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Canadian Intellectual Property Office

CA 3013463 C 2024/05/21

(11)(21) 3 013 463

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2017/02/03
(87) Date publication PCT/PCT Publication Date: 2017/08/10
(45) Date de délivrance/Issue Date: 2024/05/21
(85) Entrée phase nationale/National Entry: 2018/08/01
(86) N° demande PCT/PCT Application No.: US 2017/016407
(87) N° publication PCT/PCT Publication No.: 2017/136659
(30) Priorité/Priority: 2016/02/04 (US62/291,143)

(51) Cl.Int./Int.Cl. *A61K 39/395* (2006.01), *C07K 16/28* (2006.01), *C07K 16/46* (2006.01), *C07K 19/00* (2006.01), *C12N 15/13* (2006.01)

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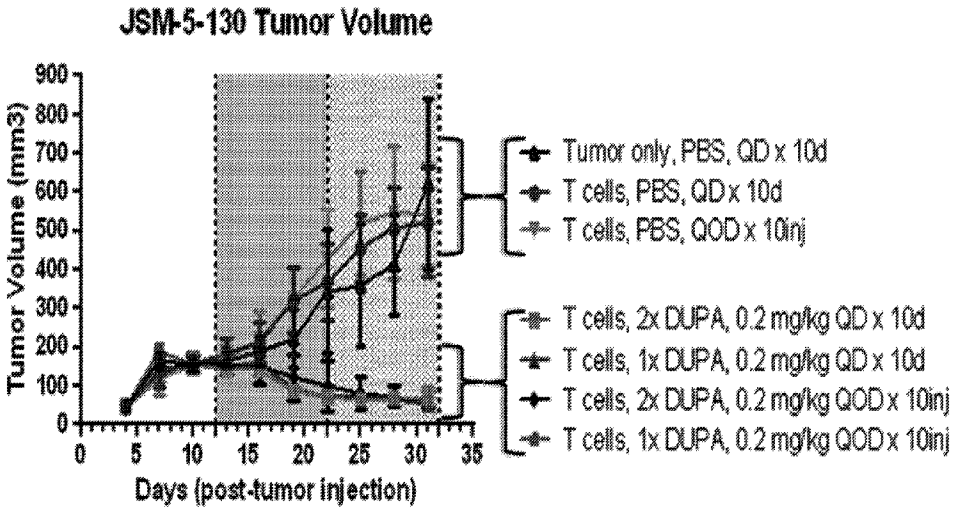
(54) Titre : ANTICORPS HUMANISES ANTI-CD3, CONJUGUES ET LEURS UTILISATIONS
(54) Title: HUMANIZED ANTI-CD3 ANTIBODIES, CONJUGATES AND USES THEREOF



(57) Abrégé/Abstract:
The present invention provides for humanized anti-CD3 antibodies and conjugates thereof. These conjugates may be useful in the treatment of conditions such as prostate cancer.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 August 2017 (10.08.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/136659 A3

(51) International Patent Classification:
A61K 39/395 (2006.01) *C07K 19/00* (2006.01)
C07K 16/28 (2006.01) *C12N 15/13* (2006.01)
C07K 16/46 (2006.01)

(21) International Application Number:
PCT/US2017/016407

(22) International Filing Date:
3 February 2017 (03.02.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/291,143 4 February 2016 (04.02.2016) US

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

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

Declarations under Rule 4.17:

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

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

Published:

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

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

(88) Date of publication of the international search report:
8 September 2017

(54) Title: HUMANIZED ANTI-CD3 ANTIBODIES, CONJUGATES AND USES THEREOF

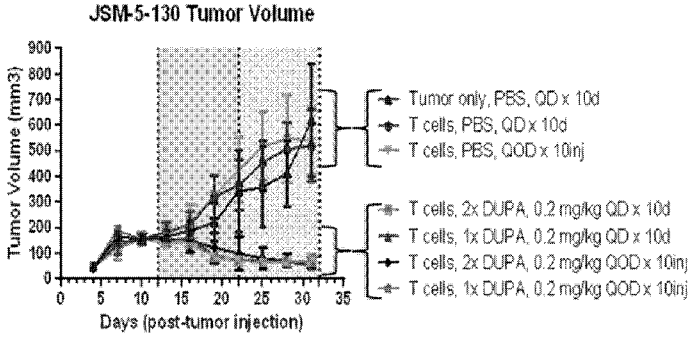

FIG. 36B

(57) Abstract: The present invention provides for humanized anti-CD3 antibodies and conjugates thereof. These conjugates may be useful in the treatment of conditions such as prostate cancer.

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HUMANIZED ANTI-CD3 ANTIBODIES, CONJUGATES AND USES THEREOF

[001]

BACKGROUND OF THE INVENTION

[002] Antibody drug conjugates (ADCs) are a promising class of therapeutics that leverage the unique properties of both biologics and small molecule drugs. By tethering antibodies to drugs through a linker, these conjugates may gain high target specificity, increased serum stability, or improved cell permeability relative to their unconjugated forms. Key variables for tuning the properties and efficacy of these conjugates include the chemical site of linker attachment (both on the antibody and the drug), the antibody structure, and the linker composition/length.

[003] In some cancers, overexpression of specific cell surface receptors can allow selective targeting of cancerous cells with small molecule drugs, while minimizing effects on healthy cells. For example, prostate cancer-specific membrane antigen (PMSA)-targeting 2-[3-(1, 3-dicarboxy propyl)-ureido] pentanedioic acid (DUPA) can be conjugated to a T-cell surface antigen (αCD3) binding antibody to selectively recruit cytotoxic T-cells to kill prostate cancer cells. N-(4-{[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino}benzoyl)-L-glutamic acid (folic acid) can also be used as a targeting agent to bind to the folate receptor (FR) antigen, which is overexpressed on FR+ cancer cell lines.

SUMMARY OF THE INVENTION

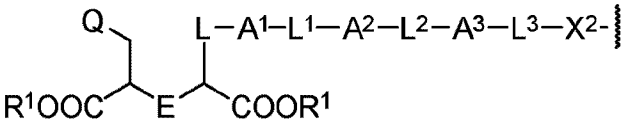
[004] In one aspect of the disclosure, provided are antibody drug conjugates (ADCs) that target cancerous cells expressing cell surface receptors, such as PSMA and FR antigen, with a small molecule. Further provided are antibodies specific for the cluster of differentiation 3 (CD3) T-cell co-receptor, which may be used in an ADC to target T-cell mediated lysis to cancerous cells expressing particular cell surface receptors. Exemplary ADCs provided herein comprise an anti-CD3 antibody conjugated to a PMSA targeting molecule. Such ADCs may be useful for the treatment of prostate cancer. Other exemplary ADCs comprise an anti-CD3 antibody conjugated to folic acid, and are thus useful in the treatment of cancers having overexpression of FR+.

[005] In one aspect, provided herein is an antibody comprising: a first amino acid sequence comprising SEQ ID NO: 74, and a second amino acid sequence comprising one or more of SEQ ID NOS: 54-56. In some embodiments, the first amino acid sequence comprises one or more of SEQ ID NOS: 51-53. In some embodiments, the first amino acid sequence comprises one or more of SEQ ID NOS: 97, 59, or 110. In some embodiments, the first amino acid sequence comprises SEQ ID NO:

Date Reçue/Date Received 2023-06-13

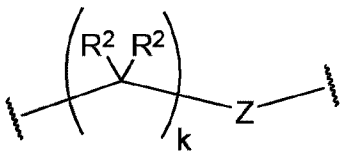
32. In some embodiments, the first amino acid sequence comprises one or more of SEQ ID NOS: 114-123. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 111 or 112. In some embodiments, the second amino acid sequence comprises one or more of SEQ ID NOS: 87, 92, 96, or 124. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 27. In some embodiments, the second amino acid sequence comprises one or more of SEQ ID NOS: 99-108. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 86 or 98. In some embodiments, the first amino acid sequence comprises a light chain constant domain sequence, the second amino acid sequence comprises a light chain constant domain sequence, or both the first and the second amino acid sequence each comprises a light chain constant domain sequence. In some embodiments, the first amino acid sequence comprises a heavy chain constant domain sequence, the second amino acid sequence comprises a heavy chain constant domain sequence, or both the first and the second amino acid sequence each comprises a heavy chain constant domain sequence. In some embodiments, a composition is provided comprising: a first portion comprising the antibody and a second portion comprising a second antibody or antibody fragment.

[006] In some embodiments: (a) one or more amino acid of the first amino acid sequence is an unnatural amino acid; (b) one or more amino acid of the second amino acid sequence is an unnatural amino acid; or (c) one or more amino acid of the first amino acid sequence is an unnatural amino acid, and one or more amino acid of the second amino acid sequence is an unnatural amino acid. In some embodiments, the antibody comprises an unnatural amino acid located within: a light chain constant domain sequence of the first amino acid sequence, a heavy chain constant domain sequence of the second amino acid sequence, or the light chain constant domain sequence of the first amino acid sequence and the heavy chain constant domain sequence of the first amino acid sequence. In some embodiments, the heavy chain constant domain sequence comprises: (a) an amino acid sequence selected from SEQ ID NOS: 86 and 98; or (b) an amino acid sequence selected from one or more of SEQ ID NOS: 99-109. In some embodiments, the light chain constant domain sequence comprises an amino acid selected from: SEQ ID NOS: 111 and 112; or (b) an amino acid sequence selected from one or more of SEQ ID NOS: 113-123. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, a composition is provided comprising the antibody and a cell-targeting molecule. In some embodiments, a composition is provided comprising a cell-targeting molecule connected to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA) or folate receptor. In some embodiments, the composition comprises a compound of Formula (III):

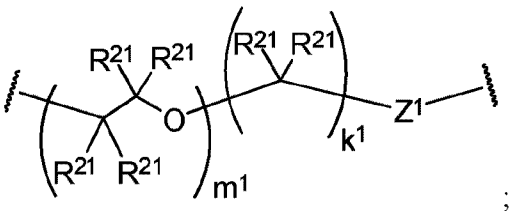
[007] 

Formula (III)

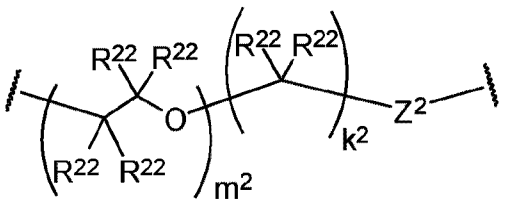
[008] wherein:

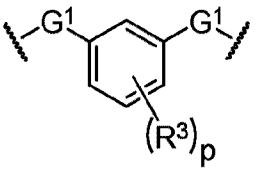
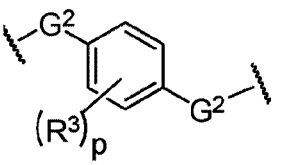
[009] L is  ;

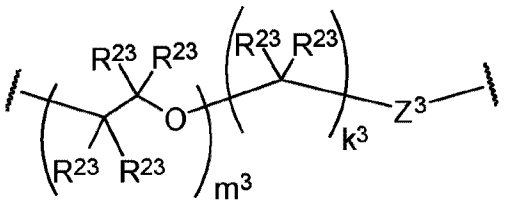
[010] A^1 is selected from the group consisting of an aryl, a 5- to 6-membered heteroaryl, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

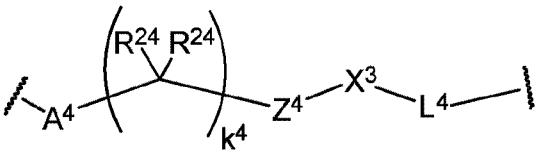
[011] L^1 is  ;

[012] A^2 is selected from the group consisting of a bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

[013] L^2 is 

[014] A^3 is a bond,  , or  ;

[015] L^3 is  ;

[016] X^2 is  ;

[017] A^4 is selected from the group consisting of a bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

[018] each R^1 is independently selected from H, alkyl, and haloalkyl;

[019] each R^2, R^{21}, R^{22}, R^{23}, and R^{24} is independently selected from H, halo, -OR^1, -CN, -SR^1, alkyl, cycloalkyl, haloalkyl, arylalkyl, and heteroarylalkyl;

[020] each R^3 is independently selected from halo, $-OR^1$, -CN, $-SR^1$, alkyl, cycloalkyl, haloalkyl, arylalkyl, heteroarylalkyl, $-NO_2$, and NR^1R^1;

[021] each G^1 and G^2 is independently selected from the group consisting of a bond, -C(O)-, $-N(R^1)-$, -O-, $-C(O)N(R^1)-$, $-N(R^1)C(O)-$, $-S(O)_{1,2}N(R^1)-$, and $-N(R^1)S(O)_{1,2}-$;

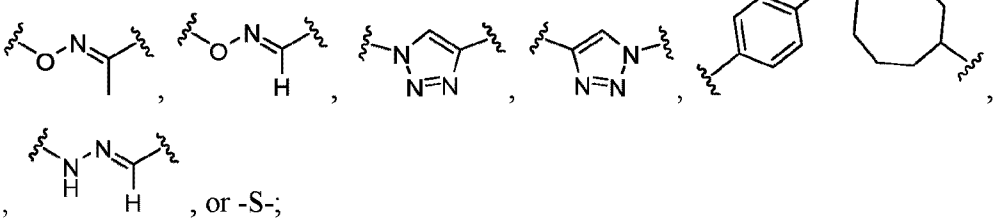
[022] each Z, Z^1, Z^2, and Z^3 is independently selected from the group consisting of a bond, -O-, and $-N(R^1)-$;

[023] Z^4 is selected from a bond, aryl, and a 5- to 6-membered heteroaryl;

[024] k, k^1, k^2, k^3, and k^4 are each independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

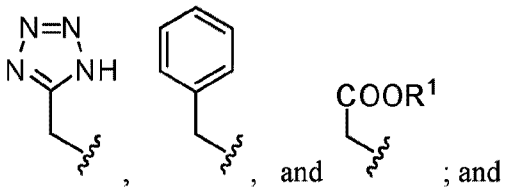
[025] m^1, m^2, and m^3 are each independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

[026] p is 0, 1, 2, 3 or 4;

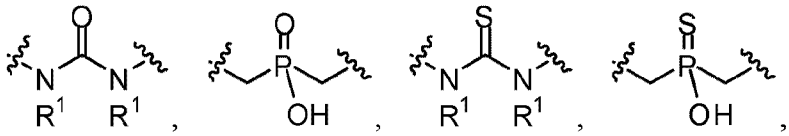
[027] X^3 is  , or -S-;

[028] L^4 is a bond directly attached to a modified amino acid, or a linker bound to a modified amino acid, wherein the modified amino acid is part of the antibody;

[029] Q is selected from the group consisting of:

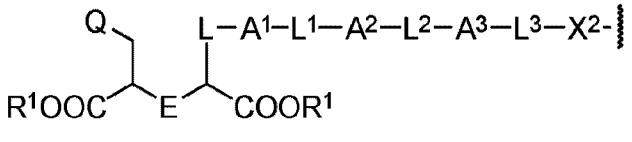
 ; and

[030] E is selected from the group consisting of:

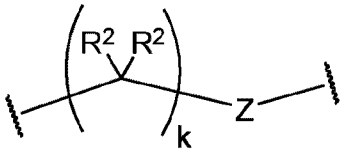
[031]  , and a stereoisomer thereof.

