFNSL (SEQ ID NO: 119)	200-208	HLA-B40
LMP2	RRRWRLTV (SEQ ID NO: 120)	236-244	HLA-B27
LMP2	RRWRRLTVC (SEQ ID NO: 121)	237-245	HLA-B14
LMP2	RRLTVCGGIMF (SEQ ID NO: 122)	240-250	HLA-B27
LMP2	TVCGGIMFL (SEQ ID NO: 123)	243-251	HLA-A1
LMP2	MFLACVLVLIVDAV (SEQ ID NO: 124)	249-262	MHC Class I
LMP2	LIVDAVLQL (SEQ ID NO: 125)	257-265	HLA-A2
LMP2	GLGTLGAAI (SEQ ID NO: 126)	293-301	HLA-A2
LMP2	LLWTLVVLL (SEQ ID NO: 127)	329-337	HLA-A2
LMP2	SSCSSCPLSKI (SEQ ID NO: 128)	340-350	HLA-A11
LMP2	ILLARLFY (SEQ ID NO: 129)	349-358	HLA-A29
LMP2	FLYALALL (SEQ ID NO: 130)	356-364	HLA-A2
LMP2	TYGPVFMCL (SEQ ID NO: 131)	419-427	HLA-A24
LMP2	CLGGLLMV (SEQ ID NO: 132)	426-434	HLA-A2
LMP2	VMSNTLLSAW (SEQ ID NO: 133)	442-451	HLA-A25
LMP2	LTAGFLIFL (SEQ ID NO: 134)	453-461	HLA-A2
LMP2	LLSAWILTA (SEQ ID NO: 135)	447-455	HLA-A2
BRLF1	LVSDYCNVLNKEFT (SEQ ID NO: 136)	25-39	MHC Class I
BRLF1	LVSDYCNVL (SEQ ID NO: 137)	25-33	HLA-A2
BRLF1	DYCNVLNKEF (SEQ ID NO: 138)	28-37	HLA-A24
BRLF1	AENAGNDAC (SEQ ID NO: 139)	91-99	HLA-B45
BRLF1	IACPIVMRYVLDHLI (SEQ ID NO: 140)	101-115	HLA-A24/C2

BRLF1	YVLDHLIVV (SEQ ID NO: 141)	109-117	HLA-A2
BRLF1	FFIQAPSNRVMIPAT (SEQ ID NO: 142)	121-135	MHC Class I
BRLF1	ATIGTAMYK (SEQ ID NO: 143)	134-142	HLA-A11
BRLF1	KHSRVRAYTYSKVLG (SEQ ID NO: 144)	145-159	HLA-A3
BRLF1	RALIKTLPRASYSSH (SEQ ID NO: 145)	225-239	HLA-A2
BRLF1	ERPIFPHPSKPTFLP (SEQ ID NO: 146)	393-407	HLA-C4
BRLF1	QKEEAAICGQMDLS (SEQ ID NO: 147)	529-543	HLA-B61
BRLF1	EVCQPKRIRPFHPPG (SEQ ID NO: 148)	441-455	MHC Class I
BZLF1	LPEPLPQGQLTAY (SEQ ID NO: 149)	52-64	MHC Class I
BZLF1	EPLPQGQLTAY (SEQ ID NO: 150)	54-63	HLA-B35
BZLF1	APENAYQAY (SEQ ID NO: 151)	81-89	HLA-B35
BZLF1	LQHYREVAA (SEQ ID NO: 152)	101-115	HLA-C8
BZLF1	DSELEIKRYKNR (SEQ ID NO: 153)	172-183	HLA-B18
BZLF1	RKCCRAKFKQLLQHYR (SEQ ID NO: 154)	186-201	HLA-C6
BZLF1	RAKFKQLL (SEQ ID NO: 155)	190-197	HLA-B8
BZLF1	SENDRLRLL (SEQ ID NO: 156)	209-217	HLA-B60
Influenza A Virus			
PR8	CTELKLSDY (SEQ ID NO: 157)	44-52	HLA-A1
PB1	VSDGGPNLY (SEQ ID NO: 158)	591-599	HLA-A1
MP	GILGFVFTL (SEQ ID NO: 159)	58-66	HLA-A2
MP	ILGFVFTLTV (SEQ ID NO: 160)	59-68	HLA-A2
PR8	ILRGsvAHK (SEQ ID NO: 161)	265-274	HLA-A3
MP	SIIPSGPLK (SEQ ID NO: 162)	13-21	HLA-A11
MP1	RMVLASTTAK (SEQ ID NO: 163)	178-187	HLA-A11
MP2	KSMREEYRK (SEQ ID NO: 164)	70-78	HLA-A11
NP	SPIVPSFDM (SEQ ID NO: 165)	473-481	HLA-B7
PB1	QPEWFRNVL (SEQ ID NO: 166)	329-337	HLA-B7
NP	SRYWAIRTR (SEQ ID NO: 167)	383-391	HLA-B27

F. Targeting Moiety

[0092] The targeting moiety functions in the TPEC by delivering the TPEC to the local environment of the unwanted cells, enabling a localized

treatment strategy. In certain embodiments, the targeting moiety targets the unwanted cells by specifically binding to the unwanted cells. In some instances, the targeting moiety specifically binds the unwanted cells even while the plurality of T-cell epitopes are conjugated to the targeting moiety.

[0093] In certain embodiments, the targeting moiety is an antibody or functional part thereof.

[0094] Certain antibody targets (with examples of unwanted cell types in parentheses) may include: Her2/Neu (Epithelial malignancies); CD22 (B cells, autoimmune or malignant); EpCAM (CD326) (Epithelial malignancies); EGFR (epithelial malignancies); PMSA (Prostate Carcinoma); CD30 (B cell malignancies); CD20 (B cells, autoimmune, allergic or malignant); CD33 (Myeloid malignancies); membrane IgE (Allergic B cells); IgE Receptor (CD23) (Mast cells or B cells in allergic disease), CD80 (B cells, autoimmune, allergic or malignant); CD86 (B cells, autoimmune, allergic or malignant); CD2 (T cell or NK cell lymphomas); CA125 (multiple cancers including Ovarian carcinoma); Carbonic Anhydrase IX (multiple cancers including Renal Cell Carcinoma); CD70 (B cells, autoimmune, allergic or malignant); CD74 (B cells, autoimmune, allergic or malignant); CD56 (T cell or NK cell lymphomas); CD40 (B cells, autoimmune, allergic or malignant); CD19 (B cells, autoimmune, allergic or malignant); c-met/HGFR (Gastrointestinal tract and hepatic malignancies); TRAIL-R1 (multiple malignancies including ovarian and colorectal carcinoma); DRS (multiple malignancies including ovarian and colorectal carcinoma); PD-1 (B cells, autoimmune, allergic or malignant); PD1L (Multiple malignancies including epithelial adenocarcinoma); IGF-1R (Most malignancies including epithelial adenocarcinoma); VEGF-R2 (The vasculature associated with the majority of malignancies including epithelial adenocarcinomas; Prostate stem cell antigen (PSCA) (Prostate Adenocarcinoma); MUC1 (Epithelial malignancies); CanAg (tumors such as carcinomas of the colon and pancreas); Mesothelin (many tumors including mesothelioma and ovarian and pancreatic adenocarcinoma); P-cadherin (Epithelial malignancies, including breast adenocarcinoma); Myostatin (GDF8) (many tumors including sarcoma and ovarian and pancreatic adenocarcinoma); Cripto (TDGF1) (Epithelial malignancies including colon, breast, lung, ovarian,

and pancreatic cancers); ACVRL 1/ALK1 (multiple malignancies including leukaemias and lymphomas); MUC5AC (Epithelial malignancies, including breast adenocarcinoma); CEACAM (Epithelial malignancies, including breast adenocarcinoma); CD137 (B cells or T cells, autoimmune, allergic or malignant); CXCR4 (B cells or T cells, autoimmune, allergic or malignant); Neuropilin 1 (Epithelial malignancies, including lung cancer); Glypicans (multiple cancers including liver, brain and breast cancers); HER3/EGFR (Epithelial malignancies); PDGFRa (Epithelial malignancies); EphA2 (multiple cancers including neuroblastoma, melanoma, breast cancer, and small cell lung carcinoma); CD38 (Myeloma); CD138 (Myeloma); α 4-integrin (AML, myeloma, CLL, and most lymphomas).

[0095] In certain modes, antibodies include an anti-epidermal growth factor receptor antibody such as Cetuximab, an anti-Her2 antibody, an anti-CD20 antibody such as Rituximab, an anti-CD22 antibody such as Inotuzumab, G544 or BU59, an anti-CD70 antibody, an antiCD33 antibody such as hp67.6 or Gemtuzumab, an anti-MUC1 antibody such as GP1.4 and SM3, an anti-CD40 antibody, an anti-CD74 antibody, an anti-P-cadherin antibody, an anti-EpCAM antibody, an anti-CD138 antibody, an anti-E-cadherin antibody, an antiCEA antibody, an anti-FGFR3 antibody, and an anti α 4-integrin antibody such as natalizumab.

G. Cleavage Sites

[0096] The cleavage sites function to release the T-cell epitope from the targeting moiety and, in some embodiments, to release the T-cell epitopes from each other. Releasing the T-cell epitopes into single epitopes allows them to most effectively label the unwanted cell for immune attack.

[0097] In some instances, the cleavage site may be a separate sequence and in other instances the cleavage site may be integrated into the T-cell epitope such that one sequence serves the function of both elements. This may apply to any of the embodiments described herein and is determined by the selection of the epitope sequences.

[0098] The cleavage sites can function in different ways to release the T-cell epitopes in the microenvironment of the unwanted cells. The cleavage may

occur inside the unwanted cell or outside the unwanted cell, depending on the strategy employed. If cleavage occurs outside the unwanted cell, the T-cell epitope peptides can be presented without first being internalized into a cell and being engaged in the classical antigen-processing pathways. If cleavage occurs outside the unwanted cell, it may occur in the microenvironment surrounding the cell, including at the cell surface. For example, when the unwanted cell is a cancer cell, the cleavage may occur in the tumor microenvironment (outside of an in the vicinity of the cancer cell), including at the surface of the cancer cell.

[0099] In certain embodiments, at least one cleavage site may be cleaved by an enzyme expressed by the unwanted cells. Cancer cells, for instance, are known to express certain enzymes, such as proteases, and these may be employed in the TPEC strategy to cleave the TPEC's cleavage site. By way of nonlimiting example, cathepsin B cleaves FR, FK, VA and VR amongst others; cathepsin D cleaves PRSFFRLGK (SEQ ID NO: 179), ADAM28 cleaves KPAKFFRL (SEQ ID NO: 180), DPAKFFRL (SEQ ID NO: 181), KPMKFFRL (SEQ ID NO: 182) and LPAKFFRL (SEQ ID NO: 183); and MMP2 cleaves AIPVSLR (SEQ ID NO: 184).

[00100] In some embodiments, at least one cleavage site may be cleaved through a pH-sensitive cleavage reaction inside the unwanted cell. If the TPEC is internalized into the cell, the cleavage reaction may occur inside the cell and may be triggered by a change in pH between the microenvironment outside the unwanted cell and the interior of the cell. Specifically, some cancer types are known to have acidic environments in the interior of the cancer cells. Such an approach may be employed when the interior unwanted cell type has a characteristically different pH from the extracellular microenvironment, such as particularly the glycocalyx. Because pH cleavage can occur in all cells in the lysozymes, selection of a targeting agent when using a pH-sensitive cleavage site may require, when desired, more specificity. For example, when a pH-sensitive cleavage site is used, a targeting agent that binds only or highly preferably to cancer cells may be desired (such as, for example, an antibody binding to mesothelin for treatment of lung cancer).

[00101] In certain embodiments, at least one cleavage site may be cleaved by a complement-dependent cleavage reaction. Once TPECs bind to the unwanted cell, the patient's complement cascade may be triggered. In such a case, the complement cascade may also be used to cleave the T-cell epitope from the targeting agent by using a cleavage site sensitive to a complement protease. For example, C1r and C1s and the C3 convertases (C4B,2a and C3b,Bb) are serine proteases. C3/C5 and C5 are also complement proteases. Mannose-associated binding proteins (MASP), serine proteases also involved in the complement cascade and responsible for cleaving C4 and C2 into C4b2b (a C3 convertase) may also be used. For example, and without limitation, C1s cleaves YLGRSYKV (SEQ ID NO: 175) and MQLGRX (SEQ ID NO: 176). MASP2 is believed to cleave SLGRKIQI (SEQ ID NO: 177). Complement component C2a and complement factor Bb are believed to cleave GLARSNLDE (SEQ ID NO: 178).

[00102] In some embodiments, at least one cleavage site may be cleaved by a protease that is colocalized to the unwanted cell by a targeting moiety that is the same or different from the targeting moiety in the TPEC. For example, any protease may be simultaneously directed to the microenvironment of the unwanted cells by conjugating the protease to a targeting agent that delivers the protease to that location. The targeting agent may be any targeting agent described herein. The protease may be affixed to the targeting agent through a peptide or chemical linker and may maintain sufficient enzymatic activity when bound to the targeting agent.

[00103] In some embodiments, the TPEC has a plurality of cleavage sites that are the same. In other embodiments, the TPEC has a plurality of cleavage sites that are not all identical, either in sequence and/or in type, as described above.

H. Preparation of the T-Cell Epitopes and Cleavage Sites

1. Preparation of Individual T-Cell Epitopes and Cleavage Sites to be Separately Conjugated to Targeting Agent

[00104] Individual T-cell epitopes conjugated to cleavage sites may be prepared through standard peptide synthesis chemistry, such as by coupling the carboxyl group of the incoming amino acid to the N-terminus of the growing

peptide chain using a N-terminal protecting group addition strategy, such as tert-butoxycarbonyl (Boc) and 9-fluorenylmethoxycarbonyl (Fmoc), with their respective deprotection agents TFA and piperidine. Peptide synthesis may occur by hand or in an automated machine. Alternatively, services may be employed that prepare peptides upon order.

2. Preparation of Linear Polytopes

[00105] Polytopes up to ~50 amino acids could be made by standard peptide synthesis. This would allow up to about 5 epitopes to be incorporated into the polytope. In instances where more epitopes in the polytope are desired, or when human protein domains flank the epitopes in the polytope, then recombinant production of the polytope may occur. The DNA sequence for the polytope would be incorporated into a vector that would allow a cell line such as Chinese hamster ovary (CHO) cells to express the protein. The protein would be secreted by the cell line and could be purified from the cell culture supernatant. The purified polytope could then be conjugated to a targeting moiety through a chemical linker, as described herein. In another embodiment, nucleic acids encoding the polytope could be added on to an end of the DNA sequence encoding the targeting moiety to make one continuous polypeptide chain incorporating the targeting moiety and polytope. This could then be expressed in a cell line in the same way as the previous embodiment.