[032] In another aspect, provided herein is an antibody comprising a first amino acid sequence comprising: (a) one or more of SEQ ID NOS: 54-56; and (b) SEQ ID NO: 86, SEQ ID NO: 98, or an amino acid sequence having an unnatural amino acid replacing one or more amino acid residues of SEQ ID NO: 86. In some embodiments, the first amino acid sequence comprises one or more of SEQ ID NOS: 87, 92, 96, or 124. In some embodiments, the antibody further comprising a second amino acid sequence comprising: (a) one or more of SEQ ID NOS: 51-53, (b) one or more of SEQ ID NOS: 97, 59, 74, 110, or (c) a combination of (a) and (b). In some embodiments, provided is a composition comprising the antibody and a cell-targeting molecule. In some embodiments, the cell-

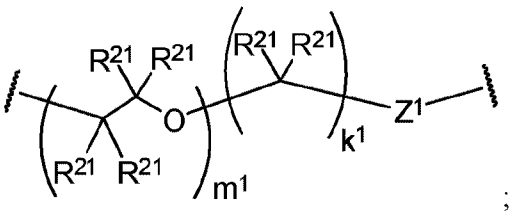
targeting molecule interacts with prostate-specific membrane antigen (PSMA) or a folate receptor. In some embodiments, provided is a composition comprising the antibody, the composition comprising a compound of Formula (III):

[033]  Formula (III)

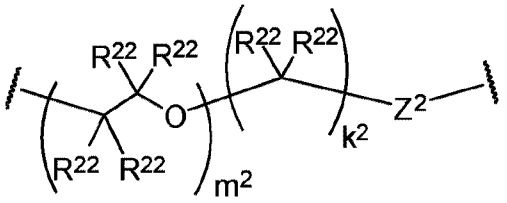
[034] wherein:

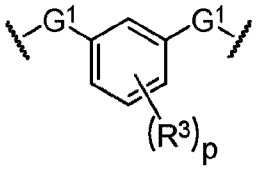
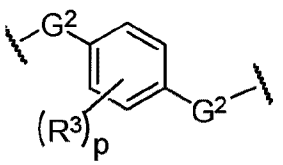
[035] L is  ;

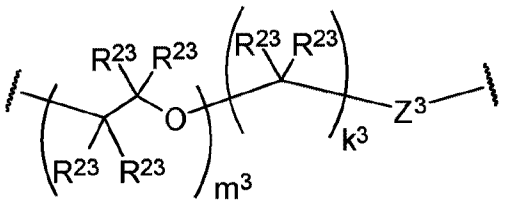
[036] A^1 is selected from the group consisting of an aryl, a 5- to 6-membered heteroaryl, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

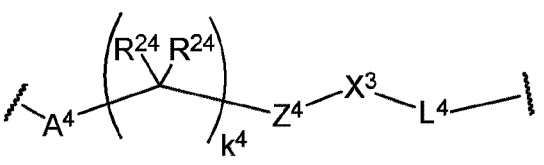
[037] L^1 is  ;

[038] A^2 is selected from the group consisting of a bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

[039] L^2 is 

[040] A^3 is a bond,  , or  ;

[041] L^3 is  ;

[042] X^2 is  ;

[043] A^4 is selected from the group consisting of a bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

[044] each R^1 is independently selected from H, alkyl, and haloalkyl;

[045] each R^2, R^{21}, R^{22}, R^{23}, and R^{24} is independently selected from H, halo, -OR^1, -CN, -SR^1, alkyl, cycloalkyl, haloalkyl, arylalkyl, and heteroarylalkyl;

[046] each R^3 is independently selected from halo, -OR^1, -CN, -SR^1, alkyl, cycloalkyl, haloalkyl, arylalkyl, heteroarylalkyl, -NO_2, and NR^1R^1;

[047] each G^1 and G^2 is independently selected from the group consisting of a bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

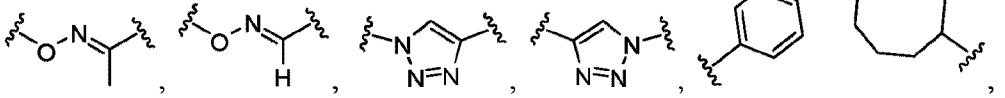
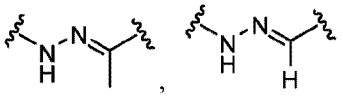
[048] each Z, Z^1, Z^2, and Z^3 is independently selected from the group consisting of a bond, -O-, and -N(R^1)-;

[049] Z^4 is selected from a bond, aryl, and a 5- to 6-membered heteroaryl;

[050] k, k^1, k^2, k^3, and k^4 are each independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

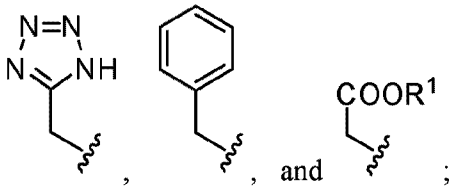
[051] m^1, m^2, and m^3 are each independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

[052] p is 0, 1, 2, 3, or 4;

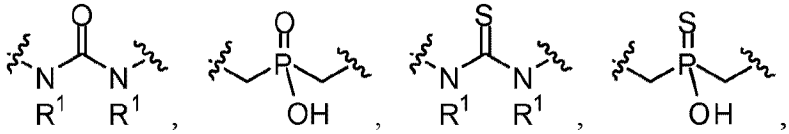
[053] X^3 is  ,  , or -S-;

[054] L^4 is a bond directly attached to a modified amino acid, or a linker bound to a modified amino acid, wherein the modified amino acid is part of the antibody;

[055] Q is selected from the group consisting of:

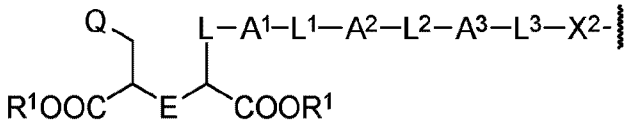
 ; and

[056] E is selected from the group consisting of:

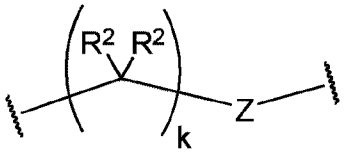
[057]  , and a stereoisomer thereof.

[058] In another aspect, provided herein is an antibody comprising: (a) a first amino acid sequence comprising one or more of SEQ ID NOS: 54-56; and (b) an unnatural amino acid. In some embodiments, the first amino acid sequence comprises one or more of SEQ ID NOS: 87, 92, 96, or

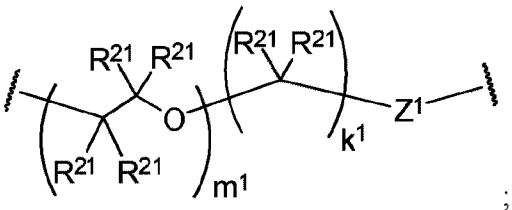
124. In some embodiments, the first amino acid sequence comprises one or more of SEQ ID NOS: 99-108. In some embodiments, the first amino acid sequence comprises SEQ ID 98. In some embodiments, the first amino acid sequence comprises the unnatural amino acid. In some embodiments, the antibody further comprises a second amino acid sequence comprising: (a) one or more of SEQ ID NOS: 51-53, (b) one or more of SEQ ID NOS: 97, 59, 74, or 110, or (c) a combination of (a) and (b). In some embodiments, provided is a composition comprising the antibody and a cell-targeting molecule. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA) or a folate receptor. In some embodiments, a composition is provided comprising an antibody of any of claims 29-36, comprising a compound of Formula (III):

[059]  Formula (III)

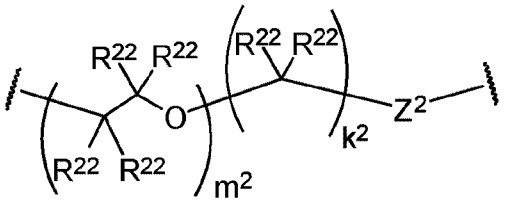
[060] wherein:

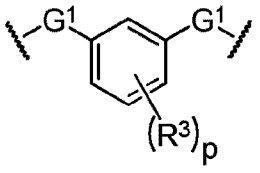
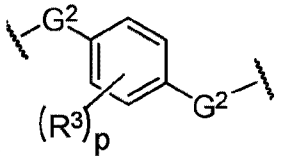
[061] L is  ;

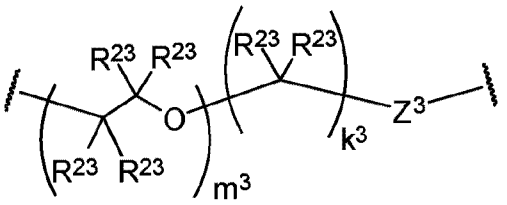
[062] A^1 is selected from the group consisting of an aryl, a 5- to 6-membered heteroaryl, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

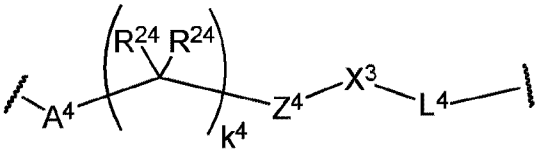
[063] L^1 is  ;

[064] A^2 is selected from the group consisting of a bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

[065] L^2 is 

[066] A^3 is a bond,  , or  ;

[067] L^3 is  ;

[068] X^2 is  ;

[069] A^4 is selected from the group consisting of a bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

[070] each R^1 is independently selected from H, alkyl, and haloalkyl;

[071] each R^2, R^{21}, R^{22}, R^{23} and R^{24} is independently selected from H, halo, -OR^1, -CN, -SR^1, alkyl, cycloalkyl, haloalkyl, arylalkyl, and heteroarylalkyl;

[072] each R^3 is independently selected from halo, -OR^1, -CN, -SR^1, alkyl, cycloalkyl, haloalkyl, arylalkyl, or heteroarylalkyl, -NO_2, and NR^1R^1;

[073] each G^1 and G^2 is independently selected from the group consisting of a bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

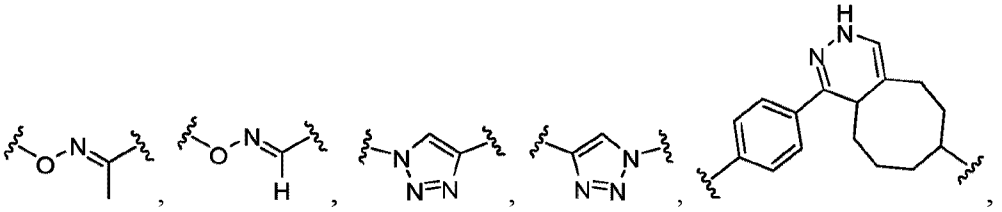
[074] each Z, Z^1, Z^2, and Z^3 is independently selected from the group consisting of a bond, -O-, and -N(R^1)-;

[075] Z^4 is selected from a bond, aryl, and a 5- to 6-membered heteroaryl;

[076] k, k^1, k^2, k^3, and k^4 are each independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

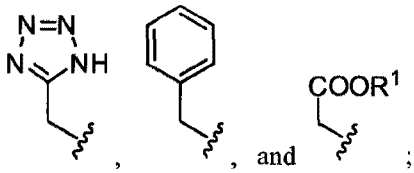
[077] m^1, m^2 and m^3 are each independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

[078] p is 0, 1, 2, 3 or 4;

[079] X^3 is  , or -S-;

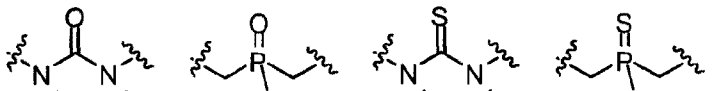
[080] L^4 is a bond directly attached to a modified amino acid, or a linker bound to a modified amino acid, wherein the modified amino acid is part of the antibody;

[081] Q is selected from the group consisting of:



, , and ; and

[082] E is selected from the group consisting of:

[083] 

, and a stereoisomer thereof.

[084] In another aspect, provided is a composition comprising: (a) an amino acid sequence comprising one or more of SEQ ID NOS: 51-56 and an unnatural amino acid; and (b) a cell-targeting molecule linked to the amino acid sequence via the unnatural amino acid. In some embodiments, the unnatural amino acid is located within a heavy chain constant domain sequence of the amino acid sequence. In some embodiments, the amino acid sequence comprises one or more of SEQ ID NOS: 87, 92, 96, or 124. In some embodiments, the amino acid sequence comprises: one or more of SEQ ID NOS: 99-108. In some embodiments, the amino acid sequence comprises: SEQ ID NO: 86, SEQ ID NO: 98, or an amino acid sequence having the unnatural amino acid replace one or more amino acid residues of SEQ ID NO: 86. In some embodiments, the antibody comprises a second amino acid sequence comprising: (a) one or more of SEQ ID NOS: 51-53; (b) one or more of SEQ ID NOS: 97, 59, 74, 110; or (c) a combination of (a) and (b). In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA) or folate receptor.

BRIEF DESCRIPTION OF THE DRAWINGS

[085] **FIG. 1A** shows a schematic synthesis of an anti-CD3 antibody (e.g., huL5H2) single mutant DUPA conjugate attached via a linker (L) to the heavy chain.

[086] **FIG. 1B** shows a schematic synthesis of an anti-CD3 antibody (e.g., huL5H2) double mutant DUPA conjugate attached via two linkers (L) to both the heavy and light chains.

[087] **FIG. 1C** shows a schematic synthesis of an anti-CD3 antibody (e.g., huL5H2) double mutant folate conjugate attached via linker (L) to both heavy and light chains.

[088] **FIG. 1D** shows a schematic synthesis of an anti-CD3 antibody (e.g., huL5H2) single mutant folate conjugate attached via linker (L) to the heavy chain.

[089] **FIG. 2** shows alignments of anti-CD3 variable heavy chain and light chain amino acid sequences, where the hypervariable regions are denoted by LCDR1, LCDR2, LCDR3, HCDR1, HCDR2, and HCDR3, corresponding to SEQ ID NOS: 51-56, respectively. VH hu1 and VH hu2 correspond to SEQ ID NOS: 25 and 26, respectively. VL hu1 through VL hu 10 correspond to SEQ ID NOS: 28-37, respectively. Nucleic acid sequences encoding these sequences were individually cloned into the pFUSE vector under the IL2 signal peptide sequence. FIG. 2 discloses murine anti-CD3 VH and VL as SEQ ID NOS 24 and 23, respectively, in order of appearance, IGHV3-73 as SEQ ID NO: 125, and IGLV7-46 as SEQ ID NO: 126.

[090] **FIG. 3** shows superior binding of antibody huL5H2 (heavy chain SEQ ID NO: 41 and light chain SEQ ID NO: 39) to human CD3, which was comparable to murine anti-CD3 on human T cells in a fluorescence-based flow cytometry assay.

[091] **FIG. 4** shows antibody huL5H2 (heavy chain SEQ ID NO: 41 and light chain SEQ ID NO: 39) binding to human CD3 was comparable to murine anti-CD3 binding to cynomolgus T cells in a fluorescence-based flow cytometry assay.

[092] **FIG. 5** shows an SDS-PAGE gel of purified anti-CD3 antibodies (kappa = SEQ ID NOS: 39, 41) lambda = SEQ ID NOS: 38, 41) containing the pAcF non-canonical amino acid. The anti-CD3 antibody having a Fab with a kappa constant region yielded approximately 4-fold higher expression levels than the Fab composed of a lambda constant region.

[093] **FIG. 6A** shows completion of the conjugation reaction of huL5H2 (SEQ ID NOS: 40, 42) with p-TriA to as confirmed by QTOF mass spectrometry after excess linkers were removed by size filtration (AMICON®, 10K and 30K). huL5H2-pTriA has two DUPA molecules (2xDUPA), one conjugated to each light chain and one conjugated to each heavy chain.

[094] **FIG. 6B** shows an SDS-PAGE gel of purified anti-CD3 Fab (SEQ ID NOS: 40, 42), before and after conjugation with p-TriA at the heavy and light chain to generate huL5H2 (2xDUPA) double mutant.

[095] **FIG. 7** shows a flow cytometry fluorescence assay where huL5H2 (SEQ ID NOS: 40, 42) and UCHT-1 (SEQ ID NOS: 84, 85) antibodies and p-TriA (2xDUPA) conjugates (SEQ ID NOS: 40, 42 conjugated to p-TriA, "huL5H2-p-TriA (2xDUPA)") demonstrated comparable cell-surface binding to Jurkat (human) T cells and C4-2 (PSMA-positive) cells, respectively, with minimal non-specific binding to DU145 (PSMA-negative) cells.

[096] **FIG. 8A** shows huL5H2-p-TriA (2xDUPA) and UCHT-1-p-TriA (2xDUPA) antibody conjugates selectively redirected human PBMCs against C4-2 (PSMA-positive) cells with comparable potency in a cytotoxicity assay.

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[097] **FIG. 8B** shows huL5H2- and UCHT-1-p-TriA (2xDUPA) antibody conjugates induced minimal non-specific killing of DU145 (PSMA-negative) cells.

[098] **FIG. 9** shows only huL5H2-p-TriA (2xDUPA) induced lysis of C4-2 cells with cynomolgus PBMCs, with an EC_{50} = 60.5 pM.

[099] **FIG. 10A** shows both p-TriA conjugates of huL5H2 and UCHT-1 (2xDUPA) induced a similar level of T cell activation in PSMA-positive cells.

[0100] **FIG. 10B** shows p-TriA of huL5H2 and UCHT-1 (2xDUPA) conjugates induced minimal T cell activation in PSMA-negative cells.

[0101] FIG. 11A shows both p-TriA huL5H2 and UCHT-1 (2xDUPA) conjugates induced similar T cell proliferation in PSMA-positive cells in a flow cytometry assay.

[0102] FIG. 11B shows both p-TriA huL5H2 and UCHT-1 (2xDUPA) conjugates induced minimal T cell proliferation in PSMA-negative cells in a flow cytometry assay.

[0103] FIG. 12A shows both p-TriA huL5H2 and UCHT-1 (2xDUPA) conjugates induced comparable levels of inflammatory cytokines from human T cells in the presence of PSMA-positive C4-2 cells.

[0104] FIG. 12B shows both p-TriA huL5H2 and UCHT-1 (2xDUPA) conjugates induced minimal levels of inflammatory cytokines from human T cells in the presence of PSMA-negative DU145 cells.

[0105] FIG. 13A shows an experimental set up for the treatment of C4-2 xenografts in mice with huL5H2-p-TriA (2xDUPA).

[0106] FIG. 13B shows huL5H2-p-TriA (2xDUPA) demonstrated dose-dependent *in vivo* anti-tumor activity against C4-2 xenografts in a NSG mouse model reconstituted with human T cells.

[0107] FIG. 14A shows an experimental set up for the treatment of a tumor in a PCSD1 PDX (patient-derived xenograft) model with HuL5H2-DUPA (2xDUPA) and activated T cells.

[0108] FIG. 14B shows huL5H2-p-TriA (2xDUPA) in combination with PBMCs demonstrated a reduction in tumor volume for the PDX mouse model.

[0109] FIG. 15 shows introduction of four de-immunizing mutations in the variable heavy chain of SEQ ID NO: 41 predicted in-silico by Epivax software that resulted in a significantly reduced immunogenicity score. The resulting antibody heavy chain has SEQ ID NO: 43, and SEQ ID NO: 44 when configured to conjugate via pAcF.

[0110] FIG. 16 shows completion of the conjugation reaction of de-immunized (DI) DI-HuL5H2 (SEQ ID NOS: 40, 44) with p-TriA as confirmed by QTOF mass spectrometry after excess linkers were removed by size filtration (Amicon, 10K and 30K) to generate huL5H2_DI-2xDUPA.

[0111] FIG. 17A shows similar binding profiles to human T cells were observed with huL5H2 (SEQ ID NOS: 40, 42) and DI-huL5H2 (SEQ ID NOS: 40, 44), which suggest that cross-reactivity to human CD3 was retained even after introducing de-immunizing mutations.

[0112] FIG. 17B shows similar binding profiles to cynomolgus T cells were observed with huL5H2 (SEQ ID NOS: 40, 42) and DI-huL5H2 (SEQ ID NOS: 40, 44), which suggest that cross-reactivity to cynomolgus CD3 was retained even after introducing de-immunizing mutations.

[0113] FIG. 18 shows huL5H2_DI-1xDUPA (SEQ ID NOS: 39, 44) and huL5H2_DI-2xDUPA (SEQ ID NOS: 40, 44) conjugates selectively redirected human PBMCs against C4-2 (PSMA-positive) cells with comparable potency (EC50 = 3.2 pM, 3.1 pM, respectively) and induced minimal non-specific killing of DU145 (PSMA-negative) cells.

[0114] FIG. 19A and **FIG. 19B** show a representative heavy chain and light chain, respectively, of exemplary DUPA conjugates described herein. The N-terminal (SEQ ID NO:80) and C-terminal (SEQ ID NO: 81) of the heavy chain are connected by a non-canonical amino acid (pAcF) which is conjugated to DUPA via a linker. The N-terminal (SEQ ID NO:82) and C-terminal (SEQ ID NO: 83) of the light chain are connected by an unnatural amino acid which is conjugated to DUPA via a linker. The targeting agent antibody conjugates may comprise only a conjugated light chain, only a conjugated heavy chain, or both a conjugated light chain and a conjugated heavy chain.

[0115] FIG. 19C and **FIG. 19D** show a representative heavy chain and light chain, respectively, of exemplary folate conjugates described herein. The N-terminal (SEQ ID NO:80) and C-terminal (SEQ ID NO: 81) of the heavy chain are connected by an unnatural amino acid which is conjugated to folate via a linker. The N-terminal (SEQ ID NO:82) and C-terminal (SEQ ID NO: 83) of the light chain are connected by an unnatural amino acid which is conjugated to folate via a linker. The targeting agent antibody conjugates may comprise only a conjugated light chain, only a conjugated heavy chain, or both a conjugated light chain and a conjugated heavy chain.

[0116] FIG. 20A shows chemical structure of 2-[3-(1,3-dicarboxypropyl)ureido]pentanedioic acid (DUPA).

[0117] FIG. 20B shows exemplary analogs of chemical structure of 2-[3-(1,3-dicarboxypropyl)ureido]pentanedioic acid (DUPA), such as ((1-carboxy-2-mercaptoethyl)carbamoyl)glutamic acid (CMCG), ((2-(tert-butylthio)-1-carboxyethyl)carbamoyl)glutamic acid (tBuCMCG), and ((1-carboxy-3-(1H-tetrazol-5-yl)propyl)carbamoyl)glutamic acid (CTCG).

[0118] FIG. 20C shows chemical structure of N-(4-{[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino}benzoyl)-L-glutamic acid (folic acid/folate).

[0119] FIG. 20D shows exemplary analogs of chemical structure of N-(4-{[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino}benzoyl)-L-glutamic acid (folic acid/folate), such as (4-((1-(2,4-diaminopteridin-6-yl)ethyl)(methyl)amino)benzoyl)glutamic acid (denopterin), and (4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzoyl)glutamic acid (methotrexate).

[0120] FIG. 21A shows that huL5H2_DI-1xDUPA (SEQ ID NOS: 39, 44) and huL5H2_DI-2xDUPA (SEQ ID NOS: 40, 44) conjugates are internalized into PSMA+ cells with similar rates.

[0121] FIG. 21B and **FIG. 21C** show calculation of the internalization rate constants (linear-fit slopes) for L5H2_DI-1xDUPA and L5H2_DI-2xDUPA, respectively.

[0122] FIG. 22 shows that both huL5H2_DI 1xDUPA and huL5H2_DI 2xDUPA, in the presence of PBMCs, were cytotoxic against PSMA+ C4-2 cells (10:1 ratio of PBMC:C4-2 cells).

[0123] FIG. 23 shows that huL5H2_DI 1xDUPA and huL5H2_DI 2xDUPA demonstrated cytokine release levels in C4-2 cancer cells. Samples were obtained from media used in the cytoxicity study in **FIG. 22.**

[0124] FIG. 24 shows that huL5H2_DI 1xDUPA and huL5H2_DI 2xDUPA demonstrated significant PSMA+ dependent upregulation of activation markers CD25/CD69 in human PBMCs.

[0125] FIG. 25 shows that huL5H2_DI 1xDUPA and huL5H2_DI 2xDUPA demonstrated significant T-cell proliferation activity when using human PBMCs.

[0126] FIG. 26 shows huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA demonstrated dose-dependent cytotoxicity across VCaP, C4-2, and LNCaP cells lines.

[0127] FIG. 27 shows huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA demonstrated dose-dependent cytotoxicity using the 22Rv-1 cell line.

[0128] FIG. 28 shows huL5H2_DI-1xDUPA (batch ID P00925) and huL5H2_DI-2xDUPA (batch ID P00774) were not significantly inhibited from activating T-cells by unbound human PSMA in the presence of human PBMCs and C4-2 cells in a Jurkat NFAT assay.

[0129] FIG. 29 shows huL5H2_DI-1xDUPA (batch ID P00925) and huL5H2_DI-2xDUPA (batch ID P00774) demonstrated no significant inhibition of cytotoxicity against C4-2 cells by unbound human PSMA using PBMC donor 5053 cells.

[0130] FIG. 30 shows a comparison of different batches (ID = P0####) of DUPA conjugates for T-cell activation in a fluorescence-based Jurkat (NFAT-Luc) assay, in the presence of C4-2 cells.

[0131] FIG. 31A shows positive controls PMA and lonomycin activated T-cells in a fluorescence-based Jurkat (NFAT-Luc) assay.

[0132] FIG. 31B shows internal positive control recombinant luciferase produced a consistent signal across batches of DUPA conjugates.

[0133] FIG. 32 shows huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA had no appreciable change in structure or mass by LCMS-QTOF mass spectrometry after 48 h incubation in different serums.

[0134] FIG. 33A shows huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA had no appreciable change in cytotoxicity against C4-2 PSMA+ cells after 48 h incubation in mouse or human serum.

[0135] FIG. 33B shows huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA had no appreciable change in cytotoxicity against C4-2 PSMA+ cells after 48 h incubation in rat or monkey serum.

[0136] FIG. 34A shows huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA had no appreciable loss of T-cell activation in PSMA+ cells (as evidenced by cytokine release) after 48 h incubation in mouse or human serum.

[0137] FIG. 34B shows huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA had no appreciable loss of T-cells activation in PSMA+ cells (as evidenced by cytokine release) after 48 h incubation in rat or monkey serum.

[0138] FIG. 35A shows an experimental set up for the treatment of C4-2 xenografts in a NSG mouse model reconstituted with human T cells using daily injections of huL5H2_DI-2xDUPA or huL5H2_DI-1xDUPA.

[0139] FIG. 35B shows a daily injection schedule of huL5H2_DI-2xDUPA or huL5H2_DI-1xDUPA demonstrated similar dose-dependent *in vivo* anti-tumor activity in the NSG mouse model reconstituted with human T cells

[0140] FIG. 35C shows a daily injection schedule of huL5H2_DI-2xDUPA and huL5H2_DI-1xDUPA demonstrated significant body weight reduction during treatment in the NSG mouse model reconstituted with human T cells.

[0141] FIG. 36A shows an experimental set up for the treatment of C4-2 xenografts with huL5H2_DI-2xDUPA or huL5H2_DI-1xDUPA with a QOD (every other day) injection schedule.

[0142] FIG. 36B shows a QOD injection schedule of huL5H2_DI-2xDUPA or huL5H2_DI-1xDUPA demonstrated similar dose-dependent *in vivo* anti-tumor activity in the NSG mouse model reconstituted with human T cells.

[0143] FIG. 36C shows a QOD injection schedule of huL5H2_DI-2xDUPA or huL5H2_DI-1xDUPA demonstrated significant body weight reduction during treatment in NSG mouse model reconstituted with human T cells.

[0144] FIG. 37A shows huL5H2_DI-2xDUPA and huL5H2_DI-1xDUPA demonstrated significant cytokine release during treatment in the NSG mouse model reconstituted with human T cells for both QD (daily) and QOD (every other day) injection schedules.

[0145] FIG. 37B shows huL5H2_DI-2xDUPA or huL5H2_DI-1xDUPA treatment demonstrated significant mouse cytokine release during treatment in the NSG mouse model reconstituted with human T cells for both QD (daily) and QOD (every other day) injection schedules.