3. Preparation of Branched Polytope

[00106] Branched polytopes, such as shown in Figure 2C, may also be prepared. Individual T-cell epitopes to be applied to a branched polytope may be prepared according to Section I.H.1 before being conjugated to a connecting stem. In some embodiments, the connecting stem may be comprised of amino acids and may be a peptide stem.

[00107] The peptide stem may comprise amino acids that have a reactive property that can be targeted by crosslinking reagents. In some embodiments, cysteine and lysine may be used as there are a large number of crosslinking reagents that may be used at these amino acids. In one embodiment, a connecting stem may be a peptide comprising a large number of lysine or cysteine residues to facilitate linking reactions by sulfo-SMCC, for example. In

another embodiment, the connecting stem may comprise serine or threonine, so as to partner with a crosslinking agent that would link to hydroxyl groups on these amino acids.

[00108] In another embodiment, spacer amino acids may be used in between those that have a reactive property to the crosslinking reagents in order to allow for protease attack without being blocked by other peptides bound to the stem. In certain aspects, a basic spacer may include glycine and serine. In some embodiments, the incorporation of proline between reactive amino acids may be useful as proline induces a slight rotation of the peptide which may help to keep the protease cleavable peptides bound to the stem further apart.

[00109] In certain embodiments, the peptide stem may comprise from about 10 to 80, 20 to 80, or 40 to 80 amino acids. In one embodiment the peptide stem comprises from about 2 to 20 amino acids that are bound to a T-cell epitope and cleavage site.

[00110] The individual T-cell epitopes may be conjugated to the peptide stem using the chemical linker technology discussed in Section I.I below. The connecting stem comprising the individual T-cell epitopes may also be conjugated to the targeting moiety using the chemical linker technology discussed in Section I.I below. In some embodiments, a different chemical linker may be chosen for these two conjugations so as to have additional control over the conjugation process. In one embodiment, T-cell epitopes may be affixed to the connecting stem by sulfo-SMCC and the connecting stem comprising the T-cell epitopes to the targeting moiety by 3-MPA, for example.

I. Method of Conjugation to the Targeting Moiety

[00111] Different approaches may be employed to conjugate the cleavage site(s) and plurality of T-cell epitopes (whether as separate T-cell epitope and cleavage site pairs or whether as a polytope) to the targeting agent. In some aspects, a polytope comprising a plurality of cleavage sites and a plurality of T-cell epitopes are conjugated to the targeting agent using at least one peptide bond. In certain aspects, the conjugation occurs through at least one bond other than a peptide bond. Such a conjugation may be through a chemical linker. A nonpeptide

bond, such as using a chemical linker, may be employed for separately conjugated pairs of T-cell epitopes and cleavage sites or for polytopes.

[00112] In some instances, a chemical linker may be a heterobifunctional crosslinking reagent. Heterobifunctional crosslinking reagents covalently bind two proteins together by targeting different functional groups on separate proteins. The amine-to-sulphydryl crosslinking reagents contain an NHS-ester at one end of the reagent, which binds to free amine groups on a first protein/peptide, predominantly on the amino acid lysine. At the other end of the reagent, a maleimide group binds to sulphydryl group of a second protein/peptide, which can be found on the amino acid cysteine. Examples of amine-to-sulphydryl crosslinking reagents may include (a) sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (Sulfo-SMCC, which is soluble in water) or succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC, which has poor water solubility), (b) succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB), (c) succinimidyl 6-[(beta-maleimidopropionamido)hexanoate] (SMPH); and (d) Sulfosuccinimidyl 6-(3'-[2-pyridyldithio]-propionamido) hexanoate (Sulfo-LC-SPDP); or (e) maleimide such as 3-maleimidopropionic acid.

[00113] Alternatively, another class of cross-linking reagents are the sulphydryl-to-carbohydrate reagents, which contain a maleimide group at one end and a hydrazide group at the other end. The maleimide group binds to a free sulphydryl on a first protein/peptide, such as that found on the amino acid cysteine, and the hydrazide group binds to an aldehyde group formed from an oxidized carbohydrate on a second protein/peptide. This family of reagents includes (a) 3,3'-N-[ε-Maleimidocaproic acid] hydrazide, trifluoroacetic acid salt (EMCH) and (b) N-beta-Maleimidopropionic acid hydrazide-trifluoroacetic acid (BMPH).

[00114] A third group of heterobifunctional cross-linking reagents are the photocleavable reagents including sulfosuccinimidyl 2-([4,4'-azipentanamido]ethyl)-1,3'-dithiopropionate (SDAD). These reagents have an NHS-ester at one end linking to free amines (lysine) and a diazine group at the other end which can react with any amino acid side chain or peptide backbone upon activation with long-wave ultra violet light (330-370nm). There are many

other reagents from these groups and from other cross-linking groups that can be used in this technology.

J. Inclusion of Human Protein Domains

[00115] In certain embodiments, the T-cell epitopes are flanked on one or both ends by at least one human protein domain. In some embodiments, the cleavage sites reside between the T-cell epitopes and the human protein domains.

[00116] Various human protein domains may be used in this manner. For example, at least one human protein domain may be a beta barrel or a coiled coil. In some embodiments, the human protein may be a FN3 (Fibronectin type III) domain. In some embodiments, the human protein may be beta-sandwich, Lipocalin, EETI-II/AGRP, Kunitz domain (BPTI), Thioredoxin, Protein A, Ankyrin, gamma-B-crystallin/ubiquitin, CTLD3/Tetranexin, or and LDLR-A. In some embodiments, the human protein may be human serum albumin, immunoglobulin CH2 domain, or camelid VHH domain.

[00117] In certain instances, a human protein domain may be a CL, CH2, and/or CH3 domain. For example, a T-cell epitope or plurality of T-cell epitopes may be flanked by CH2 sequences. Such CH2 sequences may be within the CH2 domain of an antibody or functional part serving as the targeting moiety. Such CH2 sequences may also be an additional CH2 domain distinct from the targeting moiety. In some embodiments, such as shown in Figure 2B, framework regions may be employed in a polytope to give additional three dimensional organization to the polytope.

[00118] In some aspects, the human protein domain is not a human immunoglobulin protein domain.

[00119] In some aspects, the human protein domain displays the T-cell epitopes on its three-dimensional surface.

II. Pharmaceutical Compositions

[00120] The TPECs may be employed as pharmaceutical compositions. As such, they may be prepared along with a pharmaceutically acceptable carrier. If parenteral administration is desired, for instance, the TPECs

may be provided in sterile, pyrogen-free water for injection or sterile, pyrogen-free saline. Alternatively, the TPECs may be provided in lyophilized form for resuspension with the addition of a sterile liquid carrier.

III. Methods of Treatment

A. Reduction of Unwanted Cells, Retargeting of Immune Response, and Treatment of Cancer

[00121] The TPECs described herein may be used in a method of treating a disease in a patient characterized by the presence of unwanted cells comprising administering a TPEC composition to the patient. This may include both treating and preventing a disorder. Additionally, the TPECs described herein may also be used in a method of retargeting (i.e., redirecting) a patient's own immune response to unwanted cells comprising administering a TPEC composition to the patient.

[00122] The amount of the agent administered to the patient may be chosen by the patient's physician so as to provide an effective amount to treat the condition in question.

[00123] The patient receiving treatment may be a human. The patient may be a primate or any mammal. Alternatively, the patient may be an animal, such as a domesticated animal (for example, a dog or cat), a laboratory animal (for example, a laboratory rodent, such as a mouse, rat, or rabbit), or an animal important in agriculture (such as horses, cattle, sheep, or goats).

[00124] The condition characterized by unwanted cells may include cancer. The cancer may be a solid or non-solid malignancy. The cancer may be any cancer such as breast cancer, ovarian cancer, endometrial cancer, cervical cancer, bladder cancer, renal cancer, melanoma, lung cancer, prostate cancer, testicular cancer, thyroid cancer, brain cancer, oesophageal cancer, gastric cancer, pancreatic cancer, colorectal cancer, liver cancer, leukaemia, myeloma, nonHodgkin's lymphoma, Hodgkin's lymphoma, acute myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphoblastic leukaemia, lymphoproliferative disorder, myelodysplastic disorder, myeloproliferative disease and premalignant disease.

[00125] The condition characterized by unwanted cells may also include an allergic or autoimmune disease. For instance, autoimmune diseases may include Addison's disease, celiac disease, dermatomyositis, Graves' disease, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, pernicious anemia, reactive arthritis, rheumatoid arthritis, Sjogren syndrome, and systemic lupus erythematosus.

[00126] The TPECs may be administered alone or in conjunction with other forms of therapy, including surgery, radiation, or traditional chemotherapy. In some embodiments, the activity of the TPECs may also be enhanced by boosting the patient's immune response against one or more of the T-cell epitopes used, such as by vaccinating the subject with the T-cell epitope or by administering immunostimulatory agents.

[00127] In some embodiments, the patient receives multiple doses of the composition over at least 30, 45, 60, 75, 90, 120, 150, or more days, or on an ongoing basis. In certain modes the patient receives multiple doses of the composition over at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or on an ongoing basis. If approaches are employed to reduce the patient's immune response against the TPEC itself, additional benefits may be achieved by administering the composition to a patient in relapse who received the composition for an earlier round of therapy.

B. Reduction of Immune Response Against TPEC Itself

[00128] In certain aspects described herein, having multiple copies of the same T-cell epitope increases an immune response against the unwanted cell when the agent is administered to a patient and having a diversity of T-cell epitopes assures effectiveness across a wide variety of patients from different racial and ethnic groups. Nevertheless, if a physician desires to use a TPEC therapy over a continued period of time, optional steps may be taken to reduce the patient's own immune response against the TPEC itself.

[00129] In certain embodiments, the arrangement or composition of T-cell epitopes on the TPEC are random in nature. For example, if a mixture of T-cell epitopes are conjugated using a chemical linker to the targeting moiety, different combinations of T-cell epitopes may affix to the targeting moieties. This

makes it significantly more difficult for the patient to mount an immune response against the TPECs themselves. Even in instances where the plurality of T-cell epitopes are the same, affixing them separately to the targeting moiety through a chemical linker may also result in a random placement of the T-cell epitopes along the targeting moiety, likewise damping the patient's own immune response against the TPEC.

Such approaches may ensure that the patient does not develop an immune response against the TPEC composition sufficient to inactivate the TPEC composition upon subsequent administrations, meaning that no more than about 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the composition is inactivated by the patient's own antibodies to the TPEC.

[00130] Immunogenicity of a composition may be measured in an ELISA assay using human serum. For example, test compositions may be immobilized on a 96-well plate, blocked and diluted serum samples incubated for 1 hour. Antibodies against the test composition present in the human sera may bind to the test compositions on the plate. Biotinylated test agent or a biotinylated secondary antibody such as an anti-human IgG/IgA/IgM in combination with streptavidin-HRP and TMB could be used to generate a signal.

[00131] The present compositions are expected to be at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% less immunogenic than prior compositions.

EXAMPLES

Example 1. Preparation of T-cell Epitopes and Cleavage Sites

[00132] Peptides comprising T-cell epitopes and cleavage sites were ordered from external companies who synthesized them in standard solid phase peptide synthesis. The peptides were dissolved in DMSO to a final concentration of 10mg/ml to be used for conjugation.

Example 2. Standard operating protocol for conjugation of polytope using sulfo-SMCC

[00133] The following procedure was used as a standard-operating protocol for conjugation of polytope peptide (comprising a plurality of T-cell

epitopes and cleavage sites) to an antibody serving as a targeting moiety by using sulfo-SMCC.

[00134] 1. Cysteinylated peptides dissolved in DMSO to final concentration of 10mg/ml.

[00135] 2. Weigh 1mg Sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (Sulfo-SMCC) and dissolve in 200µl phosphate buffered saline (PBS) (final concentration 5mg/ml).

[00136] 3. Add 100µl antibody (10mg/ml, 1mg antibody) to 10µl dissolved Sulfo-SMCC (5mg/ml) and incubate at room temperature for 30 minutes.

[00137] 4. Wash a ZebaSpin desalting column (Pierce) by firstly spinning the column at 1500g for 60 seconds to remove the ethanol (storage buffer).

[00138] 5. Add 300µl PBS and spin at 1500g for 60 seconds. Remove eluate and repeat a further three times.

[00139] 6. Add up to 125µl antibody-SMCC to each column, and centrifuge at 1500g for 120 seconds, collecting the eluate.

[00140] 7. To 100µl antibody-SMCC conjugate, add 3µl cysteinylated peptide (10mg/ml) and incubate at room temperature for 30 minutes.

[00141] 8. Wash a Protein A column (GE Healthcare) by firstly spinning the column at 100g for 30 seconds to remove the ethanol (storage buffer).

[00142] 9. Add 500µl PBS and spin at 100g for 30 seconds. Remove eluate and repeat a further two times.

[00143] 10. Dilute antibody-peptide conjugate to 500µl in PBS and add it to the protein A column, mixing the antibody with the beads. Leave at room temperature for 20 minutes, shaking.

[00144] 11. Spin the column at 100g and remove the eluate, the antibody-peptide complex should still be coupled to the beads.

[00145] 12. Wash the column by adding 500µl PBS and mixing the beads well before spinning at 100g for 30 seconds and removing eluate. Repeat this step a further three times.

[00146] 13. To elute the bound antibody, add 200µl 0.1M citric acid to the beads and incubate for 5 minutes at room temperature. Place column in a 2ml eppendorf containing 25µl 1M Tris pH9 and spin at 100g for 30 seconds; collect eluate.

[00147] 14. Optionally repeat step 13.

[00148] 15. Store antibody-peptide complex at 4°C.

Example 3. Standard operating protocol for production of a TPEC comprising separately-conjugated epitopes using sulfo-SMCC

[00149] The following procedure was used as a standard-operating protocol for separately conjugating a plurality of T-cell epitopes and their corresponding cleavage sites to an antibody serving as a targeting moiety by using sulfo-SMCC.

[00150] 1. Cysteinyated peptides dissolved in DMSO to final concentration of 10mg/ml.

[00151] 2. Weigh 1mg Sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (Sulfo-SMCC) and dissolve in 200µl phosphate buffered saline (PBS) (final concentration 5mg/ml).

[00152] 3. Add 100µl antibody (10mg/ml, 1mg antibody) to 10µl dissolved Sulfo-SMCC (5mg/ml) and incubate at room temperature for 30 minutes.

[00153] 4. Wash a ZebaSpin desalting column (Pierce) by firstly spinning the column at 1500g for 60 seconds to remove the ethanol (storage buffer).

[00154] 5. Add 300µl PBS and spin at 1500g for 60 seconds. Remove eluate and repeat a further three times.

[00155] 6. Add up to 125µl antibody-SMCC to each column, and centrifuge at 1500g for 120 seconds, collecting the eluate.

[00156] 7. Generate a mixture or required peptides in a tube so that there is a total of 30µg of total peptide. Add the peptide mixture to 100µl of antibody-SMCC complex and leave at room temperature for 30minutes.

[00157] 8. Wash a Protein A column (GE Healthcare) by firstly spinning the column at 100g for 30 seconds to remove the ethanol (storage buffer).