[0146] FIG. 38A shows huL5H2_DI-2xDUPA treatment led to a greater reduction in body weight than huL5H2_DI-1xDUPA with a QD (daily) injection schedule.

[0147] FIG. 38B shows huL5H2_DI-2xDUPA treatment led to a greater reduction in body weight than huL5H2_DI-1xDUPA with a QOD (every other day) injection schedule.

[0148] FIG. 38C shows huL5H2_DI-2xDUPA treatment led to a greater reduction in body weight than huL5H2_DI-1xDUPA in the absence of tumor cells.

[0149] FIG. 39A shows an experimental set up for the treatment of C4-2 xenografts with daily injections of huL5H2_DI-2xDUPA or huL5H2_DI-1xDUPA, without the addition of human T-cells.

[0150] FIG. 39B and **FIG. 39C** show huL5H2_DI-2xDUPA and huL5H2_DI-1xDUPA demonstrated no significant loss in body weight in the absence of T cells, which suggests that toxicity is due to T cell activation.

[0151] FIG. 40A, FIG. 40B, FIG. 40C, FIG. 40D, and FIG 40E show huL5H2_DI-2xDUPA and huL5H2_DI-1xDUPA demonstrated no significant blood toxicity in the absence of T-cells.

[0152] FIG. 41 shows an experimental set up for the treatment of C4-2 xenografts, with post-treatment analysis of T cell counts and cytokine levels.

[0153] FIG. 42 shows huL5H2_DI-1xDUPA demonstrated dose-dependent *in vivo* anti-tumor activity in the NSG mouse model reconstituted with human PBLs, with a slight delay in anti-tumor activity relative to experiments using PBMCs in the C4-2 xenograft mouse model.

[0154] FIG. 43 shows weight loss was caused by tumor burden. PBL and huL5H2_DI-1xDUPA did not demonstrate significant weight loss in the absence of tumor.

[0155] FIG. 44A, FIG. 44B, and FIG. 44C show huL5H2_DI-2xDUPA and huL5H2_DI-1xDUPA demonstrated no significant blood toxicity in the NSG mouse model reconstituted with human PBLs.

[0156] FIG. 45A and FIG. 45B show treatment with huL5H2_DI-2xDUPA or huL5H2_DI-1xDUPA demonstrated a decrease in peripheral human T cells, which indicated recruitment to the tumor.

[0157] FIG. 45C, FIG. 45D, and FIG. 45E show treatment with huL5H2_DI-2xDUPA or huL5H2_DI-1xDUPA preserved renal and liver function as measured from blood plasma samples.

[0158] FIG. 46 shows that huL5H2_DI-2xDUPA demonstrated a prolonged exposure compared with huL5H2_DI-1xDUPA in a mouse model.

DETAILED DESCRIPTION OF THE INVENTION

[0159] Disclosed herein are humanized anti-CD3 antibodies and their respective targeting agent antibody conjugates. These antibodies are humanized with additional mutations introduced to reduce potential immunogenicity in humans and optimize binding to T cells. Examples provided herein demonstrate humanization, optimization of binding, and reduction of immunogenicity of a murine cross-species reactive anti-CD3 antibody. Examples provided herein also demonstrate conjugation of the resulting humanized antibody to 2-[3-(1,3-dicarboxypropyl)ureido]pentanedioic acid (DUPA), which binds prostate-specific membrane antigen (PSMA). Exemplary schematics of DUPA conjugations are shown in **FIG.1A and FIG. 1B**. The humanized anti-CD3 antibody DUPA conjugate can bind both T cells and PSMA-positive cells, directing T cells and their cytotoxic activity to PSMA positive cells, as demonstrated in xenograft models herein. This indicates these conjugates may be useful in the treatment of prostate cancer in humans. In addition, these humanized anti-CD3 antibodies may be conjugated to other targeting agents (e.g., folic acid) to be used for the treatment of other cancers or conditions.

[0160] In one aspect, humanized anti-CD3 antibody sequences are provided having CDRs from a murine anti-CD3 antibody (e.g., SEQ ID NOS: 51-56). For example, the humanized antibody clone with light chain variant 5 and heavy chain variant 2, referred to herein as "huL5H2" (SEQ ID NOS:

39, 41) demonstrated binding activity to both human and cynomolgus monkey T cells that was comparable with that of the murine antibody. In addition, introducing four point mutations within the framework region of the heavy chain of HuL5H2, referred to herein as "huL5H2_DI" or "DI-huL5H2" (SEQ ID NOS: 39, 43) significantly reduced its in-silico immunogenicity score (K19R, S41P K89R, T90A). These mutations did not affect the antibody's expression levels, binding affinity to human and cynomolgus monkey T cells, or *in vitro* activity. huL5H2_DI conjugated with a single DUPA molecule ("huL5H2_DI-1xDUPA", SEQ ID NOS: 39, 44) demonstrated similar biophysical and pharmacological properties when compared with the double conjugate (huL5H2_DI-2xDUPA, SEQ ID NOS: 40, 44).

I. Antibodies

[0161] In one aspect, provided herein are antibodies and conjugates and/or fusions thereof. In a non-limiting example, an antibody is an anti-CD3 antibody, and further provided are conjugates and fusions of the anti-CD3 antibody. Exemplary antibody conjugates comprise an anti-CD3 antibody and a cell targeting molecule. Exemplary antibody fusions comprise an anti-CD3 antibody and a second amino acid molecule, such as another antibody or portion thereof. In some embodiments, an antibody fusion comprises at least one chain of the anti-CD3 antibody linked to the second amino acid molecule via a peptide linker.

[0162] Antibodies include functional domains or other fragments of an antibody, including: antigen binding (Fab) region, Fab', F(ab')2, F(ab')3, Fab', fragment crystallizable (Fc) region, single chain variable fragment (scFv), di-scFv, single domain immunoglobulin, trifunctional immunoglobulin, chemically linked F(ab')2, and combinations thereof. In some cases, reference to an antibody includes an antibody fragment thereof. In some cases, an antibody fragment is referred to as an antibody, for example, a Fab or scFv may be referred to as an antibody or antibody fragment. An antibody fragment further includes a complementarity determining regions (CDR), framework regions, heavy chain constant domain (e.g., CH1, CH2, CH3), light chain constant domain (CL), or any combination thereof. Non-limiting examples of heavy chain constant domain sequences of antibodies provided herein include SEQ ID NOS: 86 and 98-109. Non-limiting examples of light chain constant domain sequences of antibodies provided herein include SEQ ID NOS: 111-123. An antibody fragment includes an antigen binding fragment of an antibody.

[0163] In some instances, the antibody is a mammalian antibody or derived or modified from a mammalian antibody. The antibody may be a chimeric antibody. The antibody may be an engineered antibody. The antibody may be a recombinant antibody. The antibody may be selected from a humanized, human engineered, or fully human antibody.

[0164] As used herein, antibody and immunoglobulin may be interchangeable. The immunoglobulin may be selected from an IgA, IgD, IgE, IgG, IgM, IgY, and IgW.

[0165] Provided herein are humanized antibodies. The humanized antibody may comprise a human antibody, wherein at least one CDR of the human antibody is replaced or modified with a CDR from an antibody produced in a non-human species. The humanized antibody may comprise a human antibody, wherein at least one CDR of the human antibody is at least partially replaced or modified with a CDR from an antibody produced in a non-human species. The humanized antibody may comprise a human antibody, wherein between 1 CDR and 6 CDRs of the human antibody are at least partially replaced or modified with between 1 CDR and 6 CDRs from an antibody produced in a non-human species. The humanized antibody may comprise a human antibody, wherein at least one CDR of the human antibody is at least partially replaced or modified with a CDR from an antibody that binds an antigen in a non-human species. The antibody that binds an antigen in a non-human species or the antibody produced in a non-human species may be referred to herein as a "donor antibody." The CDR of the human antibody and/or the donor antibody may be a light chain CDR (CDRL). The CDR of the human antibody and/or the donor antibody may be a heavy chain CDR (CDRH). The CDR of the human antibody and/or the donor antibody may be selected from CDRL1 (e.g., SEQ ID NO: 51), CDRL2 (e.g., SEQ ID NO: 52), CDRL3 (e.g., SEQ ID NO: 53), CDRH1 (e.g., SEQ ID NO: 54), CDRH2 (e.g., SEQ ID NO: 55), and CDRH3 (e.g., SEQ ID NO: 56). The CDR of the human antibody may be a CDR of a human lambda light chain variable domain. The donor antibody may be cross-species reactive. The donor antibody may be cross-species reactive with two or more species selected from, by way of non-limiting example, human, mouse, monkey (e.g., cynomolgus monkey), rabbit, sheep, rat, guinea pig, goat, donkey, chicken, and hamster. The non-human species may be cross-species reactive with mouse, human, and cynomolgus monkey.

[0166] The antibodies and fragments thereof disclosed herein may comprise a lambda light chain or portion thereof. The antibodies and antibody fragments disclosed herein may comprise a kappa light chain or portion thereof. In some cases, a portion thereof includes between about 5 amino acids and about 10 amino acids, between about 5 amino acids and about 10 amino acids, or between 5 amino acids and about 15 amino acids. In some cases, a portion thereof includes at least 5 amino acids, at least about 10 amino acids, at least about 15 amino acids, at least about 20 amino acids, at least about 25 amino acids, at least about 30 amino acids, at least about 35 amino acids, at least about 40 amino acids, and at least about 50 amino acids. The antibodies and antibody fragments disclosed herein may comprise a heavy chain selected from a gamma heavy chain, a delta heavy chain, an alpha heavy chain, a mu heavy chain, an epsilon heavy chain, and portions thereof. The antibodies and antibody fragments disclosed herein may comprise a combination of a portion of the lambda light chain and a portion of the kappa light chain. The antibodies and antibody fragments disclosed herein may comprise a human kappa light chain variable domain and a human lambda light chain constant domain. The antibodies and antibody fragments disclosed herein may comprise a human lambda

light chain variable domain and a human kappa light chain constant domain. The antibodies and antibody fragments disclosed herein may comprise at least a portion of a light chain lambda variable domain and/or at least a portion of a light chain kappa constant domain. In some cases, at least a portion of the antibody fragment includes at least 5 amino acids, at least about 10 amino acids, at least about 15 amino acids, at least about 20 amino acids, at least about 25 amino acids, at least about 30 amino acids, at least about 35 amino acids, at least about 40 amino acids, and at least about 50 amino acids.

[0167] An antibody or antibody fragment provided herein may comprise two or more amino acid sequences. A first amino acid sequence may make up a first antibody chain and a second amino acid sequence may make up a second antibody chain. A first antibody chain may comprise a first amino acid sequence, and a second antibody chain may comprise a second amino acid sequence. A chain of an antibody may refer to an antibody heavy chain, an antibody light chain, or a combination of a region or all of an antibody heavy chain and a region or all of an antibody light chain. As a non-limiting example, an antibody provided herein comprises a heavy chain or fragment thereof, and a light chain or fragment thereof. Two amino acid sequences of an antibody, including two antibody chains, may be connected by one or more disulfide bonds, a chemical linker, a peptide linker, or a combination thereof. A chemical linker includes a linker via an unnatural amino acid. A chemical linker includes a chemical conjugate. A peptide linker includes any amino acid sequence joining the two amino acid sequences. In some cases, a peptide linker comprises at least about 1, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 or more amino acids. In some cases, a peptide linker may be a portion of any antibody, including a domain of an antibody, such as a variable domain, CH1, CH2, CH3, and/or CL domain. In some cases, a heavy and a light chain are connected, for example, via a peptide linker to make a single chain variable fragment (scFv). In some cases, a heavy chain and a light chain are connected, for example, by one or more disulfide bonds.

[0168] The antibodies and antibody fragments may be reactive with an antigen on an effector cell. For example, the effector cell is an immune cell. However, cells not traditionally categorized as immune cells (e.g. fibroblasts, pluripotent stem cells, adipocytes) are optionally (genetically) modified to have immune cell activity (e.g. cytotoxic activity). The immune cell may be capable of exerting a cytotoxic activity on another cell. The immune cell may be a leukocyte. The immune cell may be a lymphocyte. The immune cell may be selected from a macrophage, an erythrocyte, a thrombocyte, a neutrophil, a monocyte, a macrophage, an eosinophil, a basophil, a mast cell, a NK cell, a B-cell, or a T-cell. The immune cell may be a T cell. The T cell may be a cytotoxic T cell. The T cell may be a natural killer T cell. The effector cell may be a genetically modified cell. The effector cell may be genetically modified to have cytotoxic activity. The effector cell may be

genetically modified to have enhanced cytotoxic activity. The effector cell may be modified to have decreased cytotoxic activity.

[0169] The antibody or antibody fragment may interact with a receptor on a T-cell. The receptor may be a T-cell receptor (TCR). The TCR may comprise TCR alpha, TCR beta, TCR gamma, and/or TCR delta. The receptor may be a T-cell receptor zeta.

[0170] The antibody or antibody fragment may bind to a receptor on a lymphocyte, dendritic cell, B-cell, macrophage, monocytes, neutrophils and/or NK cells. The receptor may be an Fc receptor. The Fc receptor may be an Fc-gamma receptor, Fc-alpha receptor, and/or Fc-epsilon receptor. Fc-gamma receptors include, but are not limited to, FcγRI (CD64), FcγRIIA (CD32), FcγRIIB (CD32), FcγRIIIA (CD16a), and FcγRIIIB (CD16b). Fc-alpha receptors include, but are not limited to, FcαRI. Fc-epsilon receptors include, but are not limited to, FcεRI and FcεRII. The receptor may be CD89 (Fc fragment of IgA receptor or FCAR). The targeting agent may be selected from an anti-viral drug, an antibiotic, and an anti-parasitic drug. For example, the targeting agent antibody conjugate may bind specifically to pathogenic bacteria or fungi when the targeting agent antibody conjugate comprises a Fc receptor-binding antibody.

[0171] The antibody or antibody fragment may interact with a cluster of differentiation protein (CD) on a T cell. The CD may be selected from, by way of non-limiting example, CD3, CD8, CD25, CD45, and CD154.

[0172] The antibody or antibody fragment may interact with a co-receptor on a T-cell. The co-receptor may be selected from CD3, CD4, and CD8. CD8 may comprise CD8-alpha and/or CD8-beta chains. The antibody or antibody fragment may interact with a CD3 co-receptor. The CD3 co-receptor may be selected from CD3-gamma, CD3-delta and CD3-epsilon.