[00158] 9. Add 500µl PBS and spin at 100g for 30 seconds. Remove eluate and repeat a further two times.

[00159] 10. Dilute antibody-peptide conjugate to 500µl in PBS and add it to the protein A column, mixing the antibody with the beads. Leave at room temperature for 20 minutes, shaking.

[00160] 11. Spin the column at 100g and remove the eluate, the antibody-peptide complex should still be coupled to the beads.

[00161] 12. Wash the column by adding 500µl PBS and mixing the beads well before spinning at 100g for 30 seconds and removing eluate. Repeat this step a further three times.

[00162] 13. To elute the bound antibody, add 200µl 0.1M citric acid to the beads and incubate for 5 minutes at room temperature. Place column in a 2ml eppendorf containing 25µl 1M Tris pH9 and spin at 100g for 30 seconds; collect eluate.

[00163] 14. Optionally repeat step 13.

[00164] 15. Store antibody-peptide complex at 4°C.

Example 4. Standard operating protocol for production of polytope TPEC using 3-maleimidopropionic acid linked peptide

[00165] The following procedure was used as a standard-operating protocol for separately conjugating a polytope to an antibody serving as a targeting moiety by using 3-MPA.

[00166] 1. 3-maleimidopropioinc acid linked peptides dissolved in DMSO to final concentration of 10mg/ml.

[00167] 2. To 100µl antibody (diluted in PBS if required), add 3µl polytope peptide (10mg/ml) and incubate at room temperature for 30 minutes.

[00168] 3. Wash a Protein A column (GE Healthcare) by firstly spinning the column at 100g for 30 seconds to remove the ethanol (storage buffer).

[00169] 4. Add 500µl PBS and spin at 100g for 30 seconds. Remove eluate and repeat a further two times.

[00170] 5. Dilute antibody-peptide conjugate to 500µl in PBS and add it to the protein A column, mixing the antibody with the beads. Leave at room temperature for 20 minutes, shaking.

[00171] 6. Spin the column at 100g and remove the eluate, the antibody-peptide complex should still be coupled to the beads.

[00172] 7. Wash the column by adding 500µl PBS and mixing the beads well before spinning at 100g for 30 seconds and removing eluate. Repeat this step a further three times.

[00173] 8. To elute the bound antibody, add 200µl 0.1M citric acid to the beads and incubate for 5 minutes at room temperature. Place column in a 2ml eppendorf containing 25µl 1M Tris pH9 and spin at 100g for 30 seconds; collect eluate.

[00174] 9. Optionally repeat step 8.

[00175] 10. Store antibody-peptide complex at 4°C.

Example 5. Standard operating protocol for production of a TPEC comprising separately-conjugated epitopes using 3-maleimidopropionic acid linked peptide

[00176] The following procedure was used as a standard-operating protocol for separately conjugating a plurality of T-cell epitopes and their corresponding cleavage sites to an antibody serving as a targeting moiety by using 3-MPA.

[00177] 1. 3-maleimidopropionic acid linked peptides dissolved in DMSO to final concentration of 10mg/ml.

[00178] 2. Generate a mixture or required peptides in a tube so that there is a total of 30µg of total peptide, add to 100µl of antibody (diluted in PBS if required) and incubate at room temperature for 30 minutes.

[00179] 3. Wash a Protein A column (GE Healthcare) by firstly spinning the column at 100g for 30 seconds to remove the ethanol (storage buffer).

[00180] 4. Add 500µl PBS and spin at 100g for 30 seconds. Remove eluate and repeat a further two times.

[00181] 5. Dilute antibody-peptide conjugate to 500µl in PBS and add it to the protein A column, mixing the antibody with the beads. Leave at room temperature for 20 minutes, shaking.

[00182] 6. Spin the column at 100g and remove the eluate, the antibody-peptide complex should still be coupled to the beads.

[00183] 7. Wash the column by adding 500µl PBS and mixing the beads well before spinning at 100g for 30 seconds and removing eluate. Repeat this step a further three times.

[00184] 8. To elute the bound antibody, add 200µl 0.1M citric acid to the beads and incubate for 5 minutes at room temperature. Place column in a 2ml eppendorf containing 25µl 1M Tris pH9 and spin at 100g for 30 seconds; collect eluate.

[00185] Optionally repeat step 8.

[00186] Store antibody-peptide complex at 4°C.

Example 6. TPEC Compositions Comprising Two Cytomegalovirus Peptides and Evaluation in B-Lymphoblastoid Cells

[00187] We contacted B-lymphoblastoid cells (B-LCL) with an agent comprising Rituximab conjugated to two different cytomegalovirus peptides NLVPMVATV (SEQ ID NO: 1) and RIPHERNGFTVL (SEQ ID NO: 2) each with a cleavage site for cathepsin B. Subsequent exposure to peptide-specific T cells resulted in the generation of a T cell response to the B-LCL.

[00188] Rituximab was conjugated with the peptides NLVPMVATVASGV{CIT}GC (SEQ ID NO: 3) and RIPHERNGFTVLASGFKGC (SEQ ID NO: 4) at the following ratios from Table 3, wherein CIT represents citrulline, a type of amino acid that is similar to arginine.

Table 3: Peptide Ratios		
TPEC	NLVPMVATVASGV {CIT}GC (SEQ ID NO: 3)	RPIERNNGFTVLAS GFKGC (SEQ ID NO: 4)
1	0%	100%
2	10%	90%
3	20%	80%
4	30%	70%
5	40%	60%
6	50%	50%
7	60%	40%
8	70%	30%
9	80%	20%
10	90%	10%
11	100%	0%

[00189] After staining the cells with Rituximab conjugated to the mixture of peptides, labeled cells were washed and cultured overnight at 37°C in the presence of peptide-specific T cells. Supernatant was harvested and assayed for the presence of IFN- γ .

[00190] Using NLV-specific T cells, TPEC #11 was recognized strongly as seen by a large production of IFN- γ whereas TPEC #1 is not recognized at all as there was no production of IFN- γ . See Figure 3A. Conversely, using RPH-specific T cells TPEC #1 was recognized strongly as seen by a large production of IFN- γ whereas TPEC #11 is not recognized at all as there was no production of IFN- γ . See Figure 3B. There is production of IFN- γ by both NLV- and RPH-specific T cells towards TPEC #2-10, demonstrating the notion that conjugating more than one peptide to the antibody can allow one to engage with more than one specificity of peptide-specific T cell.

Example 7. TPEC Compositions Comprising Three Cytomegalovirus Peptides and Evaluation in B-Lymphoblastoid Cells

[00191] We contacted B-lymphoblastoid cells (B-LCL) with an agent comprising Rituximab conjugated to three different cytomegalovirus peptides VLEETSVML (SEQ ID NO: 5), BRVLBBYVL (SEQ ID NO: 6) and YILEETSVML (SEQ ID NO: 7) each with a cleavage site for cathepsin B.

Subsequent exposure to peptide-specific T cells resulted in the generation of a T cell response to the B-LCL.

[00192] Rituximab was conjugated with VLEETSVMLASGFKGC (SEQ ID NO: 8), BRVLBBYVLASGFKGC (SEQ ID NO: 9) where B is amino butyric acid, a homolog for cysteine, and YILEETSVMASGFKGC (SEQ ID NO: 10) at equal amounts of peptide. B-LCL cells were labeled with Rituximab TPEC and after incubation, washed to remove excess TPEC. Labeled target cells were incubated overnight at 37°C in the presence of VLE-specific, CRV-specific or YIL-specific T cells. Supernatant from the cultures was used to assess the presence of IFN- γ .

[00193] Results are shown in Figure 4. There was IFN- γ released by all of the different peptide-specific T cells in response to the TPEC-labeled target cells suggesting that each of the three conjugated peptides could be released from the antibody and used to stimulate peptide-specific T cells.

Example 8. TPEC Compositions Comprising A Single Peptide Having Two Different Cytomegalovirus Peptides and Evaluation in B-Lymphoblastoid Cells

[00194] We contacted B-lymphoblastoid cells (B-LCL) with an agent comprising Rituximab conjugated to a single peptide which comprised two different cytomegalovirus peptides: (i) NLVPMVATV (SEQ ID NO: 1) and RPHERNGFTVL (SEQ ID NO: 2) or (ii) NLVPMVATV (SEQ ID NO: 1) and VLEETSVML (SEQ ID NO: 5) each separated from the other by a cleavage site for cathepsin B. Subsequent exposure to NLV peptide-specific T cells resulted in the generation of a T cell response to the B-LCL.

[00195] Rituximab was conjugated with the peptides (i) CGVANLVPMVATVAVAVLEETSVML (SEQ ID NO: 11), (ii) CVARPHERNGFTVLVANLVPMVATV (SEQ ID NO: 12) and (iii) CGVANLVPMVATVARPHERNGFTVL (SEQ ID NO: 13). Target cells were labeled with TPEC and washed after incubation to remove excess TPEC. Target cells were cultured overnight at 37°C in the presence of NLV-specific T cells and the supernatant assayed for the presence of IFN- γ the following day.

[00196] NLV-specific T cells were able to recognize target cells labeled with each of the three TPECs tested as determined by IFN- γ release. This demonstrates the ability of a peptide containing more than one cytomegalovirus derived peptide, separated by a protease cleavage site, to stimulate peptide-specific T cells. Furthermore, the position of the NLVPMVATV (SEQ ID NO: 1) peptide in the polytope appears to affect the magnitude of the response with the NLV peptide being furthest from the antibody CVARPHERNGFTVLVANLVPMVATV (SEQ ID NO: 12) producing the largest response. However, both polytope peptides containing the NLV peptide closest to the antibody CGVANLVPMVATVAVAVLEETSVML (SEQ ID NO: 11) and CGVANLVPMVATVARPHERNGFTVL (SEQ ID NO: 13) also demonstrate a T cell response above background.

[00197] Results are shown in Figure 5.

Example 9. Preparation of a TPEC comprising polytope branched peptide

[00198] A TPEC comprising polytope branched peptide was prepared. A stem peptide that contains four azidonorleucine residues was provided along with peptides (branches) that contain propargyl glycine at the amino terminus (Figure 6A). Each branched peptide has a NH₂ cap on the C-terminal end which can aid in peptide serum stability. The sequence of the stem peptide was SEEZSEEZSEEZSEEZ, wherein Z is Azidonorleucine (SEQ ID NO: 166). For Branched TPEC 1, the sequences of the branched peptides were BKPAKFFRLTPRVTGGGAM-nh₂, wherein B is propargyl glycine (SEQ ID NO: 167), BKPAKFFRLRPHERNGFTVL-nh₂, wherein B is propargyl glycine (SEQ ID NO: 168), BKPAKFFRLRELRRKMMYM-nh₂, wherein B is propargyl glycine (SEQ ID NO: 169), and BKPAKFFRLNLVPMVATV-nh₂, wherein B is propargyl glycine (SEQ ID NO: 170).

[00199] And for Branched TPEC 3, the sequences of the branched peptides were BAIPVSLRTPRVTGGGAM-nh₂, wherein B is propargyl glycine (SEQ ID NO: 171), BAIPVSLRRPHERNGFTVL-nh₂, wherein B is propargyl glycine (SEQ ID NO: 172), BAIPVSLRELRRKMMYM-nh₂, wherein B is

propargyl glycine (SEQ ID NO: 173), and BAIPVSLVTEHDTLLY-nh₂, wherein B is propargyl glycine (SEQ ID NO: 174).

[00200] Incubation of equimolar concentrations of the branches with the stem peptide in DMSO in the presence of 10mg in 4ml of the CuSO₄·5H₂O catalyst mixed with 10mg in 4 ml ascorbic acid overnight resulted in the formation of the branched peptide used for conjugation. The final concentration of DMSO for the reaction was 50%. The branched peptides were purified using HPLC and verified using mass spectrometry (Figure 6B).

[00201] The branched peptide was conjugated to either Rituximab or Cetuximab by firstly reducing the antibody in the presence of approximately 0.1mM (tris(2-carboxyethyl)phosphine (TCEP) for 90 minutes. The branched peptide is then added at 3x the concentration as TCEP for 60 minutes and N-acetyl cysteine is added to quench the free peptide using the same concentration as the branched peptide for 30 minutes. The free peptide is removed using protein A sepharose beads to bind the TPEC whilst the free peptide is washed off. The TPEC is then collected from the protein A beads by eluting using 0.1M glycine and buffered using 1m Tris pH8.

Example 10. *In vitro* targeting of a transformed B cell line using TPEC comprising polytope branched peptide

[00202] Two agents comprised of Rituximab conjugated with a branched peptide consisting of four different viral epitopes, each linked to a single stem peptide. In Branched TPEC 1, each of the four peptides was separated from the stem peptide by an ADAM28 protease cleavage sequence. In Branched TPEC 3, each of the four peptides was separated from the stem peptide by an MMP2 cleavage sequence. The target cells were labelled with the Rituximab-TPEC before being incubated with CD8 T cells specific for one of two different viral epitopes. Specifically, TPR T cells were specific for the viral epitope TPRVTGGGAM (SEQ ID NO: 49) and this epitope was present in branches 1-1 and 3-1. RPH T cells were specific for the viral epitope RIPHERNGFTVL (SEQ ID NO: 2) and this epitope was present in branches 1-2 and 3-2.

[00203] Upon binding malignant cells (transformed B cell line), viral epitopes were released from the stem peptide by proteolytic cleavage releasing the viral epitopes TPRVTGGGAM (SEQ ID NO: 49), RIPHERNGFTVL (SEQ ID NO: 2), ELRRKMMYM (SEQ ID NO: 24) and VTEHDTLLY (SEQ ID NO: 53). Upon release, the peptides were presented on MHC class I molecules on the malignant cells where they could be recognized by the CD8 T cells. Recognition of malignant cells by the CD8 T cells was measured by release of IFN- γ by the T cells which can be used as a proxy for T cell killing.

[00204] Results are shown in Figures 7A and 7B. Target lymphoma cells labeled with branched peptide TPEC are recognized by T cells specific for the RIPHERNGFTVL (SEQ ID NO: 2) and TPRVTGGGAM (SEQ ID NO: 49) peptides, especially using the Branch 1 TPEC. Untreated target cells were not recognized by the peptide-specific T cells (negative control) whereas free peptide pulsed target cells were strongly recognized by T cells (positive control).

[00205] These results demonstrate that branched TPECs can obtain positive results in a widely-recognized model for retargeting a T cell response.

Example 11. *In vitro* targeting of ovarian carcinoma cell line using TPEC comprising polytope branched peptide

[00206] Two agents comprised of Cetuximab conjugated with a branched peptide consisting of four different viral epitopes, all linked to a single stem peptide was provided. In Branched TPEC 1, each of the four peptides was separated from the stem peptide by an ADAM28 protease cleavage sequence. In Branched TPEC 3, each of the four peptides was separated from the stem peptide by an MMP2 cleavage sequence. The target cells were labelled with the Cetuximab-TPEC before being incubated with CD8 T cells specific for one of two different viral epitopes. Specifically, TPR T cells were specific for the viral epitope TPRVTGGGAM (SEQ ID NO: 49) and this epitope was present in branches 1-1 and 3-1. RPH T cells were specific for the viral epitope RIPHERNGFTVL (SEQ ID NO: 2) and this epitope was present in branches 1-2 and 3-2.