[0173] The antibody or antibody fragment may bind a cluster of differentiation 3 protein (CD3). Thus, the antibody or antibody fragment may be an anti-CD3 antibody or anti-CD3 antibody fragment. The anti-CD3 antibody or anti-CD3 antibody fragment may be a humanized anti-CD3 antibody or a humanized anti-CD3 antibody fragment. The humanized anti-CD3 antibody fragment may be a humanized anti-CD3 Fab. In mammals, CD3 is a protein complex of four distinct chains, one CD3 gamma chain, one CD3 delta chain, and two CD3 epsilon chains. Unless otherwise noted, CD3 includes any one or combination of these distinct chains. Thus, the anti-CD3 antibody or anti-CD3 antibody fragment may bind a CD3 selected from a CD3 gamma, a CD3 delta, and a CD3 epsilon. The CD3 may be a non-human CD3. The CD3 may be selected from a murine CD3, a simian CD3, and a human CD3. The anti-CD3 antibody or anti-CD3 antibody fragment may be cross-species reactive. For example, the anti-CD3 antibody or anti-CD3 antibody fragment may bind a human CD3, as well as a CD3 expressed in another species.

[0174] The antibody or antibody fragment may comprise a light chain, wherein the light chain is encoded by a nucleotide sequence selected from SEQ ID NOS: 16-18. The antibody or antibody fragment may comprise a light chain, wherein the light chain is encoded by a nucleotide sequence having at least 20 consecutive nucleotides, at least 50 consecutive nucleotides, at least 100 consecutive nucleotides, at least 200 consecutive nucleotides, at least 300 consecutive nucleotides, at least 400 consecutive nucleotides, at least 500 consecutive nucleotides, or at least 600 consecutive nucleotides, wherein the consecutive nucleotides have a sequence found in a sequence selected from SEQ ID NOS: 16-18.

[0175] The antibody or antibody fragment may comprise a light chain, wherein the light chain has an amino acid sequence selected from SEQ ID NOS: 38-40. The antibody or antibody fragment may comprise a light chain, wherein the light chain is encoded by an amino acid sequence selected from SEQ ID NOS: 38-40, wherein about 1 to about 5, about 1 to about 10, or about 1 to about 20 amino acids of SEQ ID NOS: 38-40 have been substituted with an alternate amino acid.

[0176] The antibody or antibody fragment may comprise a light chain variable domain, wherein the light chain variable domain is represented by an amino acid sequence selected from SEQ ID NOS: 28-37. The antibody or antibody fragment may comprise a light chain variable domain, wherein the light chain variable domain is represented by an amino acid of SEQ ID NO: 32. The antibody or antibody fragment may comprise a light chain variable domain, wherein the light chain variable domain is represented by an amino acid sequence selected from SEQ ID NOS: 28-37, and wherein about 1 to about 5, about 1 to about 10, or about 1 to about 20 amino acids of SEQ ID NOS: 28-37 have been substituted with an alternate amino acid. The antibody or antibody fragment may comprise a light chain variable domain, wherein the light chain variable domain is represented by an amino acid of SEQ ID NO: 32, and wherein about 1 to about 5, about 1 to about 10, or about 1 to about 20 amino acids of SEQ ID NO: 32 have been substituted with an alternate amino acid.

[0177] The antibody or antibody fragment may comprise a heavy chain, wherein the heavy chain is encoded by a nucleotide sequence selected from SEQ ID NOS: 19-22. The antibody or antibody fragment may comprise a heavy chain, wherein the heavy chain is encoded by a nucleotide sequence having at least 20 consecutive nucleotides, at least 50 consecutive nucleotides, at least 100 consecutive nucleotides, at least 200 consecutive nucleotides, at least 300 consecutive nucleotides, at least 400 consecutive nucleotides, at least 500 consecutive nucleotides, or at least 600 consecutive nucleotides, wherein the consecutive nucleotides have a sequence found in a sequence selected from SEQ ID NOS: 19-22.

[0178] The antibody or antibody fragment may comprise a heavy chain, wherein the heavy chain has an amino acid sequence selected from SEQ ID NOS: 41-44. The antibody or antibody fragment may comprise a heavy chain, wherein the heavy chain is encoded by an amino acid sequence selected

from SEQ ID NOS: 41-44, wherein about 1 to about 5, about 1 to about 10, or about 1 to about 20 amino acids of SEQ ID NOS: 41-44 have been substituted with an alternate amino acid. The antibody or antibody fragment may comprise a heavy chain, wherein the heavy chain is represented by an amino acid sequence of SEQ ID NO: 41. The heavy chain may be represented by SEQ ID NO:41, wherein at least one amino acid is replaced with an alternate amino acid. The at least one amino acid may be selected from lysine at position 19 (K19), serine at position 41 (S41), lysine at position 89 (K89), and threonine at position 90 (T90). K19 may be replaced with an arginine (K19R). S41 may be replaced with a proline (S41P). K89 may be replaced with an arginine (K89R). T90 may be replaced with an alanine (T90A). The heavy chain may be represented by SEQ ID NO: 41, wherein any combination of these replacements may be made. The heavy chain may be represented by an amino acid sequence selected from SEQ ID NOS: 45-48. The heavy chain may comprise an amino acid sequence selected from SEQ ID NOS: 49-50.

[0179] The antibody or antibody fragment may comprise a heavy chain variable domain, wherein the heavy chain variable domain is represented by an amino acid sequence selected from SEQ ID NOS: 25-27. The antibody or antibody fragment may comprise a heavy chain variable domain, wherein the heavy chain variable domain is represented by an amino acid of SEQ ID NO: 27. The antibody or antibody fragment may comprise a heavy chain variable domain, wherein the heavy chain variable domain is represented by an amino acid sequence selected from SEQ ID NOS: 25-27, and wherein about 1 to about 5, about 1 to about 10, or about 1 to about 20 amino acids of SEQ ID NOS: 25-27 have been substituted with an alternate amino acid. The antibody or antibody fragment may comprise a heavy chain variable domain, wherein the heavy chain variable domain is represented by an amino acid of SEQ ID NO: 27, and wherein about 1 to about 5, about 1 to about 10, or about 1 to about 20 amino acids of SEQ ID NO: 27 have been substituted with an alternate amino acid.

[0180] The antibody or antibody fragment may comprise a light chain, wherein the light chain comprises a variable domain. The variable domain may comprise a CDR1, a CDR2, and a CDR3, and any combination thereof. The variable domain may comprise a region between two CDRs. The region between two CDRs may be a region between the CDR1 and the CDR2, referred to herein as "LC Inter-CDR1/2 Region." The LC Inter-CDR1/2 Region may be represented by a sequence selected from SEQ ID NOS: 57-61. The LC Inter-CDR1/2 Region may be represented by a sequence of SEQ ID NO. 59. The LC Inter-CDR1/2 Region may comprise a peptide represented by SEQ ID NO. 64. The LC Inter-CDR1/2 Region may comprise a peptide represented by a sequence selected from SEQ ID NOS: 62-68. The region between two CDRs may be a region between the CDR2 and the CDR3, referred to herein as "LC Inter-CDR2/3 Region." The LC Inter-CDR2/3 Region may be represented by a sequence selected from SEQ ID NOS: 72-77.

[0181] The LC Inter-CDR1/2 Region may comprise a peptide represented by the amino acid sequence QKPDHLFR (SEQ ID NO. 64). The LC Inter-CDR1/2 Region may comprise a peptide represented by the amino acid sequence QX_1X_2DHLFR (SEQ ID NO. 65), wherein X_1 is lysine. The LC Inter-CDR1/2 Region may comprise a peptide represented by the amino acid sequence QX_1X_2DHLFR (SEQ ID NO. 65), wherein X_1 is selected from a polar amino acid and a basic amino acid. The LC Inter-CDR1/2 Region may comprise a peptide represented by the amino acid sequence QX_1X_2DHLFR (SEQ ID NO. 65), wherein X_1 is selected from a histidine and an arginine. The LC Inter-CDR1/2 Region may comprise a peptide represented by the amino acid sequence QX_1X_2DHLFR (SEQ ID NO. 65), wherein X_2 is proline. The LC Inter-CDR1/2 Region may comprise a peptide represented by the amino acid sequence QX_1X_2DHLFR (SEQ ID NO. 65), wherein X_2 is a polar amino acid. The LC Inter-CDR1/2 Region may comprise a peptide represented by the amino acid sequence QX_1X_2DHLFR (SEQ ID NO. 65), wherein X_2 is selected from a serine, a threonine, a cysteine, an asparagine and a glutamine.

[0182] The LC Inter-CDR1/2 Region may be represented by SEQ ID NO. 66 ($X_1VX_2X_3X_4X_5DHLFRGX_6X_7G$). X_1 may be tryptophan. X_2 may be glutamine. X_3 may be selected from glutamine and glutamic acid. X_4 may be lysine. X_5 may be proline. X_6 may be leucine. X_7 may be isoleucine. The LC Inter-CDR1/2 Region may be represented by SEQ ID NO. 67 ($X_1VX_2Q\ X_3X_4DHLFX_5GX_6X_7G$). X_1 may be tryptophan. X_2 may be glutamine. X_3 may be lysine. X_4 may be may be proline. X_5 may be selected from arginine and threonine. X_6 may be leucine. X_7 may be isoleucine. The LC Inter-CDR1/2 Region may be represented by SEQ ID NO. 68 ($X_1VX_2X_3X_4X_5DHLFX_6GX_7X_8G$). X_1 may be tryptophan. X_2 may be glutamine. X_3 may be selected from glutamine and glutamic acid. X_4 may be lysine. X_5 may be proline. X_5 may be selected from arginine and threonine. X_7 may be leucine. X_8 may be isoleucine. In some cases, the valine in position 2 of a sequence selected from SEQ ID NO. 66-68 is substituted with a phenylalanine.

[0183] The antibody or antibody fragment may comprise a combination of two or more peptides or polypeptides represented by the sequences disclosed herein. One of skill in the art would readily understand that a few amino acids may be substituted with alternate amino acids while maintaining the properties of the antibody or antibody fragment. A few amino acids may be about 1 to about 5 amino acids, about 1 to about 10 amino acids, or about 1 to about 20 amino acids. The next several paragraphs describe, by way of non-limiting example, the antibodies or antibody fragments disclosed herein that may comprise the combination of two or more peptides or polypeptides represented by the sequences disclosed herein.

[0184] The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region represented by a sequence selected from SEQ ID NOS: 57-61, and a heavy chain represented by an amino acid sequence selected from SEQ ID NOS: 41-44. The antibody or antibody fragment may comprise a LC

Inter-CDR1/2 Region, wherein the LC Inter-CDR1/2 Region comprises a peptide represented by a sequence selected from SEQ ID NOS: 62-68, and a heavy chain represented by an amino acid sequence selected from SEQ ID NOS: 41-44. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region represented by SEQ ID NO: 59, and a heavy chain represented by an amino acid sequence selected from SEQ ID NOS: 41-44. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region comprising a peptide represented by SEQ ID NO: 64, and a heavy chain represented by an amino acid sequence selected from SEQ ID NOS: 41-44.

[0185] The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region represented by a sequence selected from SEQ ID NOS: 57-61; and a heavy chain variable domain represented by an amino acid sequence selected from SEQ ID NOS: 25-27. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region, wherein the LC Inter-CDR1/2 Region comprises a peptide represented by a sequence selected from SEQ ID NOS: 62-68, and a heavy chain variable domain represented by an amino acid sequence selected from SEQ ID NOS: 25-27. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region represented by SEQ ID NO: 59, and a heavy chain variable domain represented by an amino acid sequence selected from SEQ ID NOS: 25-27. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region comprising a peptide represented by SEQ ID NO: 64, and a heavy chain variable domain represented by an amino acid sequence selected from SEQ ID NOS: 25-27. The heavy chain variable domain may be represented by amino acid sequence of SEQ ID NO: 27.

[0186] The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region represented by a sequence selected from SEQ ID NOS: 57-61; and a CDR represented by an amino acid sequence selected from SEQ ID NOS: 51-56. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region, wherein the LC Inter-CDR1/2 Region comprises a peptide represented by a sequence selected from SEQ ID NOS: 62-68; and a CDR represented by an amino acid sequence selected from SEQ ID NOS: 51-56. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region represented by SEQ ID NO: 59; and a CDR represented by an amino acid sequence selected from SEQ ID NOS: 51-56. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region comprising a peptide represented by SEQ ID NO: 64; and a CDR represented by an amino acid sequence selected from SEQ ID NOS: 51-56.

[0187] The antibody or antibody fragment may comprise a heavy chain, wherein the heavy chain comprises a variable domain. The variable domain may comprise a CDR1, a CDR2, a CDR3, and any combination thereof. The variable domain may comprise a region between two CDRs. The region between two CDRs may be a region between the CDR1 and the CDR2, referred to herein as "HC Inter-CDR1/2 Region." The HC Inter-CDR1/2 Region may be represented by a sequence selected from SEQ ID NOS: 70-71. The region between two CDRs may be a region between the

CDR2 and the CDR3, referred to herein as "HC Inter-CDR2/3 Region." The HC Inter-CDR2/3 Region may be represented by a sequence selected from SEQ ID NOS: 78-79. The variable domain may comprise a region next to a CDR. The region next to the CDR may be a region N-terminal to CDR1, referred to herein as "HC Pre-CDR1." The HC Pre-CDR1 Region may be represented by SEQ ID NO. 69.

[0188] The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region represented by a sequence selected from SEQ ID NOS: 57-61, and a LC Inter-CDR2/3 Region represented by a sequence selected from SEQ ID NOS: 72-77. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region, wherein the LC Inter-CDR1/2 Region comprises a peptide represented by a sequence selected from SEQ ID NOS: 62-68, and a LC Inter-CDR2/3 Region represented by a sequence selected from SEQ ID NOS: 72-77. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region represented by SEQ ID NO: 59, and a LC Inter-CDR2/3 Region represented by a sequence selected from SEQ ID NOS: 72-77. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region comprising a peptide represented by SEQ ID NO: 64, and a LC Inter-CDR2/3 Region represented by a sequence selected from SEQ ID NOS: 72-77.

[0189] The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region represented by a sequence selected from SEQ ID NOS: 57-61, and a HC Inter-CDR1/2 Region may be represented by a sequence selected from SEQ ID NOS: 70-71. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region represented by a sequence selected from SEQ ID NOS: 57-61, and a HC Inter-CDR2/3 Region may be represented by a sequence selected from SEQ ID NOS: 78-79. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region represented by a sequence selected from SEQ ID NOS: 57-61, and a HC Pre-CDR1 Region may be represented by SEQ ID NO. 69.