[00207] Upon binding malignant cells (ovarian carcinoma cell line), viral epitopes were released from the stem peptide by proteolytic cleavage releasing the viral epitopes TPRVTGGGAM (SEQ ID NO: 49), RIPHERNGFTVL (SEQ ID NO: 2), ELRRKMMYM (SEQ ID NO: 24) and VTEHDTLLY (SEQ ID NO: 53). Upon release, the peptides were presented on MHC class I molecules on the malignant cells where they could be recognized by the CD8 T cells. Recognition of malignant cells by the CD8 T cells was measured by release of IFN- γ by the T cells.

[00208] Results are shown in Figures 8A and 8B. Target lymphoma cells labeled with branched peptide TPEC are recognized by T cells specific for the RIPHERNGFTVL (SEQ ID NO: 2) and TPRVTGGGAM (SEQ ID NO: 49) peptides, especially using the Branch 1 TPEC. Untreated target cells were not recognized by the peptide-specific T cells (negative control) whereas free peptide pulsed target cells were strongly recognized by T cells (positive control).

[00209] These results demonstrate that branched TPECs can obtain positive results in a widely-recognized model for retargeting a T cell response.

Example 12. *In vitro* targeting of ovarian carcinoma cell line using TPEC containing a bundled polytope domain

[00210] DNA for an agent comprising Cetuximab containing an additional fibronectin type 3 domain (from fibronectin 1 protein) attached to each heavy chain (depicted in Fig 2B) was synthesized (Figure 9A). The fibronectin type 3 domain was mutated at the “BC”, “DE” and “FG” loops to contain the viral epitopes TPRVTGGGAM (SEQ ID NO: 49), NLVPMVATV (SEQ ID NO: 1), and VLEETSVML (SEQ ID NO: 5). The viral epitopes were separated from the fibronectin type 3 domain by MMP2 protease cleavage sequences on both sides of the CMV epitope as depicted:

Cetuximab heavy chain--Domain--MMP2--Epitope--
MMP2--Domain--MMP2--Epitope--MMP2--Domain--
MMP2—Epitope

[00211] This would allow release of the viral epitopes from the bundled domain and targeting agent (Cetuximab) after proteolytic cleavage by

MMP2. The DNA for the bundled domain TPEC was transfected into Expi293 cells and the supernatant harvested 10 days post transfection. The bundled domain TPEC was purified from the supernatant using protein A sepharose beads. The target cells, ovarian carcinoma cell line, were labelled with the purified Cetuximab-TPEC with excess, unbound TPEC washed off. The labelled target cells were then incubated overnight in the presence of NLV-specific T cells.

[00212] Upon binding malignant cells (ovarian carcinoma cell line), viral epitopes were released from the additional protein domain by proteolytic cleavage. The released peptides were presented on MHC class I molecules on the malignant cells where they could be recognized by the CD8⁺ T cells. Recognition of malignant cells by the CD8⁺ T cells was measured by release of IFN- γ by the T cells.

[00213] Results are shown in Fig 9. Target ovarian carcinoma cells labeled with bundled domain TPEC were recognized by T cells specific for the NLVPMVATV (SEQ ID NO: 1) peptide from four different bundled domain TPEC preparations. Untreated target cells were not recognized by the peptide-specific T cells (negative control) whereas free peptide pulsed target cells were strongly recognized by T cells (positive control).

[00214] These results demonstrate that bundled domain TPECs can obtain positive results in a widely-recognized model for retargeting a T cell response.

Example 13. *In Vivo* Targeting of B Cell Lymphoma Using the Separately Conjugated T-Cell Epitopes (i.e., “hairbrush”) (e.g., chronic lymphocytic leukaemia)

[00215] An agent comprising Rituximab or an anti-CD22 antibody is conjugated with a peptide mixture containing equal parts of five different cytomegalovirus-derived peptides each containing a protease cleavage site specific for cathepsin B (i) NLVPMVATVASGV{CIT}GC (SEQ ID NO: 3), (ii) VLEETSVMLASGFKGC (SEQ ID NO: 8), (iii) RIPHERNGFTVLASGFKGC (SEQ ID NO: 4), (iv) YILEETSVMASGFKGC (SEQ ID NO: 10) and

(v) BRVLBBYVLASGFKGC (SEQ ID NO: 9). The patient is infused with the agent, which targets all B cells, healthy and malignant. Upon binding malignant cells, the agent comes into contact with proteases whereby cleavage of the protease recognition domain releases the T-cell epitopes (i) NLVPMVATV (SEQ ID NO: 1), (ii) VLEETSVML (SEQ ID NO: 5), (iii) RPHERNNGFTVL (SEQ ID NO: 2), (iv) YILEETSVM (SEQ ID NO: 7) and (v) BRVLBBYVL (SEQ ID NO: 6), which subsequently bind to HLA molecules on the surface of the malignant B cell. The malignant B cells expressing the T-cell epitopes are targeted by the host immune system for cytolysis by T cells.

Example 14. *In Vivo* Targeting of B-Cell Lymphoma Using a TPEC

Comprising Polytope (e.g., Chronic Lymphocytic Leukaemia)

[00216] An agent comprising Rituximab or an anti-CD22 antibody is conjugated with a peptide containing four different cytomegalovirus-derived epitopes (i)

CGSFRVTEHDTLLYGSFRRPHERNGFTVLGSFRELKRKMIYMGSFRN
LVPMVATV (SEQ ID NO: 14). Each of the four epitopes is separated from the next one by a protease cleavage site specific for cathepsin B in the conformation targeting agent–C-E₁-C-E₂-C-E₃-C-E₄ where C is protease cleavage and E is epitope. The patient is infused with the agent, which targets all B cells, healthy and malignant. Upon binding malignant cells, the agent comes into contact with proteases whereby cleavage of the protease recognition domain releases the T-cell epitopes (i) NLVPMVATV (SEQ ID NO: 1), (ii) VTEHDTLLY (SEQ ID NO: 53), (iii) RPHERNNGFTVL (SEQ ID NO: 2), and (iv) ELKRKMIYM (SEQ ID NO: 23), which subsequently bind to HLA molecules on the surface of the malignant B cell. The cells expressing the T-cell epitopes are then targeted by the host immune system for cytolysis by T cells.

Example 15. *In Vivo* Targeting of B Cell Lymphoma Using the Separately Conjugated T-Cell Epitopes (i.e., “hairbrush”) (e.g., chronic lymphocytic leukaemia)

[00217] An agent comprising Rituximab or an anti-CD22 antibody is conjugated with a peptide mixture containing equal parts of five different

cytomegalovirus-derived peptides each containing a protease cleavage site specific for either ADAM28 or cathepsin D (i) CKPAKFFRLNLVPMVATV (SEQ ID NO: 58), (ii) CKPAKFFRLRPHERNGFTVL (SEQ ID NO: 59), (iii) CPRSFFRLGKVLEETSVML (SEQ ID NO: 60), (iv) CKPAKFFRLELELRKMIYM (SEQ ID NO: 61) and (v) CPRSFFRLGKQIKVRVDMV (SEQ ID NO: 62). The patient is infused with the agent, which targets all B cells, healthy and malignant. Upon binding malignant cells, the agent comes into contact with proteases whereby cleavage of the protease recognition domain releases the T-cell epitopes (i) NLVPMVATV (SEQ ID NO: 1), (ii) RPHERNGFTVL (SEQ ID NO: 2), (iii) VLEETSVML (SEQ ID NO: 5), (iv) ELKRKMIYM (SEQ ID NO: 23) and (v) QIKVRVDMV (SEQ ID NO: 38), which subsequently bind to HLA molecules on the surface of the malignant B cell. The malignant B cells expressing the T-cell epitopes on their HLA molecules at the cell surface are targeted by the host immune system for cytolysis by T cells.

**Example 16. *In Vivo* Targeting of B-Cell Lymphoma Using a TPEC
Comprising Polytope (e.g., Chronic Lymphocytic Leukaemia)**

[00218] An agent comprising Rituximab or an anti-CD22 antibody is conjugated with a peptide containing four different cytomegalovirus-derived epitopes (i) CGSKPAKFFRLYSEHPTFTSQYGSFRSFFRLGKTPRVTGGGAMG SKPAKFFRLQIKVRVDMVGSPRSFFRLGKELRRKMMYM (SEQ ID NO: 63). Each of the four epitopes is separated from the next one by a protease cleavage site specific for either ADAM28 or cathepsin D in a potential conformation, but not limited to the conformation, Targeting agent—C_i-E₁-C_{ii}-E₂-C_i-E₃-C_{ii}-E₄ where C is protease cleavage and E is epitope. The patient is infused with the agent, which targets all B cells, healthy and malignant. Upon binding malignant cells, the agent comes into contact with proteases whereby cleavage of the protease recognition domain releases the T-cell epitopes (i) YSEHPTFTSQY (SEQ ID NO: 55), (ii) TPRVTGGGAM (SEQ ID NO: 49), (iii) QIKVRVDMV (SEQ ID NO: 38), and (iv) ELRRKMMYM (SEQ ID NO: 24), which

subsequently bind to HLA molecules on the surface of the malignant B cell. The cells expressing the T-cell epitopes are then targeted by the host immune system for cytolysis by T cells.

Example 17. Embodiments Described Herein

[00219] Various embodiments are described in the following nonlimiting items.

Item 1. A composition for retargeting an immune response to unwanted cells comprising a TPEC wherein:

- a. T is a targeting moiety that is capable of targeting unwanted cells;
- b. L is at least one linker capable of linkage to T, where L may be a peptide bond, at least one peptide, or a chemical linker;
- c. C is at least one cleavage site
 - i. cleaved by an enzyme expressed by the unwanted cells;
 - ii. cleaved through a pH-sensitive cleavage reaction inside the unwanted cell;
 - iii. cleaved by a complement-dependent cleavage reaction; or
 - iv. cleaved by a protease that is colocalized to the unwanted cell by a targeting moiety that is the same or different from the targeting moiety in the TPEC; and
- d. E is at least one T-cell epitope,

wherein the L, C, and E moieties are arranged in at least one of the following patterns:

- i. a plurality of L-C-E, each attached separately to T,
- ii. at least one of L-(C-E)_n, n is an integer of at least 2 (optionally from about 2 to 50) and with each C-E attached to the L in parallel, and/or
- iii. at least one of L-(C-E)_n, n is an integer of at least 2 (optionally from about 2 to 50) and with each C-E attached to the L in series.

Item 2. A composition for retargeting an immune response to unwanted cells comprising a TPEC comprising the formula $T-(L-C-E)_n$ or $T-(L-C_i-E_j)_n$, wherein:

- a. T is a targeting moiety that is capable of targeting unwanted cells;
- b. L is a linker capable of chemical linkage to T;
- c. C is a cleavage site
 - i. cleaved by an enzyme expressed by the unwanted cells;
 - ii. cleaved through a pH-sensitive cleavage reaction inside the unwanted cell;
 - iii. cleaved by a complement-dependent cleavage reaction; or
 - iv. cleaved by a protease that is colocalized to the unwanted cell by a targeting moiety that is the same or different from the targeting moiety in the TPEC; and
- d. E is a T-cell epitope

wherein n is an integer of at least 2 (optionally from about 2 to 50), i is an integer of at least 1 (optionally from about 1 to 50), and j is an integer of at least 2 (optionally from about 1 to 50).

Item 3. A composition for retargeting an immune response to unwanted cells comprising a TPEC comprising the formula $T-L-(C_i-E_j)_n$, wherein:

- a. T is a targeting moiety that is capable of targeting unwanted cells;
- b. L is an optional linker capable of chemical or peptide linkage to T;
- c. C is a cleavage site
 - i. cleaved by an enzyme expressed by the unwanted cells;
 - ii. cleaved through a pH-sensitive cleavage reaction inside the unwanted cell;
 - iii. cleaved by a complement-dependent cleavage reaction; or
 - iv. cleaved by a protease that is colocalized to the unwanted cell by a targeting moiety that is the same or different from the targeting moiety in the TPEC; and
- d. E is a T-cell epitope;

wherein n is an integer of at least 2 (optionally from about 2 to 50), i is an integer of at least 1 (optionally from about 1 to 50), and j is an integer of at least 1 (optionally from about 1 to 50).

Item 4. A composition for retargeting an immune response to unwanted cells comprising a TPEC having:

- a. a targeting moiety that is capable of targeting unwanted cells;
- b. a plurality of more than 10 T-cell epitopes conjugated to the targeting moiety with at least one cleavage site, wherein the cleavage site is
 - i. cleaved by an enzyme expressed by the unwanted cells;
 - ii. cleaved through a pH-sensitive cleavage reaction inside the unwanted cell;
 - iii. cleaved by a complement-dependent cleavage reaction; or
 - iv. cleaved by a protease that is colocalized to the unwanted cell by a targeting moiety that is the same or different from the targeting moiety in the TPEC.

Item 5. The composition of any one of items 1, 2, or 4, wherein the plurality of T-cell epitopes are individually conjugated to the targeting moiety, each T-cell epitope by a cleavage site.

Item 6. The composition of any one of items 1, 3, or 4, wherein the plurality of T-cell epitopes are conjugated as at least one bundle to the targeting moiety by one cleavage site.

Item 7. The composition of item 6, wherein the T-cell epitopes within a bundle have cleavage sites between them.

Item 8. The composition of any one of items 1, 3-4, 6-7, wherein at least two groups of a plurality of T-cell epitopes are conjugated to the targeting moiety, each group by one cleavage site.

Item 9. The composition of any one of items 1-8, wherein the plurality of T-cell epitopes are not all identical.

Item 10. The composition of any one of items 1-8, wherein the plurality of T-cell epitopes are the same.

Item 11. The composition of any one of items 1-8, wherein some the T-cell epitopes are the same and further wherein some are different.

Item 12. The composition of any one of items 1-11, wherein the agent has a plurality of cleavage sites that are the same.

Item 13. The composition of any one of items 1-11, wherein the agent has a plurality of cleavage sites that are not all identical.

Item 14. The composition of any one of items 1, 3-4, and 6-13, wherein the at least one cleavage site and the plurality of T-cell epitopes are conjugated to the targeting agent using at least one peptide bond.

Item 15. The composition of any one of items 1-13, wherein the at least one cleavage site and the plurality of T-cell epitopes are conjugated to the targeting agent through at least one bond other than a peptide bond.

Item 16. The composition of item 15, wherein the at least one cleavage site and the plurality of T-cell epitopes are conjugated to the targeting agent through a Sulfo-SMCC, SMCC, or maleimide linkage.

Item 17. The composition of any one of items 1-16 wherein at least one T-cell epitope is an MHC Class I restricted peptide.