[0190] The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region, wherein the LC Inter-CDR1/2 Region comprises a peptide represented by a sequence selected from SEQ ID NOS: 62-68, and a HC Inter-CDR2/3 Region may be represented by a sequence selected from SEQ ID NOS: 78-79. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region, wherein the LC Inter-CDR1/2 Region comprises a peptide represented by a sequence selected from SEQ ID NOS: 62-68, and a HC Inter-CDR1/2 Region may be represented by a sequence selected from SEQ ID NOS: 70-71. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region, wherein the LC Inter-CDR1/2 Region comprises a peptide represented by a sequence selected from SEQ ID NOS: 62-68, and a HC Pre-CDR1 Region may be represented by SEQ ID NO. 69.

[0191] The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region represented by SEQ ID NO: 59, and a HC Inter-CDR1/2 Region may be represented by a sequence selected from

SEQ ID NOS: 70-71. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region represented by SEQ ID NO: 59, and a HC Inter-CDR2/3 Region may be represented by a sequence selected from SEQ ID NOS: 78-79. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region represented by SEQ ID NO: 59 and a HC Pre-CDR1 Region may be represented by SEQ ID NO. 69.

[0192] The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region comprising a peptide represented by SEQ ID NO: 64, and a HC Inter-CDR1/2 Region may be represented by a sequence selected from SEQ ID NOS: 70-71. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region comprising a peptide represented by SEQ ID NO: 64, and a HC Inter-CDR2/3 Region may be represented by a sequence selected from SEQ ID NOS: 78-79. The antibody or antibody fragment may comprise a LC Inter-CDR1/2 Region comprising a peptide represented by SEQ ID NO: 64 and a HC Pre-CDR1 Region may be represented by SEQ ID NO: 69.

[0193] *Humanized anti-CD3* antibodies *and fragments thereof*

[0194] The antibody or antibody fragment may be a humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment. The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may comprise a lambda light chain or portion thereof. The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may comprise a kappa light chain or portion thereof. The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may comprise a heavy chain selected from a gamma heavy chain, a delta heavy chain, an alpha heavy chain, a mu heavy chain, and an epsilon heavy chain. The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may comprise a combination of a portion of the lambda light chain and a portion of the kappa light chain. The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may comprise a human kappa light chain variable domain and a human lambda light chain constant domain. The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may comprise a human lambda light chain variable domain and a human kappa light chain constant domain.

[0195] The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may have a heavy chain variable region (VH) encoded by a nucleotide sequence selected from SEQ ID NOS: 3-5. The VH may be encoded by at least about 50, about 100, about 150, about 200, about 250, or about 300 consecutive nucleotides of SEQ ID NOS: 3-5. The VH may be encoded by a nucleotide sequence similar to SEQ ID NOS: 3-5. The nucleotide sequence similar to SEQ ID NOS: 3-5 may be SEQ ID NOS: 3-5 with about 1 to about 5, about 1 to about 10, about 1 to about 20, or about 1 to about 30 nucleotide substitutions. The substitutions may be an alternative nucleotide for the nucleotide in SEQ ID NOS: 3-5.

[0196] The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may have a heavy chain variable region (VH) encoded by an amino acid sequence selected from SEQ ID NOS: 25-27. The antibody or antibody fragment may comprise a VH, wherein the VH is encoded by an amino acid sequence selected from SEQ ID NOS: 25-27, wherein SEQ ID NOS: 25-27 have 1 to about 10 amino acids substituted with an alternate amino acid.

[0197] The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may have a light chain variable region (VL) encoded by a nucleotide sequence selected from SEQ ID NOS: 6-15. The VL may be encoded by at least about 50, about 100, about 150, about 200, about 250, or about 300 consecutive nucleotides of SEQ ID NOS: 6-15. The VL may be encoded by a nucleotide sequence similar to SEQ ID NOS: 6-15. The nucleotide sequence similar to SEQ ID NOS: 6-15 may be SEQ ID NOS: 6-15 with about 1 to about 5, about 1 to about 10, about 1 to about 20, or about 1 to about 30 nucleotide substitutions. The substitutions may be an alternative nucleotide for the nucleotide in SEQ ID NOS: 6-15.

[0198] The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may have a light chain variable region (VL) encoded by an amino acid sequence selected from SEQ ID NOS: 28-37. The antibody or antibody fragment may comprise a VL, wherein the VL is encoded by an amino acid sequence selected from SEQ ID NOS: 28-37, wherein SEQ ID NOS: 28-37 have about 1 to about 10 amino acids substituted with an alternate amino acid.

[0199] The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may have a light chain (LC) encoded by a nucleotide sequence selected from SEQ ID NOS: 16-18. The LC may be encoded by at least about 20 consecutive nucleotides, at least 50 consecutive nucleotides, at least 100 consecutive nucleotides, at least 200 consecutive nucleotides, at least 300 consecutive nucleotides, at least 400 consecutive nucleotides, at least 500 consecutive nucleotides, or at least 600 consecutive nucleotides of SEQ ID NOS: 16-18. The LC may be encoded by a nucleotide sequence similar to SEQ ID NOS: 16-18. The nucleotide sequence similar to SEQ ID NOS: 16-18 may be SEQ ID NOS: 16-18 with about 1 to about 10, about 1 to about 20, about 1 to about 30, about 1 to about 40, about 1 to about 50, or about 1 to about 60 substitutions. The substitutions may be an alternative nucleotide for the nucleotide in SEQ ID NOS: 16-18.

[0200] The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may have a light chain (LC) encoded by an amino acid sequence selected from SEQ ID NOS: 38-40. The antibody or antibody fragment may comprise a LC, wherein the LC is encoded by an amino acid sequence selected from SEQ ID NOS: 38-40, wherein SEQ ID NOS: 38-40 have about 1 to about 5, about 1 to about 10, or about 1 to about 20 amino acids substituted with an alternate amino acid.

[0201] The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may have a heavy chain (HC) encoded by a nucleotide sequence selected from SEQ ID NOS: 19-22. The HC

may be encoded by at least about 20 consecutive nucleotides, at least 50 consecutive nucleotides, at least 100 consecutive nucleotides, at least 200 consecutive nucleotides, at least 300 consecutive nucleotides, at least 400 consecutive nucleotides, at least 500 consecutive nucleotides, or at least 600 consecutive nucleotides of SEQ ID NOS: 19-22. The HC may be encoded by a nucleotide sequence similar to SEQ ID NOS: 19-22. The nucleotide sequence similar to SEQ ID NOS: 19-22 may be SEQ ID NOS: 19-22 with about 1 to about 10, about 1 to about 20, about 1 to about 30, about 1 to about 40, about 1 to about 50, or about 1 to about 60 substitutions. The substitutions may be an alternative nucleotide for the nucleotide in SEQ ID NOS: 19-22.

[0202] The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may have a heavy chain (HC) encoded by an amino acid sequence selected from SEQ ID NOS: 41-44. The antibody or antibody fragment may comprise a HC, wherein the HC is encoded by an amino acid sequence selected from SEQ ID NOS: 41-44, wherein SEQ ID NOS: 41-44 have about 1 to about 5, about 1 to about 10, or about 1 to about 20 amino acids substituted with an alternate amino acid.

[0203] The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may have a light chain encoded by a nucleotide sequence selected from SEQ ID NOS: 17 and 18 and a heavy chain encoded by a nucleotide sequence selected from SEQ ID NOS: 21 and 22. The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment has a light chain encoded by SEQ ID NO: 17 and a heavy chain encoded by SEQ ID NO: 21. The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment has a light chain encoded by SEQ ID NO: 17 and a heavy chain encoded by SEQ ID NO: 22. The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment has a light chain encoded by SEQ ID NO: 18 and a heavy chain encoded by SEQ ID NO: 21. The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment has a light chain encoded by SEQ ID NO: 18 and a heavy chain encoded by SEQ ID NO: 22.

[0204] The light chain may be encoded by a nucleotide sequence similar to SEQ ID NOS: 17 and 18. The nucleotide sequence similar to SEQ ID NOS: 17 and 18 may be SEQ ID NOS: 17 and 18 with about 1 to about 10, about 1 to about 20, about 1 to about 30, about 1 to about 40, about 1 to about 50, or about 1 to about 60 substitutions. The substitutions may be an alternative nucleotide for the nucleotide in SEQ ID NOS: 17 and 18. The heavy chain may be encoded by a nucleotide sequence similar to SEQ ID NOS: 21 and 22. The nucleotide sequence similar to SEQ ID NOS: 21 and 22 may be SEQ ID NOS: 21 and 22 with about 1 to about 10, about 1 to about 20, about 1 to about 30, about 1 to about 40, about 1 to about 50, or about 1 to about 60 substitutions. The substitutions may be an alternative nucleotide for the nucleotide in SEQ ID NOS: 21 and 22.

[0205] In one aspect, disclosed herein is an antibody comprising an amino acid sequence comprising SEQ ID NOS: 55 and 96. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some

embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0206] In another aspect, disclosed herein is an antibody comprising an amino acid sequence comprising SEQ ID NOS: 54 and 55, and one or more of SEQ ID NOS: 87 and 96. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody

of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0207] In another aspect, disclosed herein is an antibody comprising: (a) a first amino acid sequence comprising SEQ ID NO: 96; and (b) a second amino acid sequence comprising SEQ ID NO: 51, and one or more of SEQ ID NOS: 97, 59, and 74. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab

domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0208] In another aspect, disclosed herein is an antibody comprising: (a) a first amino acid sequence comprising SEQ ID NO: 96; and (b) a second amino acid sequence comprising one or more of SEQ ID NOS: 59 and 74. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed

herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0209] In another aspect, disclosed herein is an antibody comprising: (a) a first amino acid sequence comprising SEQ ID NO: 54; and (b) a second amino acid sequence comprising SEQ ID NO: 51 and one or more of SEQ ID NOS: 97, 59, and 74. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a

chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0210] In another aspect, disclosed herein is an antibody comprising: (a) a first amino acid sequence comprising SEQ ID NO: 54; and (b) a second amino acid sequence comprising one or more of SEQ ID NOS: 59 and 74. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some

embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0211] In another aspect, disclosed herein is an antibody comprising: (a) a first amino acid sequence comprising SEQ ID NOS: 54 and 55; and (b) a second amino acid sequence comprising one or more of SEQ ID NOS: 97, 59, and 74. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA , or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some

embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0212] In another aspect, disclosed herein is an antibody comprising: (a) a first amino acid sequence comprising SEQ ID NOS: 54 and 92; and (b) a second amino acid sequence comprising one or more of SEQ ID NOS: 97, 59, and 74. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody

specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0213] In another aspect, disclosed herein is an antibody comprising: (a) a first amino acid sequence comprising SEQ ID NO: 55; and (b) a second amino acid sequence comprising one or more of SEQ ID NOS: 59 and 74. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule

interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0214] In another aspect, disclosed herein is an antibody comprising: (a) a first amino acid sequence comprising SEQ ID NOS: 92 and 96; and (b) a second amino acid sequence comprising one or more of SEQ ID NOS: 97, 59, and 74. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some

embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0215] In another aspect, disclosed herein is an antibody comprising: (a) a first amino acid sequence comprising one or more of SEQ ID NOS: 54, 55, 56, and 96; and (b) a second amino acid sequence comprising one or more of SEQ ID NOS: 59 and 74. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the

unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0216] In another aspect, disclosed herein is an antibody comprising: an amino acid sequence comprising one or more of SEQ ID NOS: 51, 52, and 53, and one or more of SEQ ID NOS: 59 and 74. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 92. In some

embodiments, the second amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the second amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0217] In another aspect, disclosed herein is an antibody comprising: an amino acid sequence comprising SEQ ID NO: 74. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 54. In some

embodiments, the second amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the second amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0218] In another aspect, disclosed herein is an antibody comprising: (a) a first amino acid sequence comprising SEQ ID NOS: 54, 55, 56, 87, 92, and 96; and (b) a second amino acid sequence comprising SEQ ID NOS: 51, 52, 53, 97, 59, and 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some

embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0219] In another aspect, disclosed herein is an antibody comprising: an amino acid sequence comprising one or more of SEQ ID NOS: 54-56, 69-71, 78, 79, and 87-96. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 69. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 70. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 71. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 78. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 79. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 88. In some embodiments, the first amino acid sequence comprises

SEQ ID NO: 89. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 90. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 91. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 93. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 94. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 95. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 57. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 58. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 60. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 61. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 62. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 63. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 64. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 65. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 66. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 67. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 68. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 72. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 73. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 75. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 76. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 77. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some

embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0220] In another aspect, disclosed herein is an antibody comprising: an amino acid sequence comprising one or more of SEQ ID NOS: 51-53, 57-68, 72-77, and 97. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 57. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 58. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 60. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 61. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 62. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 63. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 64. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 65. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 66. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 67. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 68. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 72. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 73. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 75. In some embodiments, the first amino acid

sequence comprises SEQ ID NO: 76. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 77. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 69. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 70. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 71. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 78. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 79. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 88. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 89. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 90. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 91. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 93. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 94. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 95. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the antibody comprises an unnatural amino acid. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the second amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a

chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0221] In another aspect, disclosed herein is an antibody comprising: (a) an amino acid sequence comprising one or more of SEQ ID NOS: 54, 55, and 56; and (b) an unnatural amino acid. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some

embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0222] In another aspect, disclosed herein is an antibody comprising: (a) an amino acid sequence comprising SEQ ID NO: 96; and (b) an unnatural amino acid. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate,

or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0223] In another aspect, disclosed herein is an antibody comprising: (a) an amino acid sequence comprising one or more of SEQ ID NOS: 54, 55, 56, and 96; and (b) an unnatural amino acid. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some

embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0224] In another aspect, disclosed herein is an antibody comprising: (a) an amino acid sequence comprising one or more of SEQ ID NOS: 51, 52, and 53; and (b) an unnatural amino acid. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In

some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the second amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0225] In another aspect, disclosed herein is an antibody comprising: (a) an amino acid sequence comprising one or more of SEQ ID NOS: 97, 59, and 74; and (b) an unnatural amino acid. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 87. In some

embodiments, the second amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the second amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0226] In another aspect, disclosed herein is an antibody comprising: (a) an amino acid sequence comprising one or more of SEQ ID NOS: 51, 52, 53, 97, 59, and 74; and (b) an unnatural amino acid. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the first amino acid

sequence comprises SEQ ID NO: 74. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the second amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0227] In another aspect, disclosed herein is an antibody comprising: (a) one or more of SEQ ID NOS: 54, 55, 56, 96, 51, 52, 53, 97, 59, and 74; and (b) an unnatural amino acid. In some embodiments, the antibody comprises a first amino acid sequence and a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some

embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0228] In another aspect, disclosed herein is an antibody comprising: (a) an amino acid sequence comprising one or more of SEQ ID NOS: 54-56, 69-71, 78, 79, and 87-96; and (b) an unnatural amino acid. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 69. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 70. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 71. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 78. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 79. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 88. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 89. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 90. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 91. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 93. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 94. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 95. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 57. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 58. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 60. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 61. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 62. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 63. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 64. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 65. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 66. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 67. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 68. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 72. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 73. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 75. In some embodiments,

the second amino acid sequence comprises SEQ ID NO: 76. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 77. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0229] In another aspect, disclosed herein is an antibody comprising: (a) an amino acid sequence comprising one or more of SEQ ID NOS: 51-53, 57-68, 72-77, and 97; and (b) an unnatural amino acid. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 57. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 58. In some embodiments, the

first amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 60. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 61. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 62. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 63. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 64. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 65. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 66. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 67. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 68. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 72. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 73. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 75. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 76. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 77. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 69. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 70. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 71. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 78. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 79. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 88. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 89. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 90. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 91. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 93. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 94. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 95. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the second amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the antibody comprises a cell-targeting molecule linked to

the antibody via the unnatural amino acid. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the antibody specifically binds CD3. In some embodiments, the antibody is cross-species reactive. In some embodiments, the antibody is cross-species reactive with human and monkey antigens. In some embodiments, the antibody comprises a cross-species reactive CDR. In some embodiments, the antibody is a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the antibody of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the antibody of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the antibody of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the antibody of any of the preceding embodiments to a patient.

[0230] In another aspect, disclosed herein is a composition comprising: (a) an amino acid sequence comprising one or more of SEQ ID NOS: 54, 55, and 56, and an unnatural amino acid; and (b) a cell-targeting molecule linked to the amino acid sequence via the unnatural amino acid. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the composition further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments,

the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the composition specifically binds CD3. In some embodiments, the composition is cross-species reactive. In some embodiments, the composition is cross-species reactive with human and monkey antigens. In some embodiments, the composition comprises a cross-species reactive CDR. In some embodiments, the composition comprises a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the composition of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the composition of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the composition of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the composition of any of the preceding embodiments to a patient.

[0231] In another aspect, disclosed herein is a composition comprising: (a) an amino acid sequence comprising SEQ ID NO: 96, and an unnatural amino acid; and (b) a cell-targeting molecule linked to the amino acid sequence via the unnatural amino acid. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second

amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the composition specifically binds CD3. In some embodiments, the composition is cross-species reactive. In some embodiments, the composition is cross-species reactive with human and monkey antigens. In some embodiments, the composition comprises a cross-species reactive CDR. In some embodiments, the composition comprises a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the composition of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the composition of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the composition of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the composition of any of the preceding embodiments to a patient.

[0232] In another aspect, disclosed herein is a composition comprising: (a) an amino acid sequence comprising one or more of SEQ ID NOS: 54, 55, 56, and 96, and an unnatural amino acid; and (b) a cell-targeting molecule linked to the amino acid sequence via the unnatural amino acid. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the composition further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the first amino acid

sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the composition specifically binds CD3. In some embodiments, the composition is cross-species reactive. In some embodiments, the composition is cross-species reactive with human and monkey antigens. In some embodiments, the composition comprises a cross-species reactive CDR. In some embodiments, the composition comprises a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the composition of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the composition of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the composition of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the composition of any of the preceding embodiments to a patient.

[0233] In another aspect, disclosed herein is a composition comprising: (a) a first amino acid sequence comprising one or more of SEQ ID NOS: 51, 52, and 53; (b) a second amino acid sequence comprising an unnatural amino acid; and (c) a cell-targeting molecule linked to the second amino acid sequence via the unnatural amino acid. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the first amino acid sequence comprises SEQ ID

NO: 52. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the second amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the composition specifically binds CD3. In some embodiments, the composition is cross-species reactive. In some embodiments, the composition is cross-species reactive with human and monkey antigens. In some embodiments, the composition comprises a cross-species reactive CDR. In some embodiments, the composition comprises a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the composition of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the composition of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the composition of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the composition of any of the preceding embodiments to a patient.

[0234] In another aspect, disclosed herein is a composition comprising: (a) a first amino acid sequence comprising one or more of SEQ ID NOS: 97, 59, and 74; (b) a second amino acid sequence

comprising an unnatural amino acid; and (c) a cell-targeting molecule linked to the second amino acid sequence via the unnatural amino acid. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the second amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the composition specifically binds CD3. In some embodiments, the composition is cross-species reactive. In some embodiments, the composition is cross-species reactive with human and monkey antigens. In some embodiments, the composition comprises a cross-species reactive CDR. In some embodiments, the composition comprises a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the composition of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the composition of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the composition of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method

for treating cancer by administering the composition of any of the preceding embodiments to a patient.

[0235] In another aspect, disclosed herein is a composition comprising: (a) a first amino acid sequence comprising one or more of SEQ ID NOS: 51, 52, 53, 97, 59, and 74; (b) a second amino acid sequence comprising an unnatural amino acid; and (c) a cell-targeting molecule linked to the second amino acid sequence via the unnatural amino acid. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the second amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the composition specifically binds CD3. In some embodiments, the composition is cross-species reactive. In some embodiments, the composition is cross-species reactive with human and monkey antigens. In some embodiments, the composition comprises a cross-species reactive CDR. In some embodiments, the composition comprises a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the composition of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the

composition of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the composition of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the composition of any of the preceding embodiments to a patient.

[0236] In another aspect, disclosed herein is an composition comprising: (a) an amino acid sequence comprising one or more of SEQ ID NOS: 54-56, 69-71, 78, 79, and 87-96, and an unnatural amino acid; and (b) a cell-targeting molecule linked to the amino acid sequence via the unnatural amino acid. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 69. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 70. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 71. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 78. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 79. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 88. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 89. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 90. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 91. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 93. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 94. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 95. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 57. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 58. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 60. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 61. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 62. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 63. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 64. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 65. In some embodiments, the second

amino acid sequence comprises SEQ ID NO: 66. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 67. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 68. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 72. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 73. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 75. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 76. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 77. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the first amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the composition specifically binds CD3. In some embodiments, the composition is cross-species reactive. In some embodiments, the composition is cross-species reactive with human and monkey antigens. In some embodiments, the composition comprises a cross-species reactive CDR. In some embodiments, the composition comprises a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the composition of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the composition of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the composition of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the composition of any of the preceding embodiments to a patient.

[0237] In another aspect, disclosed herein is a composition comprising: (a) an amino acid sequence comprising one or more of SEQ ID NOS: 51-53, 57-68, 72-77, and 97, and an unnatural amino acid;

and (b) a cell-targeting molecule linked to the amino acid sequence via the unnatural amino acid. In some embodiments, the amino acid sequence is a first amino acid sequence, wherein the antibody further comprises a second amino acid sequence. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 51. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 52. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 53. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 57. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 58. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 59. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 60. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 61. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 62. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 63. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 64. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 65. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 66. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 67. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 68. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 72. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 73. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 74. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 75. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 76. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 77. In some embodiments, the first amino acid sequence comprises SEQ ID NO: 97. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 54. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 55. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 56. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 69. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 70. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 71. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 78. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 79. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 87. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 88. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 89. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 90. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 91. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 92. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 93. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 94. In some embodiments, the second amino acid sequence comprises SEQ ID NO: 95. In

some embodiments, the second amino acid sequence comprises SEQ ID NO: 96. In some embodiments, the first amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 39. In some embodiments, the second amino acid sequence comprises at least 95% sequence identity with SEQ ID NO: 44. In some embodiments, the unnatural amino acid is para-acetylphenylalanine. In some embodiments, the unnatural amino acid is located within a CH1 sequence of the second amino acid sequence. In some embodiments, the CH1 sequence comprises SEQ ID NO: 86. In some embodiments, the cell-targeting molecule is a non-peptide compound. In some embodiments, the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA). In some embodiments, the cell-targeting molecule is DUPA. In some embodiments, the composition specifically binds CD3, or a derivative there. In some embodiments, the cell-targeting molecule is folate, or a derivative thereof. In some embodiments, the composition is cross-species reactive. In some embodiments, the composition is cross-species reactive with human and monkey antigens. In some embodiments, the composition comprises a cross-species reactive CDR. In some embodiments, the composition comprises a humanized antibody. In some embodiments, the first and second amino acid sequences are linked. In some embodiments, the first and second amino acid sequences are linked as a single fusion sequence. In some embodiments, the first and second amino acid sequences are linked by a chemical bond. In some embodiments, the first and second amino acid sequences are linked by a disulfide bond. In some embodiments, disclosed herein is a fusion antibody comprising at least a portion of the composition of any of the preceding embodiments and at least a portion of another antibody. In some embodiments, the fusion antibody is a bispecific antibody comprising an Fab domain of the composition of any of the preceding claims and an Fab domain of another antibody. In some embodiments, disclosed herein is a method for treating a disease by administering the composition of any of the preceding embodiments to a patient. In some embodiments, disclosed herein is a method for treating cancer by administering the composition of any of the preceding embodiments to a patient.

[0238] The anti-CD3 antibody may comprise a heavy chain region selected from SEQ ID NOS: 24-27, 42-50,54-56, 69-71, and 78-81 and a light chain region selected from SEQ ID NOS: 23, 28-40, 51-53, 57, 58-68, 72-77, and 82-83. As a non-limiting example, an antibody may comprise heavy chain SEQ ID NO: 44, and light chain SEQ ID NO: 39.

[0239] The anti-CD3 antibody may comprise any combination of sequences selected from those presented in **Tables 35-39** herein.

Sites for Conjugation

[0240] The antibody or antibody fragment may comprise one or more sites for conjugation to another molecule, for example, a non-immunoglobulin peptide, an additional antibody or additional antibody fragment, a targeting agent, a non-peptide structure, or a therapeutic compound. The one or

more sites may comprise a lysine or a cysteine. In one embodiment, the one or more sites may comprise one or more unnatural amino acids. In one embodiment, the one or more unnatural amino acids of the antibody or antibody fragment consist of p-acetylphenylalanine (pAcF). Optionally, the one or more unnatural amino acids of the antibody or antibody fragment consist of selenocysteine. Optionally, the one or more unnatural amino acids consist of (a) various substituted tyrosine and phenylalanine analogues such as *O*-methyl-L-tyrosine, *p*-amino-L-phenylalanine, 3-nitro-L-tyrosine, *p*-nitro-L-phenylalanine, *m*-methoxy-L-phenylalanine and *p*-isopropyl-L-phenylalanine; (b) amino acids with aryl azide and benzophenone groups that may be photo-cross-linked; (c) amino acids that have unique chemical reactivity including acetyl-L-phenylalanine, *m*-acetyl-L-phenylalanine, *O*-allyl-L-tyrosine, *O*-(2-propynyl)-L-tyrosine, *p*-ethylthiocarbonyl-L-phenylalanine, and *p*-(3-oxobutanoyl)-L-phenylalanine; (d) heavy-atom-containing amino acids for phasing in X-ray crystallography including *p*-iodo and *p*-bromo-L-phenylalanine; (e) the redox-active amino acid dihydroxy-L-phenylalanine; (f) glycosylated amino acids including b-N-acetylglucosamine-*O*-serine and a-N-acetylgalactosamine-*O*-threonine; (g) fluorescent amino acids with naphthyl, dansyl, and 7-aminocoumarin side chains; (h) photocleavable and photoisomerizable amino acids with azobenzene and nitrobenzyl Cys, Ser, and Tyr side chains; (i) the phosphotyrosine mimetic *p*-carboxymethyl-L-phenylalanine; (j) the glutamine homologue homoglutamine; (k) 2-aminooctanoic acid; (l) and any combination of (a) – (k) thereof. Optionally, the one or more unnatural amino acids consist of at least one oxime, carbonyl, dicarbonyl, hydroxylamine, cyclooctyne, aryl/alkyl azides, norbornene, cyclopropene, trans-cyclooctene, tetrazine group, and any combination thereof. The one or more unnatural amino acids may be genetically encoded. The one or more unnatural amino acids may be incorporated into the antibody or antibody fragment. The one or more unnatural amino acids may be site-specifically incorporated the antibody or antibody fragment. The targeting agent antibody conjugate may comprise two or more unnatural amino acids. The targeting agent antibody conjugate may comprise three or more unnatural amino acids. The targeting agent antibody conjugate may comprise four or more unnatural amino acids. The one or more unnatural amino acids may replace one or more amino acid residues in the antibody or antibody fragment. The one or more unnatural amino acids may replace an amino acid residue in a heavy chain of the antibody or antibody fragment. The one or more unnatural amino acids of the antibody or antibody fragment replace an amino acid residue in a light chain of the antibody or antibody fragment. The one or more unnatural amino acids of the antibody or antibody fragment replace an amino acid residue in a variable region of the antibody or antibody fragment.

II. Targeting Agents

[0241] In another aspect, provided herein are antibody conjugates and antibody fusions comprising an antibody or antibody fragment disclosed herein, or an antibody or antibody fragment derived or

otherwise modified from an antibody or antibody fragment disclosed herein. As a non-limiting example, provided are targeting agent antibody conjugates comprising an antibody or antibody fragment disclosed herein conjugated to a targeting agent. It should be understood that the targeting agents described herein may be slightly modified by conjugation to the antibody or antibody fragment or to a linker that connects the targeting agent to the antibody or antibody fragment, and that targeting agents as disclosed herein include these slightly modified forms, but otherwise remain structurally and functionally similar to the therapeutic agent as known in the art. The targeting agent may be selected from a small molecule, a cell-targeting molecule, a ligand, a protein, a peptide, a peptoid, a DNA aptamer, a peptide nucleic acid (PNA), a vitamin, a substrate, or a substrate analog. The peptide may comprise a cyclic peptide or a linear peptide. The targeting agent may comprise a ligand. The targeting agent may comprise at least a portion of a ligand. The ligand may be a chemical ligand. The ligand may be a hormonal ligand. The ligand may be a peptide ligand. The ligand may be a protein ligand. The targeting agent may be derivatized (e.g. with a naturally occurring protein or peptide). The targeting agent may be a compound. The targeting agent may be a (non-peptidic) small molecule. The targeting agent may bind a target cell. The targeting agent may bind a cell surface protein or a cell surface marker on a cell. The targeting agent may bind a protein, a peptide, or a biomolecule, wherein the protein, the peptide, or the biomolecule is not bound to a cell. The protein, peptide or biomolecule may be circulating in a bloodstream. The protein, peptide, or biomolecule may be a component of extracellular matrix. The protein may be an enzyme. The enzyme may have enzymatic activity. A biomolecule, by non-limiting example, may be selected from a fiber, a biopolymer (e.g. collagen), a glycan, a proteoglycan, a lipid, a sterol, a carbohydrate, a nucleic acid, and a cellular fragment.