Item 18. The composition of any one of items 1-17, wherein at least one T-cell epitope is an MHC Class II restricted peptide.

Item 19. The composition of any one of items 1-18, wherein the plurality of T-cell epitopes are from about 7 to about 14 amino acids in length.

Item 20. The composition of any one of items 1-19, wherein the plurality of T cell epitopes are about 8 to 13, about 9 to 12, about 9, or about 10 amino acids in length.

Item 21. The composition of any one of items 1-20, wherein the agent comprises about or at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 T cell epitopes, optionally from 2 to 100 T cell epitopes.

Item 22. The composition of any one of items 1-21, wherein the T-cell epitopes are chosen from CMV, influenza, EBV, hepatitis, chicken pox, mumps, measles, diphtheria, Haemophilus influenzae, rubella, pertussis, polio, pneumococcus, rotavirus, tetanus, vaccinia, and yellow fever T-cell epitopes.

Item 23. The composition of any one of items 1-22, wherein the composition comprises T-cell epitopes from at least two different infectious agents.

- Item 24. The composition of any one of items 1-23, wherein the TPEC comprises at least one CMV T-cell epitope.
- Item 25. The composition of any one of items 1-9 and 11-24, wherein using a plurality of different T-cell epitopes allows the agent to stimulate a T-cell response in at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the human population.
- Item 26. The composition of any one of items 1-25, wherein the T-cell epitopes are chosen from HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, CD1d, and MR1.
- Item 27. The composition of any one of items 1-26, wherein the T-cell epitopes are chosen from HLA-A*01, HLA-A*02, HLA-A*03, HLA-A*11, HLA-B*44, HLA-B*07, HLA-B*08, HLA-B*15, HLA-B*35, HLA-B*40, HLA-C*07, HLA-C*03, HLA-C*05, HLA-C*04, HLA-C*06, and HLA-E*0101 restricted antigens.
- Item 28. The composition of any one of items 1-9 and 11-27, wherein the composition comprises at least the following T-cell epitopes: HLA-A*02, HLA-A*01, and HLA-A*03.
- Item 29. The composition of any one of items 1-28, wherein having multiple copies of the same T-cell epitope increases an immune response against the unwanted cell when the agent is administered to a subject.
- Item 30. The composition of any one of items 1-29, wherein the T-cell epitopes comprise at least one of SEQ ID NOS: 1-2, 5-7, 15-57.
- Item 31. The composition of any one of items 1-30, wherein the targeting moiety targets the unwanted cells by specifically binding to the unwanted cells.
- Item 32. The composition of any one of items 1-32, wherein the targeting moiety specifically binds the unwanted cells even while the plurality of T-cell epitopes are conjugated to the targeting moiety.
- Item 33. The composition of any one of items 1-32, wherein the targeting moiety is an antibody or functional part thereof.
- Item 34. The composition of any one of items 1-33, wherein the T-cell epitopes are flanked on one or both ends by at least one human protein domain.

- Item 35. The composition of items 34, wherein at least one human protein domain is a beta barrel or a coiled coil.
- Item 36. The composition of any one of items 34-35, wherein the human protein domain is a CL, CH2, and/or CH3 domain.
- Item 37. The composition of any one of items 34-35, wherein the human protein domain is a FN3 domain, beta-sandwich, Lipocalin, EETI-II/AGRP, Kunitz domain (BPTI), Thioredoxin, Protein A, Ankyrin, gamma-B-crystallin/ubiquitin, CTLD3/Tetranexin LDLR-A, human serum albumin, immunoglobulin CH2 domain, or camelid VHH domain.
- Item 38. The composition of any one of item 34-35 or 37, wherein the human protein domain is not a human immunoglobulin protein domain.
- Item 39. The composition of any one of items 34-38, wherein the human protein domain displays the T-cell epitopes on its three-dimensional surface.
- Item 40. The composition of any one of items 1-39, wherein the cleavage site is capable of being cleaved outside the unwanted cells.
- Item 41. The composition of any one of items 1-40, wherein the enzyme expressed by the unwanted cells is a protease.
- Item 42. The composition of any one of items 1-41, wherein the unwanted cells are cancer cells.
- Item 43. A composition comprising a plurality of different TPECs of any one of items 1-42, wherein each targeting agent is conjugated to a plurality of identical T-cell epitopes.
- Item 44. A composition comprising a plurality of compositions of any one of items 1-42, wherein at least some of the targeting agents in the composition are conjugated to a plurality of T-cell epitopes that are not identical.
- Item 45. A composition comprising a plurality of compositions of item 44, wherein at least some of the compositions are not identical.
- Item 46. A composition comprising a plurality of compositions of any one of items 43-45, wherein the composition comprises at least about 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or more different TPECs.
- Item 47. A pharmaceutical composition comprising a composition of any one of items 1-46 and a pharmaceutically acceptable carrier.

Item 48. A method of treating a disease in a patient characterized by the presence of unwanted cells comprising administering a composition of any one of items 1-47 to the patient.

Item 49. A method of retargeting an immune response of a patient to unwanted cells comprising administering a composition of any one of items 1-47 to the patient.

Item 50. The method of any of items 48-49, wherein the patient does not develop an immune response against the composition sufficient to inactivate the composition.

Item 51. The method of any one of items 48-50, wherein the patient receives multiple doses of the composition over at least 30, 45, 60, 75, 90, 120, 150, or more days or on an ongoing basis.

Item 52. The method of any one of items 48-50, wherein the patient receives multiple doses of the composition over at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or on an ongoing basis.

Item 53. The method of any one of items 48-52, wherein the composition can be administered to a patient in relapse who received the composition for an earlier round of therapy.

Item 54. The method of any one of items 48-53, wherein the patient has cancer.

EQUIVALENTS

[00220] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the embodiments. The foregoing description and Examples detail certain embodiments and describes the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the embodiment may be practiced in many ways and should be construed in accordance with the appended claims and any equivalents thereof.

[00221] As used herein, the term about refers to a numeric value, including, for example, whole numbers, fractions, and percentages, whether or not explicitly indicated. The term about generally refers to a range of numerical values

(e.g., $\pm 5-10\%$ of the recited range) that one of ordinary skill in the art would consider equivalent to the recited value (e.g., having the same function or result). When terms such as at least and about precede a list of numerical values or ranges, the terms modify all of the values or ranges provided in the list. In some instances, the term about may include numerical values that are rounded to the nearest significant figure.

AMENDED CLAIMS**received by the International Bureau on 02 August 2016 (02.08.2016)****What is Claimed is:**

1. A composition for retargeting an immune response to unwanted cells comprising a TPEC wherein:

- a. T is a targeting moiety that is capable of targeting unwanted cells;
- b. L is at least one linker capable of linkage to T where L is a peptide bond, at least one peptide, or a chemical linker;
- c. C is at least one cleavage site
 - i. cleaved by an enzyme expressed by the unwanted cells;
 - ii. cleaved through a pH-sensitive cleavage reaction inside the unwanted cell;
 - iii. cleaved by a complement-dependent cleavage reaction; or
 - iv. cleaved by a protease that is colocalized to the unwanted cell by a targeting moiety that is the same or different from the targeting moiety in the TPEC; and
- d. E is at least one T-cell epitope,

wherein the L, C, and E moieties are arranged in at least one of the following patterns:

- i. a plurality of L-C-E, each attached separately to T,
- ii. at least one of L-(C-E)_n, wherein n is an integer of at least 2 (optionally from about 2 to 50) and with each C-E attached to the L in parallel, and/or
- iii. at least one of L-(C-E)_n, wherein n is an integer of at least 2 (optionally from about 2 to 50) and with each C-E attached to the L in series.

2. A composition for retargeting an immune response to unwanted cells comprising a TPEC having:

- a. a targeting moiety that is capable of targeting unwanted cells;
- b. a plurality of more than 10 T-cell epitopes conjugated to the targeting moiety with at least one cleavage site, wherein the cleavage site is
 - i. cleaved by an enzyme expressed by the unwanted cells;
 - ii. cleaved through a pH-sensitive cleavage reaction inside the unwanted cell;

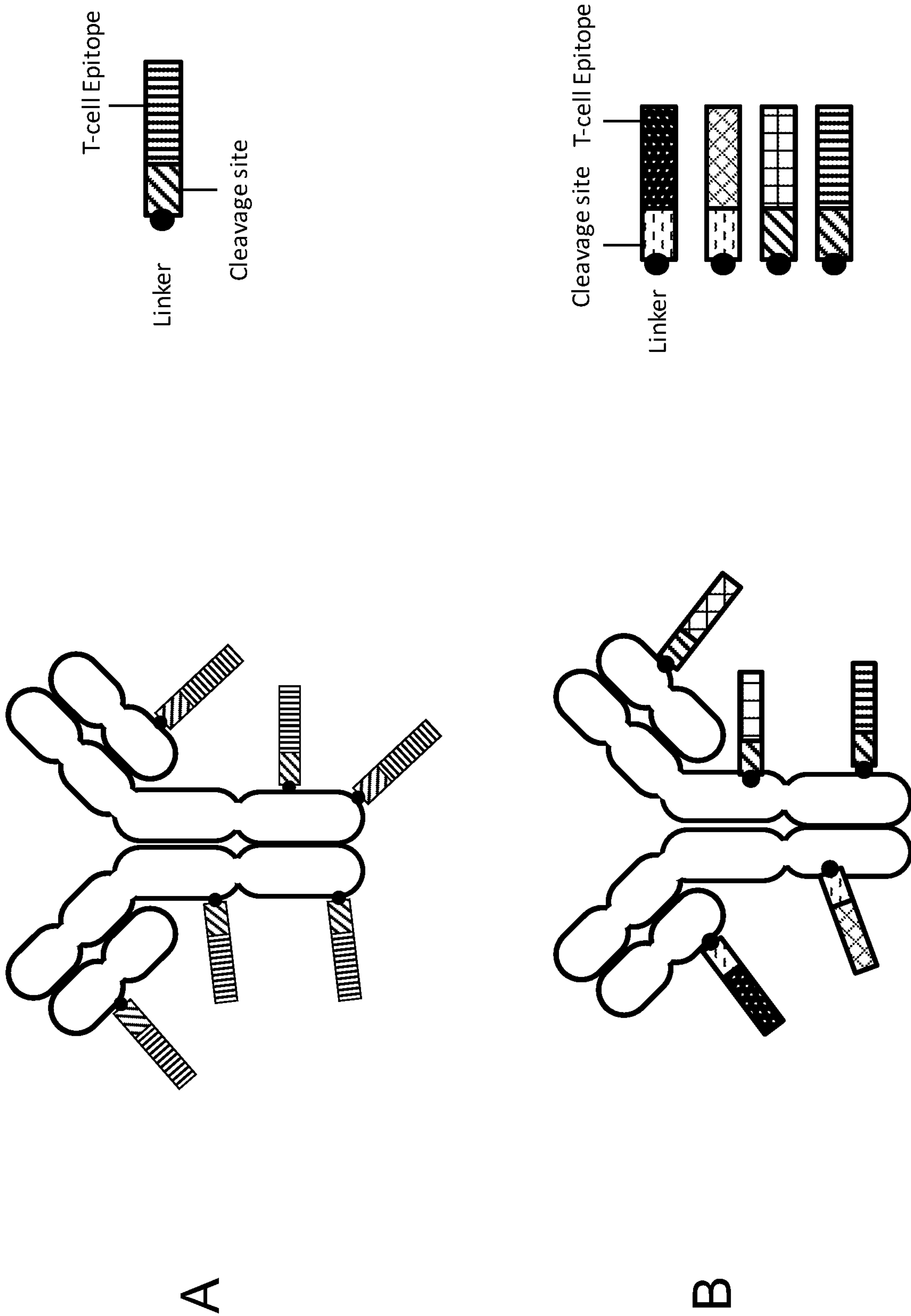
- iii. cleaved by a complement-dependent cleavage reaction; or
 - iv. cleaved by a protease that is colocalized to the unwanted cell by a targeting moiety that is the same or different from the targeting moiety in the TPEC.
3. The composition of claim 1, wherein the plurality of T-cell epitopes are individually conjugated to the targeting moiety, each T-cell epitope by a cleavage site.
 4. The composition of claim 1, wherein the plurality of T-cell epitopes are conjugated as at least one bundle to the targeting moiety by one cleavage site.
 5. The composition of claim 1, wherein the T-cell epitopes within a bundle have cleavage sites between them.
 6. The composition of claims 1, wherein the plurality of T-cell epitopes are not all identical.
 7. The composition of claim 1, wherein the at least one cleavage site and the plurality of T-cell epitopes are conjugated to the targeting agent through a Sulfo-SMCC, SMCC, or maleimide linkage.
 8. The composition of claim 1, wherein at least one T-cell epitope is an MHC Class I restricted peptide.
 9. The composition of claim 1, wherein at least one T-cell epitope is an MHC Class II restricted peptide.
 10. The composition of claim 1, wherein the plurality of T-cell epitopes are from about 7 to about 14 amino acids in length.
 11. The composition of claim 1, wherein the agent comprises at least about 2, 3, 4, 5, 6, 7, 8, 9, or 10 T cell epitopes.
 12. The composition of claim 1, wherein the T-cell epitopes are chosen from CMV, influenza, EBV, hepatitis, chicken pox, mumps, measles, diphtheria, Haemophilus influenzae, rubella, pertussis, polio, pneumococcus, rotavirus, tetanus, vaccinia, and yellow fever T-cell epitopes.
 13. The composition of claim 1, wherein the composition comprises T-cell epitopes from at least two different infectious agents.

14. The composition of claim 1, wherein the T-cell epitopes are chosen from HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, CD1d, and MR1.
15. The composition of claim 1, wherein the T-cell epitopes are chosen from HLA-A*01, HLA-A*02, HLA-A*03, HLA-A*11, HLA-B*44, HLA-B*07, HLA-B*08, HLA-B*15, HLA-B*35, HLA-B*40, HLA-C*07, HLA-C*03, HLA-C*05, HLA-C*04, HLA-C*06, and HLA-E*0101 restricted antigens.
16. The composition of claim 1, wherein the composition comprises at least the following T-cell epitopes: HLA-A*02, HLA-A*01, and HLA-A*03.
17. The composition of claim 1, wherein the T-cell epitopes comprise at least one of SEQ ID NOS: 1-2, 5-7, 15-57.
18. The composition of claim 1, wherein the targeting moiety is an antibody or functional part thereof.
19. The composition of claim 1, wherein the T-cell epitopes are flanked on one or both ends by at least one human protein domain.
20. The composition of claim 1, wherein the cleavage site is capable of being cleaved outside the unwanted cells.
21. The composition of claim 1, wherein the enzyme expressed by the unwanted cells is a protease.
22. The composition of claim 1, wherein the unwanted cells are cancer cells.
23. A method of treating a disease in a patient characterized by the presence of unwanted cells comprising administering a composition of claim 1 to the patient.
24. A method of retargeting an immune response of a patient to unwanted cells comprising administering a composition of claim 1 to the patient.
25. The composition of claim 18, wherein the antibody or functional part thereof is an anti-CEA or anti-CEACAM antibody or functional part thereof.
26. The composition of claim 2, wherein the targeting moiety is an antibody or functional part thereof.
27. The composition of claim 26, wherein the antibody or functional part thereof is an anti-CEA or anti-CEACAM antibody or functional part thereof.

28. The method of claim 23, wherein the targeting moiety is an antibody or functional part thereof and the antibody or functional part thereof is optionally an anti-CEA or anti-CEACAM antibody or functional part thereof.

29. The method of claim 24, wherein the targeting moiety is an antibody or functional part thereof and wherein the antibody or functional part thereof is optionally an anti-CEA or anti-CEACAM antibody or functional part thereof.

Figs. 1A-B



Figs. 2A-B

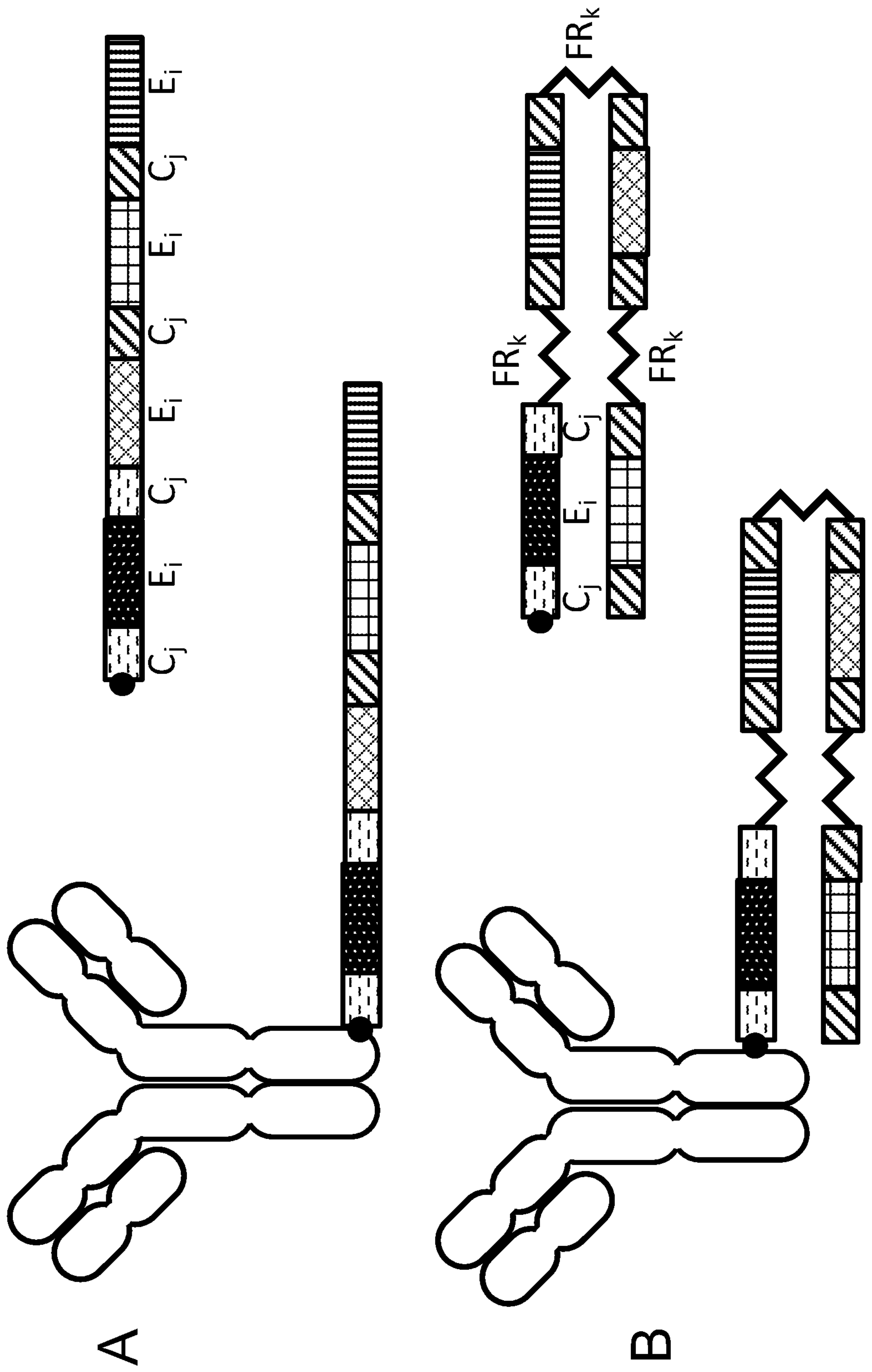
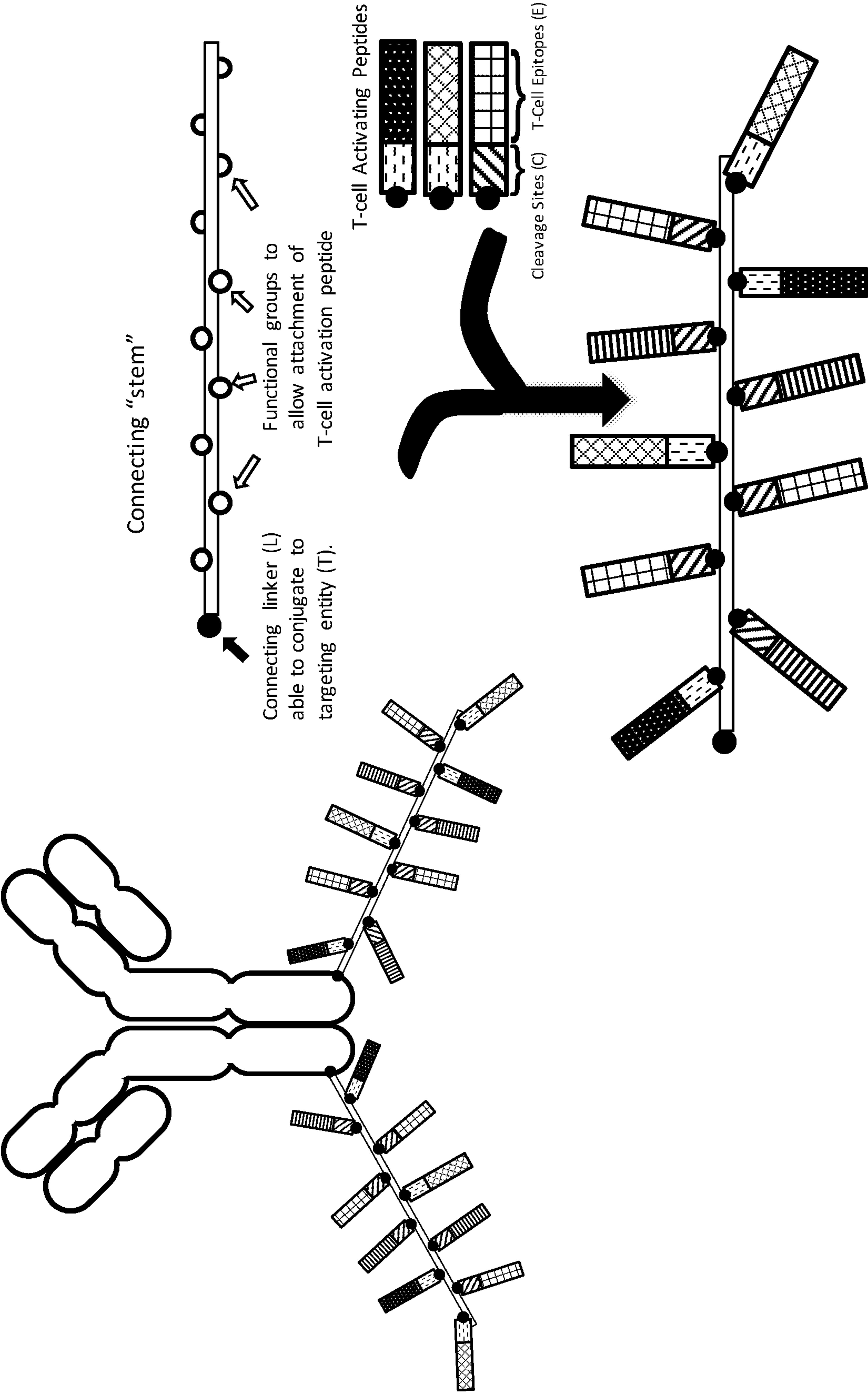


Fig. 2C



Figs. 3A-B

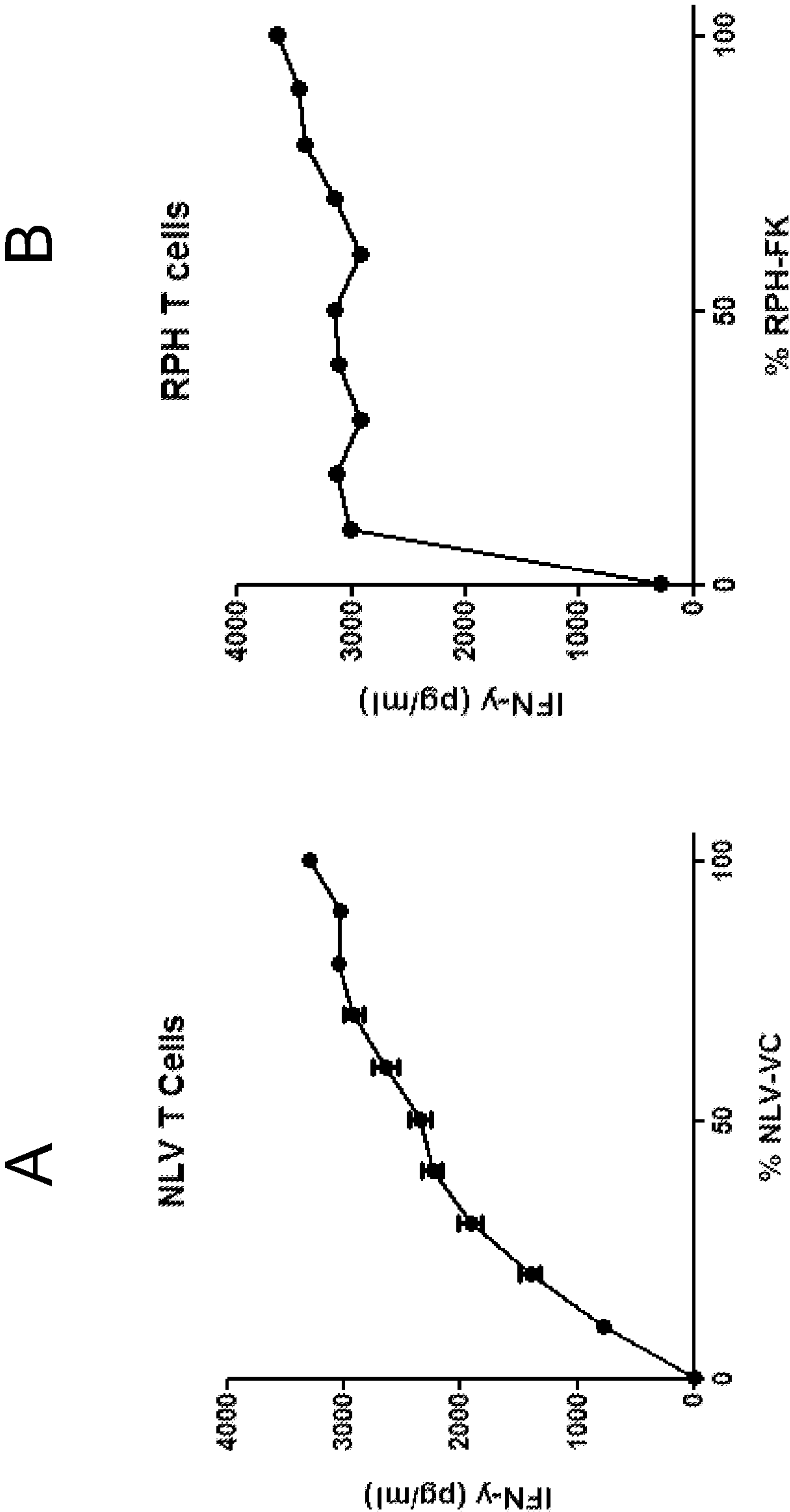


Fig. 3C

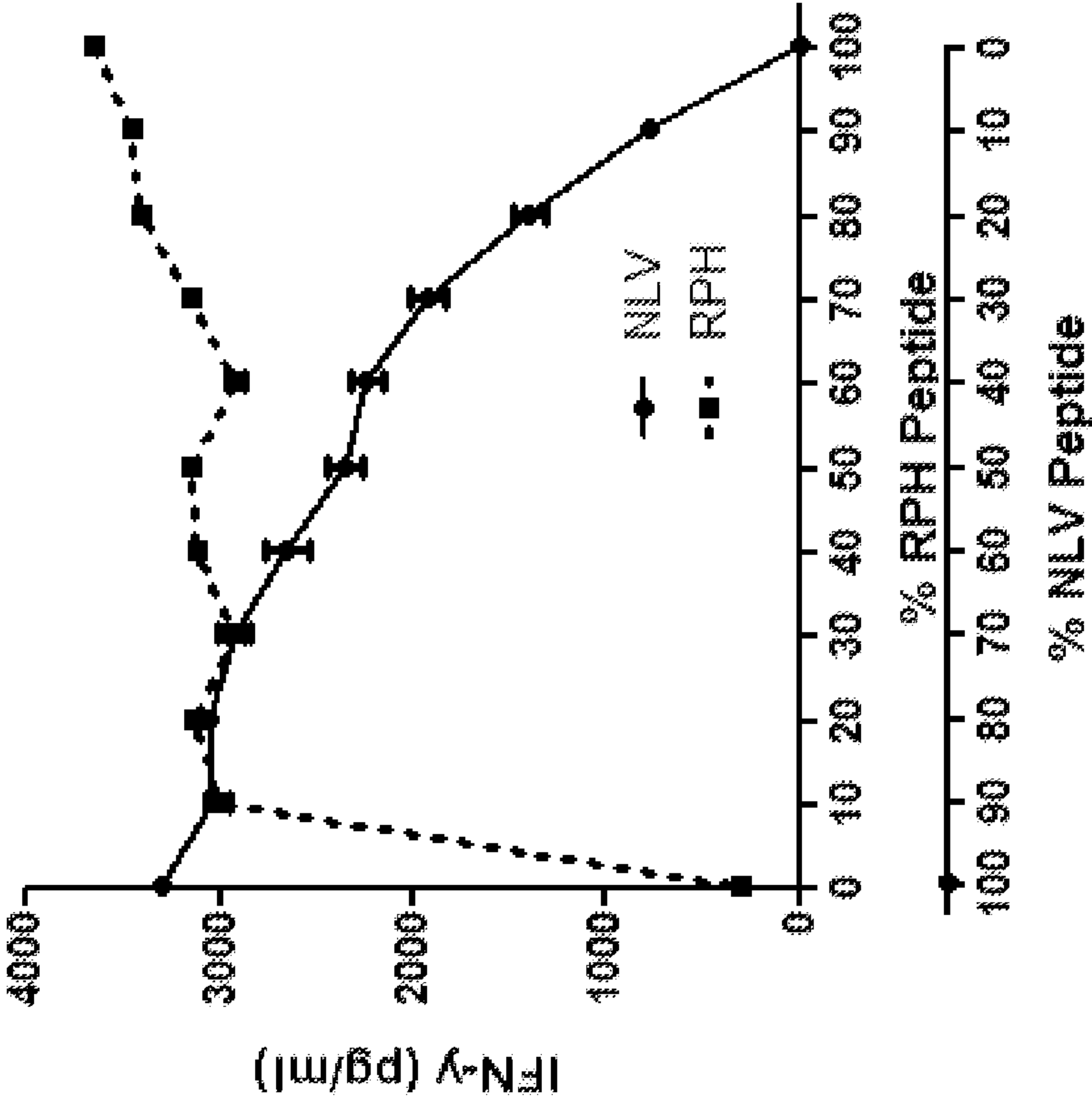


Fig. 4

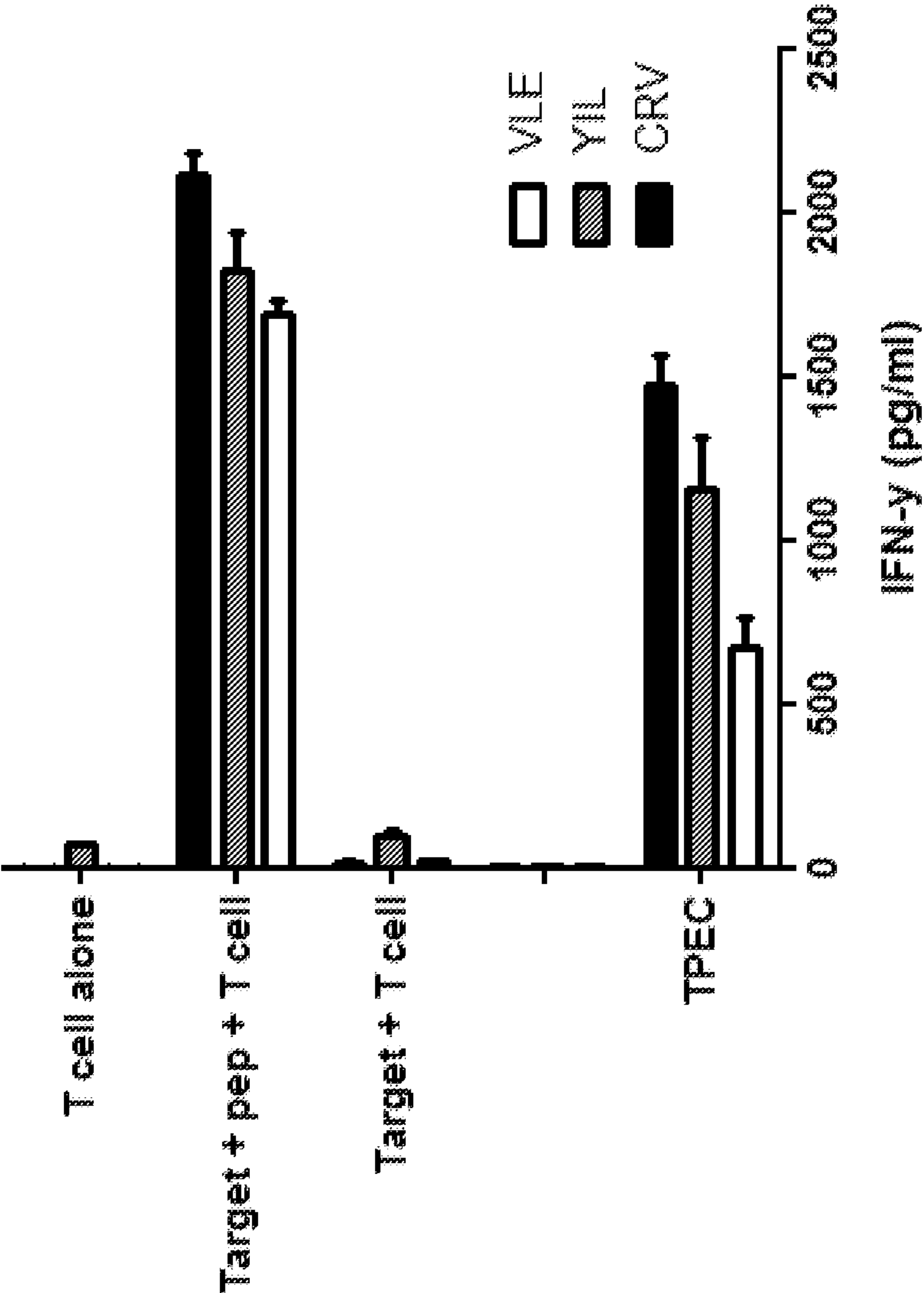


Fig. 5

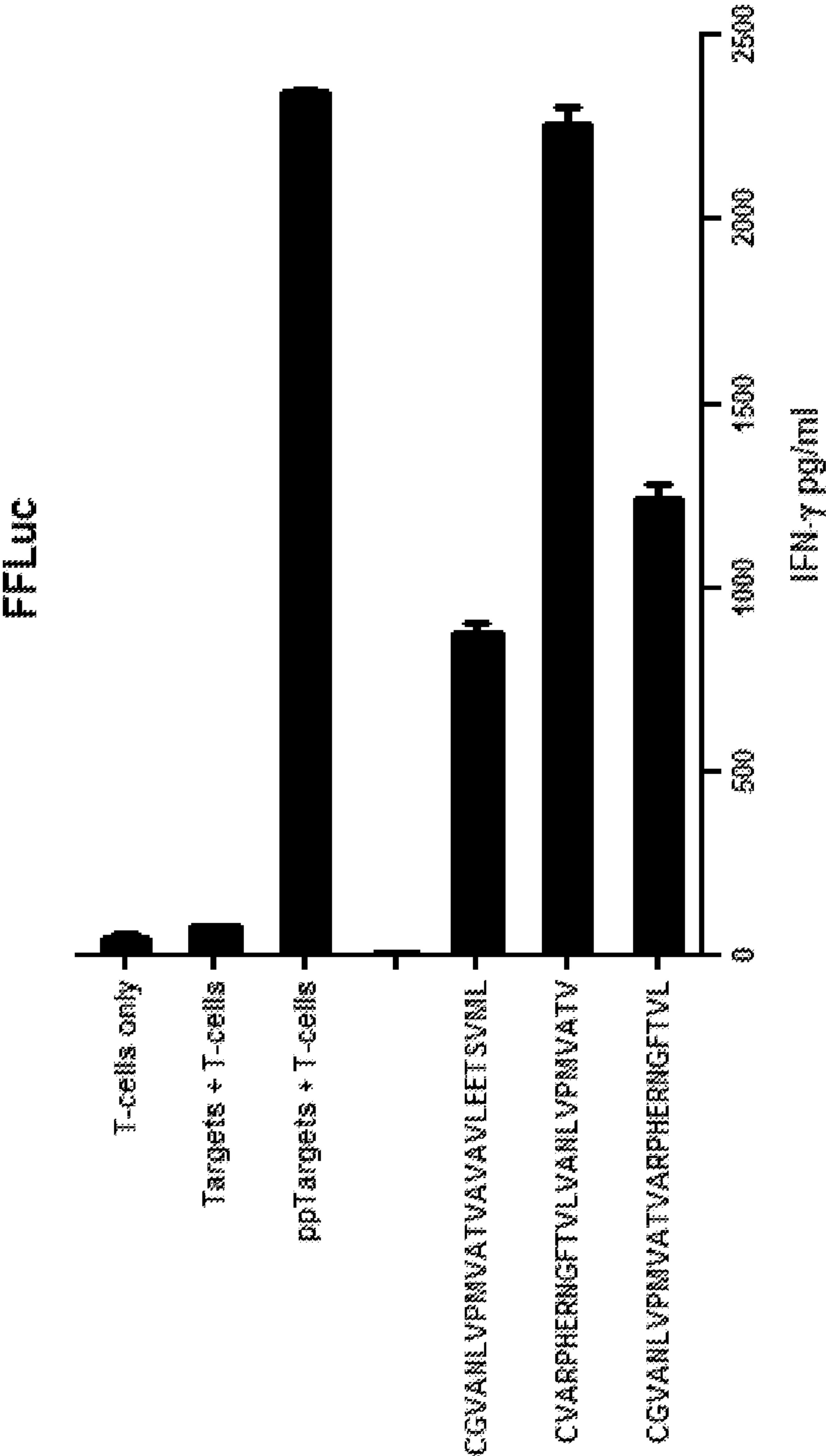


Fig. 6

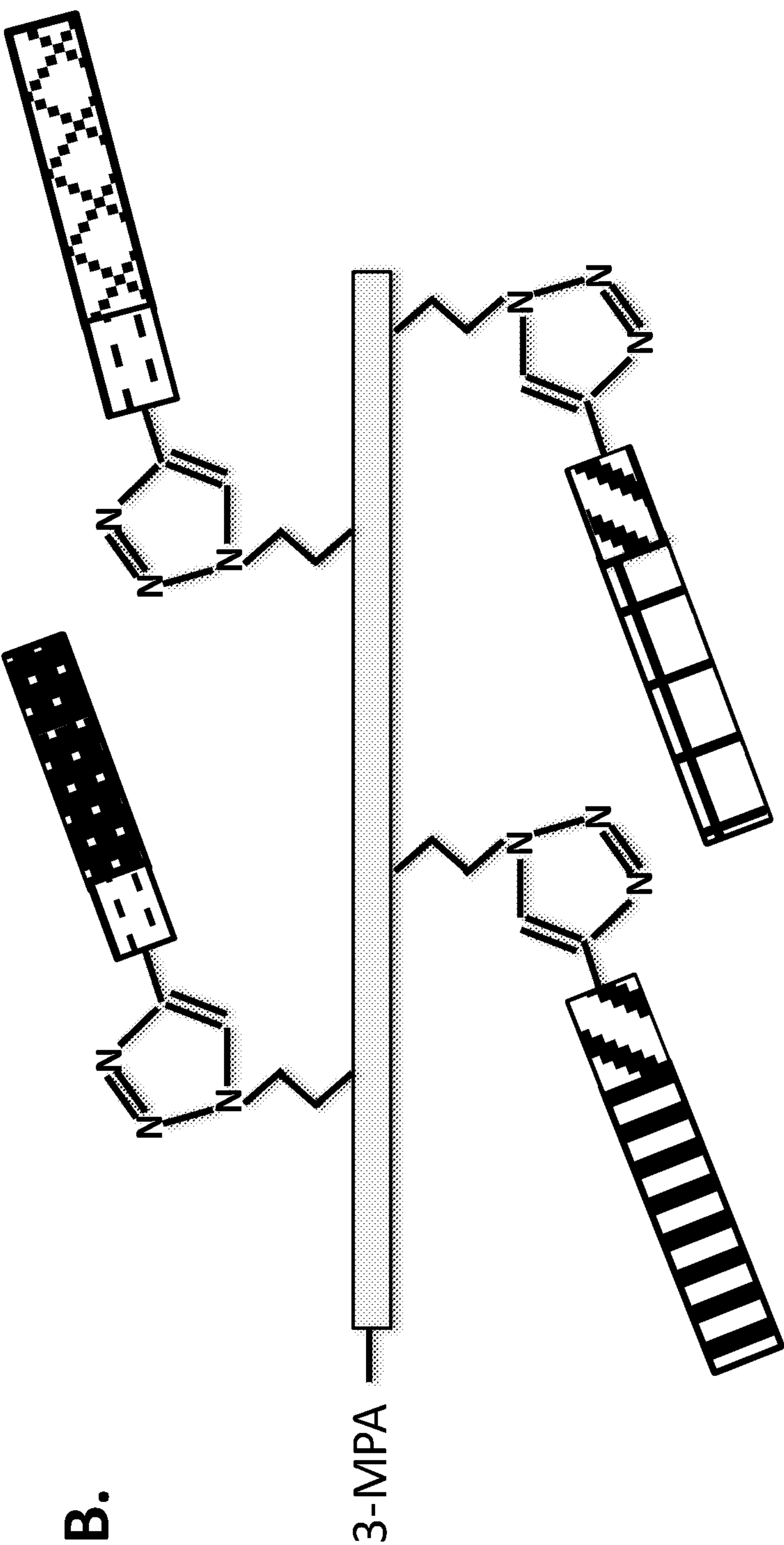
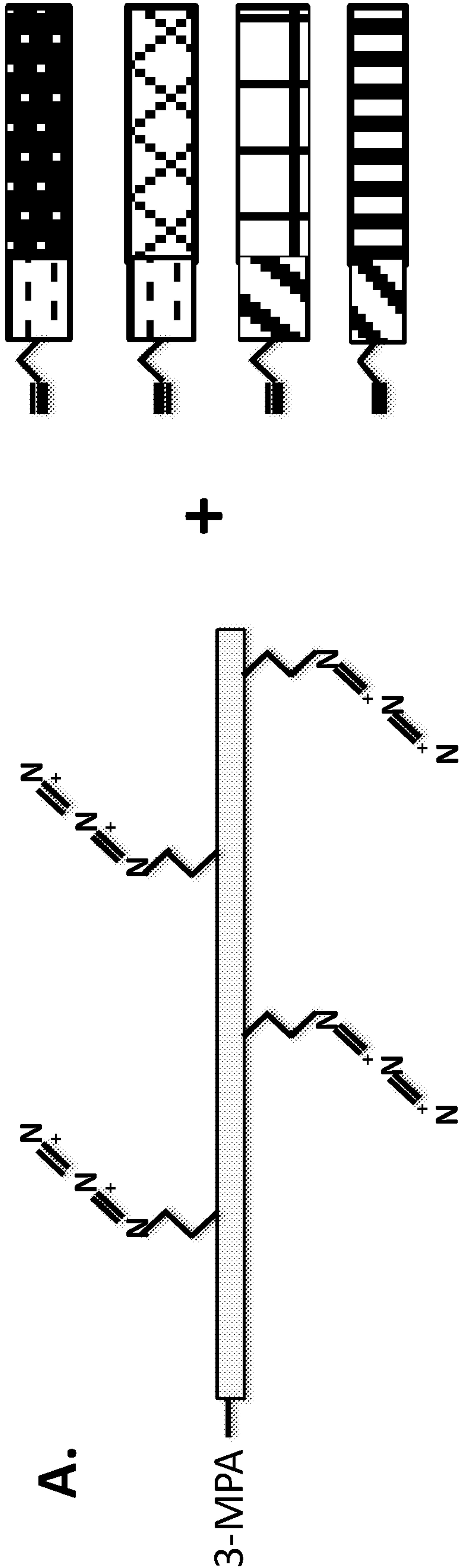


Fig. 7

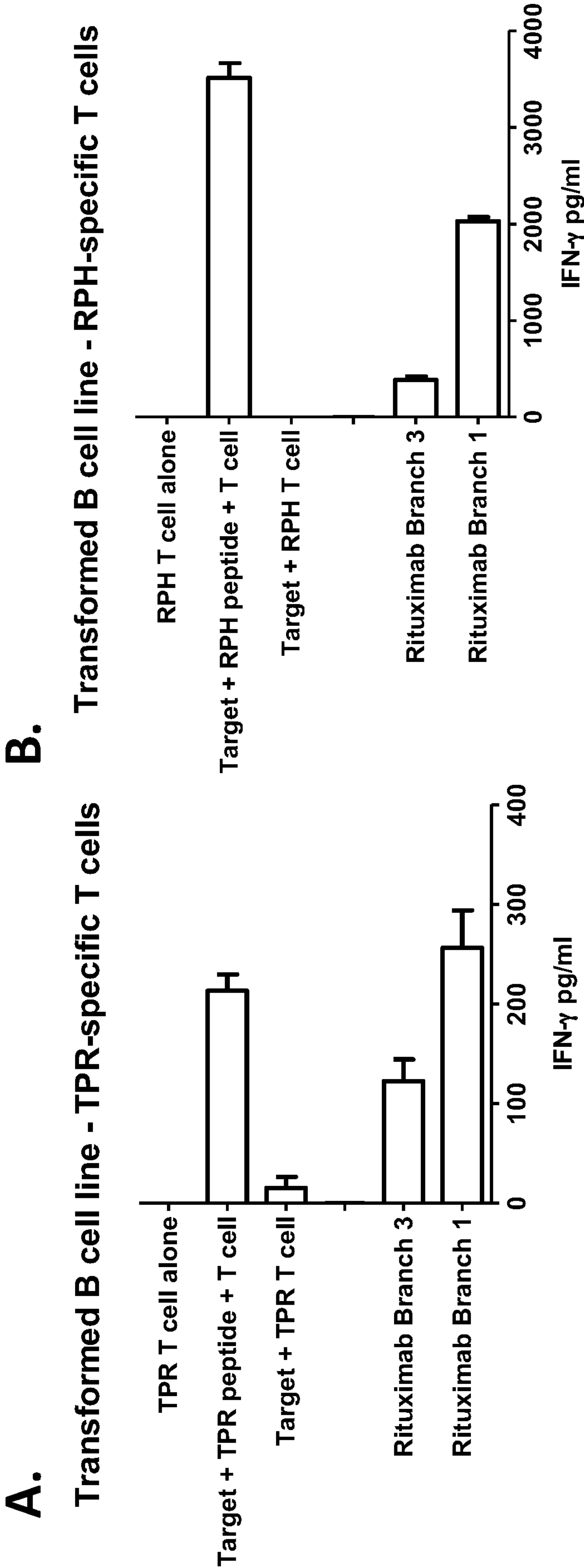
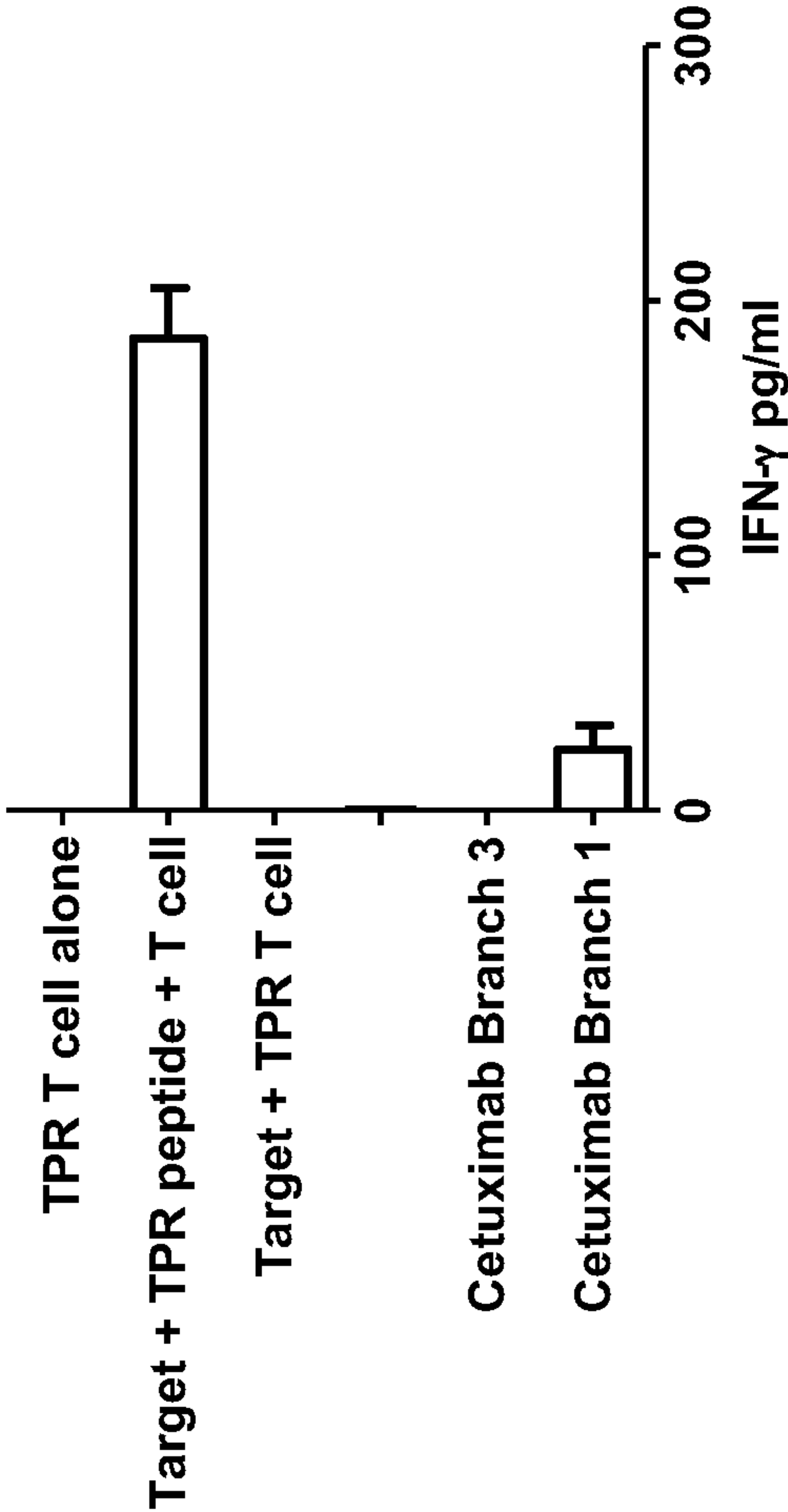


Fig. 8

A.

Ovarian Carcinoma cell line - TPR-specific T cells



B.

Ovarian Carcinoma cell line - RPH-specific T cells

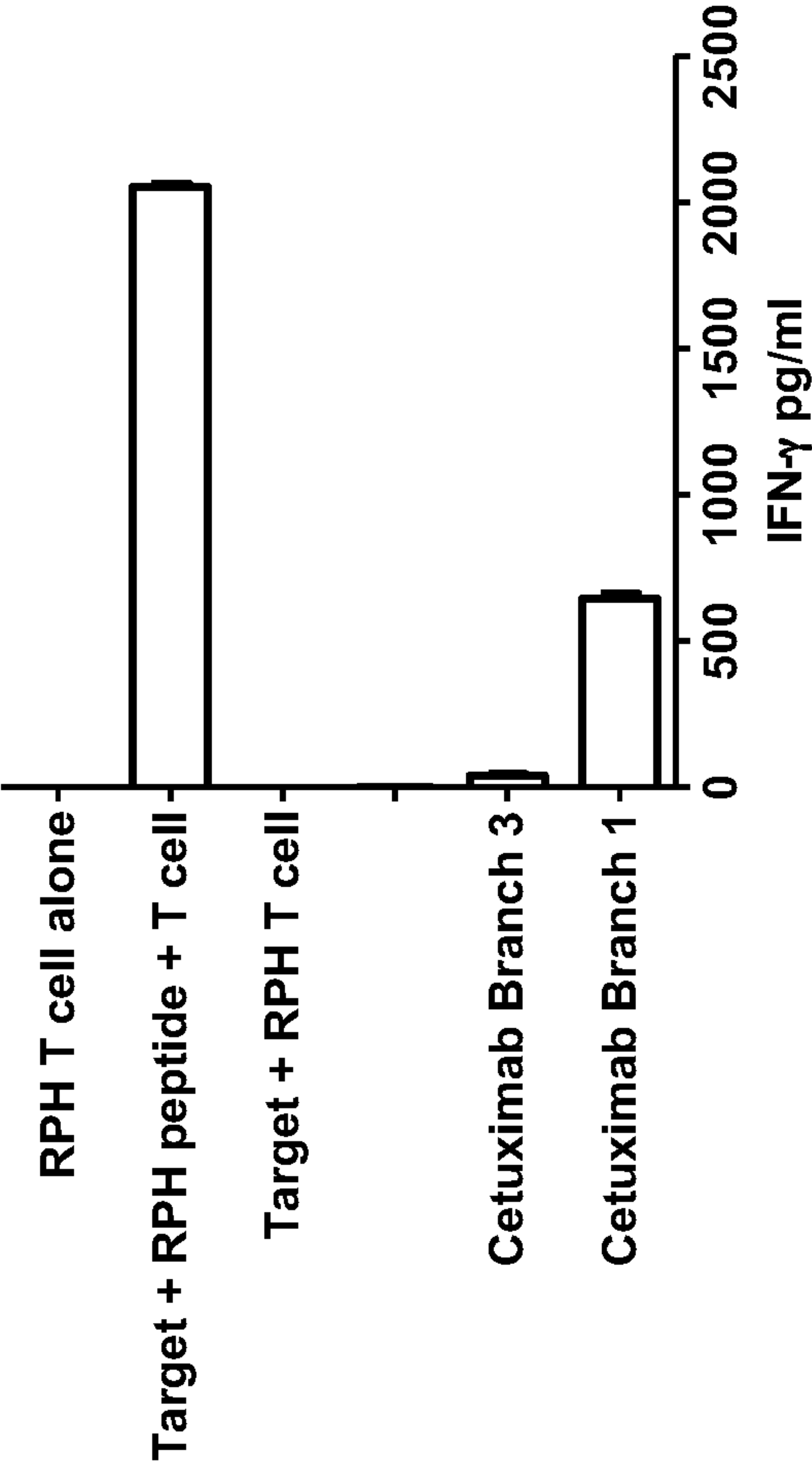


Fig. 9

A.

SASGGGGGSDVPRDLEVVAATPTSLISWDAPAVGGGGGGSTIPVSLRSTPRVTGGGAMTI
PVSLRSGGGGGGGSTVRYRYITYGETGGNSPVQEFTVPGSKSTATISGLKPGVDYTITVYAVTGGGGG
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LRSVLEETSVML (SEQ ID NO: 185)

B.

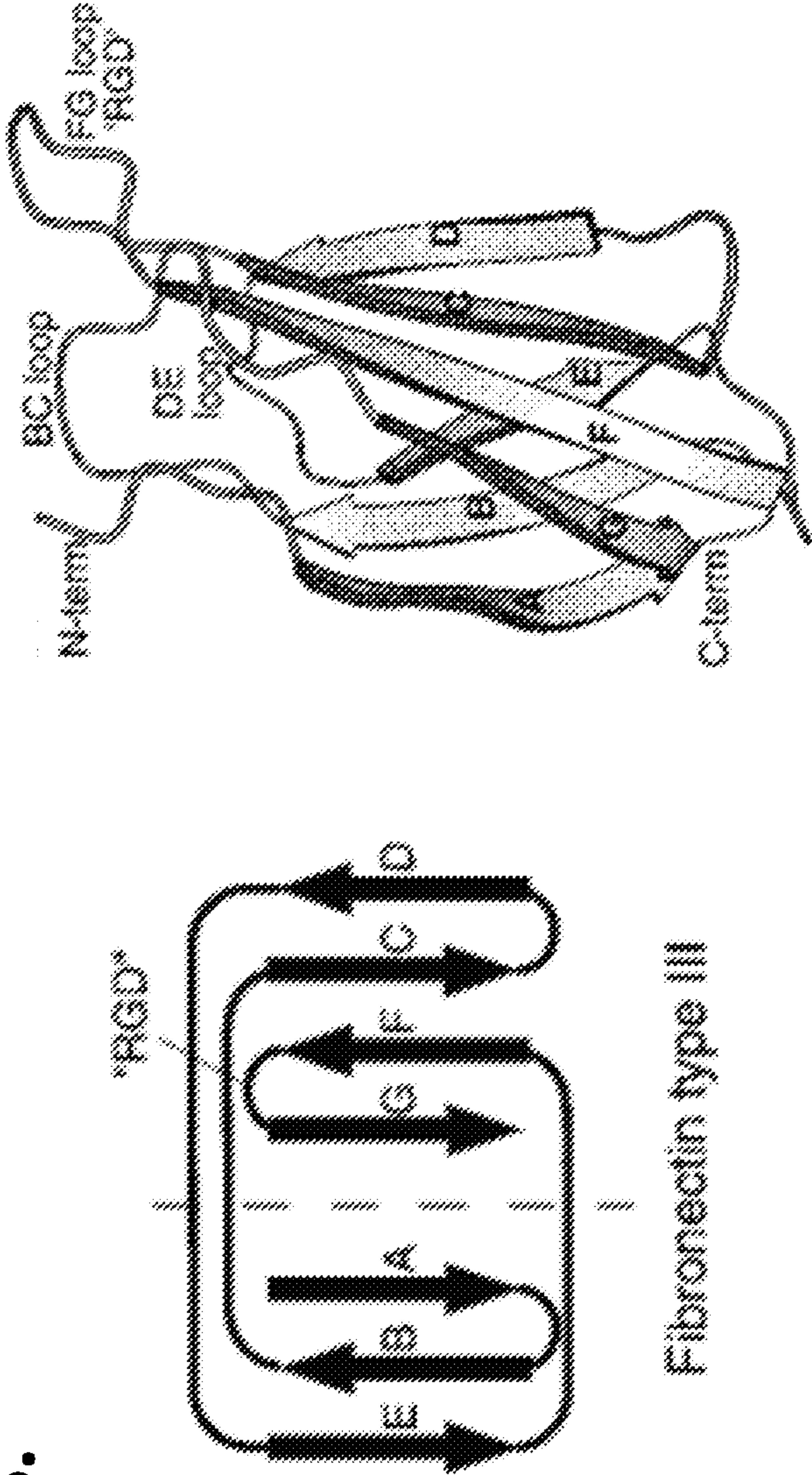


Fig. 9