[0242] The targeting agent of the targeting agent antibody conjugate may have a therapeutic effect because it brings a cytotoxic effector cell in proximity of a target cell. The therapeutic effect on the intended indication of the targeting agent antibody conjugate may be due to the targeting agent antibody conjugate recruiting a cytotoxic effector cell to the target cell. The therapeutic effect on the intended indication of the targeting agent antibody conjugate may be wholly due to the targeting agent antibody conjugate recruiting a cytotoxic effector cell to the target cell. The therapeutic effect on the intended indication of the targeting agent antibody construct may be predominantly due to the targeting agent antibody conjugate recruiting a cytotoxic effector cell to the target cell.

[0243] The therapeutic effect of the intended indication may be due to the targeting agent antibody conjugate recruiting a protein, peptide, or biomolecule to the target cell. The therapeutic effect of the intended indication may wholly due to the targeting agent antibody conjugate recruiting a protein, peptide, or biomolecule to the target cell. The therapeutic effect on the intended indication may be at

least partially due to the targeting agent antibody conjugate recruiting a protein, peptide or biomolecule to the target cell.

[0244] The targeting agent alone may be a targeting agent that has a therapeutic effect (e.g., a drug). The targeting agent alone may not be a targeting agent that has any therapeutic effect. The targeting agent alone may or may not have any therapeutic effect towards an intended indication of the targeting agent antibody conjugate. The targeting agent may or may not have a therapeutic effect towards the intended indication of the targeting agent antibody conjugate without being conjugated to the anti-CD3 antibody or antibody fragment. The dose of the therapeutic agent when administered as part of the targeting agent antibody conjugate to provide a therapeutic effect may or may not have a therapeutic effect when the therapeutic agent is administered alone at that dose. The targeting agent of the targeting agent antibody conjugate may or may not be intended to have any therapeutic effect besides recruiting the cytotoxic effector cell to the target cell. The targeting agent of the targeting agent antibody conjugate may or may not have a therapeutic effect on the target cell, wherein the therapeutic effect is negligible relative to the therapeutic effect of recruiting the cytotoxic effector cell, protein, peptide or biomolecule to the target cell. The targeting agent of the targeting agent antibody conjugate may or may not have a therapeutic effect on the target cell, wherein the therapeutic effect is less than the therapeutic effect of recruiting the cytotoxic effector cell, protein, peptide, or biomolecule to the target cell. The binding of the targeting agent to the target cell may induce an unintentional response from the target cell. The binding of the targeting agent to the target cell may induce an unintentional therapeutic effect in addition to the therapeutic effect of recruiting the cytotoxic effector cell, protein, peptide, or biomolecule to the target cell.

[0245] The targeting agent may bind a cell surface molecule on a cancer cell. The cancer cell may be selected from, by way of non-limiting example, a breast cancer cell, a brain cancer cell, a pancreatic cancer cell, a skin cancer cell, a lung cancer cell, a liver cancer cell, a gall bladder cancer cell, a colon cancer cell, an ovarian cancer cell, a prostate cancer cell, a uterine cancer cell, a bone cancer cell, and a blood cancer (leukemic) cancer cell. The cell surface molecule may be selected from, by way of non-limiting example, a G protein coupled receptor (GPCR), a kinase receptor, a cytokine receptor, and a chemokine receptor. The cell surface molecule may be selected from, by way of non-limiting example, a CD20, a CD19, a CD22, a CS1, a BCMA, a CD123, a CD33, a CLL-1, a GD-2, a EGFR, a EGRF vIII, a mesothelin, a CD38, a Her2/ErbB2, a Patched receptor (PTCH), a Smoothened receptor (SMO), a FKBP-12, an estrogen receptor, a vascular endothelial growth factor (VEGFR1, VEGFR2), an epidermal growth factor receptor, a fibroblast growth factor receptor (FGFR), a folate receptor, a cholecystokinin B receptor, a gonadotropin-releasing hormone receptor, a somatostatin receptor, a gastrin-releasing peptide receptor, a neurokinin receptor, a melanocortin receptor, a neurotensin receptor, a neuropeptide Y receptor, and an integrin.

[0246] The targeting agent may bind a prostate-specific membrane antigen (PSMA). The targeting agent that binds PSMA may be 2-[3-(1,3-dicarboxypropyl)ureido]pentanedioic acid (DUPA), or an analog thereof (*see, e.g.,* **FIGS. 20A** and **20B**). An analog thereof may be a moiety that is based on DUPA and preserves PSMA binding. The DUPA analog may preserve a significant portion of the structure of DUPA. Moreover, the DUPA analog may be a slightly modified form of DUPA because of its conjugation to a linker or an antibody/antibody fragment. For example, the DUPA analog may be slightly modified due to conjugation of a DUPA carboxyl group to the linker or antibody/antibody fragment. In addition, DUPA may be slightly modified because of its conjugation to a linker or an antibody/antibody fragment, but maintain its PSMA binding properties. As used herein, the term "DUPA" comprises 2-[3-(1,3-dicarboxypropyl)ureido]pentanedioic acid, analogs, and stereoisomers thereof as described above.

[0247] The targeting agent may bind a folate receptor protein (FR). The targeting agent that binds FR may be N-(4-{[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino}benzoyl)-L-glutamic acid (folic acid), or an analog thereof (*see, e.g.,* **FIGS. 20C** and **20D**). An analog thereof may be a moiety that is based on folic acid and preserves FR binding. The folic acid analog may preserve a significant portion of the structure of folic acid. Moreover, the folic acid analog may be a slightly modified form of folic acid because of its conjugation to a linker or an antibody/antibody fragment. For example, the folic acid analog may be slightly modified due to conjugation of a folate carboxyl group to the linker or antibody/antibody fragment. In addition, folic acid may be slightly modified because of its conjugation to a linker or an antibody/antibody fragment, but maintain its FR binding properties. As used herein, the term "folic acid" or "folate" comprises N-(4-{[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino}benzoyl)-L-glutamic acid and analogs thereof as described above.

III. Targeting Agent Antibody Conjugates

[0248] In another aspect, provided herein are targeting agent antibody conjugates comprising antibodies and antibody fragments disclosed herein conjugated to one or more targeting agents. The targeting agent antibody conjugates disclosed herein may comprise one or more antibodies or antibody fragments. The targeting agent antibody conjugates disclosed herein may comprise one or more targeting agents, which may be referred to herein as multivalent targeting agent antibody conjugates. For example, the targeting agent antibody conjugate may comprise an antibody or antibody fragment with a first targeting agent conjugated to the light chain and a second targeting agent conjugated to the heavy chain (*see, e.g.,* **FIG. 1A, FIG. 1C**). The targeting agent antibody conjugate may comprise an antibody or antibody fragment with a first targeting agent conjugated to the light chain and a second targeting agent conjugated to the light chain. The targeting agent antibody conjugate may comprise an antibody or antibody fragment with a first targeting agent

conjugated to the heavy chain and a second targeting agent conjugated to the heavy chain. The targeting agent antibody may comprise an antibody or antibody fragment with only a first targeting agent conjugated to the heavy chain, and an unconjugated light chain (*see, e.g.,* **FIG. 1B, FIG. 1D).**

[0249] The targeting agent antibody conjugates disclosed herein may comprise one or more natural amino acids, wherein the one or more targeting agents are conjugated to the one or more natural amino acids. The one or more natural amino acids, by way of non-limiting example, may be selected from a lysine and a cysteine. The targeting agent antibody conjugates disclosed herein may comprise one or more unnatural amino acids, wherein the one or more targeting agents are conjugated to the one or more unnatural amino acids. The one or more targeting agents may be conjugated to the one or more natural or unnatural amino acids via a linker.

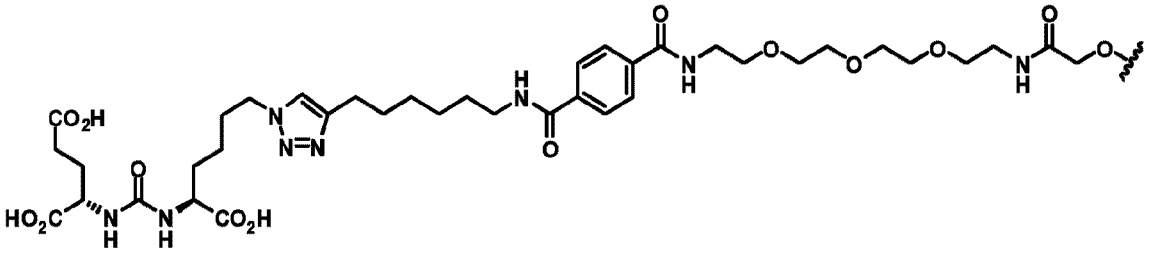
[0250] The targeting agent antibody conjugates may bring the effector cell in proximity of a target cell, such that the effector cell may have cytotoxic activity towards the target cell. The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment conjugated to a targeting agent. The humanized anti-CD3 antibody fragment may be a humanized anti-CD3 Fab. The humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment may comprise a peptide or polypeptide represented by a sequence selected from SEQ ID NOS: 23-79, and combinations thereof.

[0251] The targeting agent antibody conjugates may bring a T cell in proximity of a prostate cancer cell. The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment conjugated to a compound that binds prostate-specific membrane antigen (PSMA). The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment conjugated to a peptide that binds prostate-specific membrane antigen (PSMA). The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment conjugated to 2-[3-(1,3-dicarboxypropyl)ureido]pentanedioic acid (DUPA). The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 Fab conjugated to DUPA. The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 Fab conjugated to more than one DUPA. The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 Fab conjugated to two DUPAs, wherein a first DUPA is conjugated to a light chain of the humanized anti-CD3 antibody or humanized anti-CD3 Fab and the second DUPA is conjugated to a heavy chain of the humanized anti-CD3 antibody or humanized anti-CD3 Fab. The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 Fab conjugated to more than one DUPA and a peptide or protein represented by an amino acid sequence selected from SEQ ID NOS.

80-83. The targeting agent antibody conjugate may be depicted in **FIG. 1A and FIG. 1B**. Portions of the targeting agent antibody conjugate may be depicted in **FIG. 19A** or **FIG. 19B**.

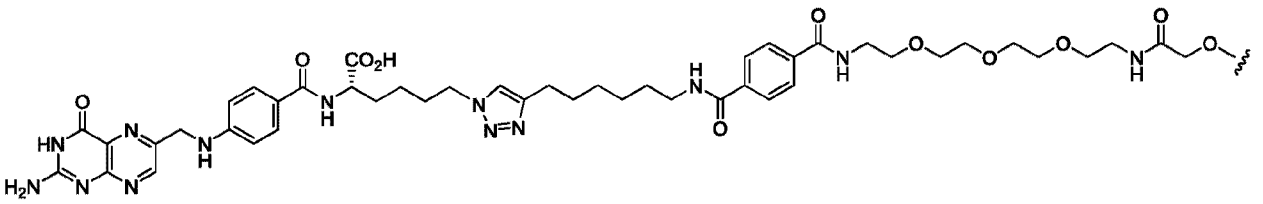
[0252] The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment conjugated to a compound that binds folate receptor protein (FR). The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment conjugated to a peptide that binds folate receptor protein. The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment conjugated to N-(4-{[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino}benzoyl)-L-glutamic acid. The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 Fab conjugated to folic acid. The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 Fab conjugated to more than one folic acid molecule. The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 Fab conjugated to two folic acids, wherein a first folic acid is conjugated to a light chain of the humanized anti-CD3 antibody or humanized anti-CD3 Fab and the second folic acid is conjugated to a heavy chain of the humanized anti-CD3 antibody or humanized anti-CD3 Fab. The targeting agent antibody conjugates may comprise a humanized anti-CD3 antibody or humanized anti-CD3 Fab conjugated to more than one folic acid and a peptide or protein represented by an amino acid sequence selected from SEQ ID NOS. 80-83. The targeting agent antibody conjugate may be depicted in **FIG. 1C and FIG. 1D**. Portions of the targeting agent antibody conjugate may be depicted in **FIG. 19C** or **FIG. 19D**.

[0253] DUPA may be conjugated to the humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment via a linker. DUPA and the linker together may be a compound of Formula (VI):



Formula (VI).

[0254] Folic acid may be conjugated to the humanized anti-CD3 antibody or humanized anti-CD3 antibody fragment via a linker. Folic acid and the linker together may be a compound of Formula (VII):



Formula (VII).

[0255] Formula (VI) may be referred to herein as "p-TriA" or "P-TriA." P-TriA may be conjugated to an antibody, e.g., a Fab, via an unnatural amino acid. The Fab may comprise a light chain encoded by a nucleotide sequence selected from SEQ ID NOS: 17 and 18 and a heavy chain encoded by a nucleotide sequence selected from SEQ ID NOS: 21 and 22 (DI-huL5H2), or selected from SEQ ID NOS: 19 and 20 (huL5H2). The resulting cross-species reactive PSMA-binding/anti-CD3 antibody conjugate is referred to herein as huL5H2-P-TriA or DI-huL5H2-P-TriA. huL5H2-P-TriA and DI-huL5H2-P-TriA showed good in vitro efficacy and selectivity, and demonstrated robust in vivo anti-tumor activity in PSMA-positive C4-2 cancer xenograft and prostate cancer patient-derived xenograft (PDX) models (see, e.g., Examples 4 and 5).

Linkers

[0256] The targeting agent antibody conjugates disclosed herein may comprise one or more linkers. The targeting agent antibody conjugates disclosed herein may comprise two or more linkers. The targeting agent antibody conjugates disclosed herein may comprise three or more linkers. The targeting agent antibody conjugates disclosed herein may comprise 4, 5, 6, 7, or more linkers.

[0257] The one or more linkers may comprise a functional group. The one or more linkers may comprise an amino acid. The one or more linkers may comprise a peptide. The one or more linkers may comprise a polymer. The polymer may be a polyethylene glycol. The one or more linkers may comprise an amide. The one or more linkers may comprise phenyl group.

[0258] One or more linkers may be formed by reaction of an amino acid on the antibody with a linker already attached to the targeting agent. One or more linkers may be formed by reaction of an amino acid or another reactive functional group on the targeting agent with a linker already attached to the antibody. One or more linkers may be formed by reaction of a linker already attached to the antibody with another linker already attached to the targeting agent. In order to form a linker already attached to the antibody or the targeting agent, a bifunctional linker, with two orthogonally reactive functional groups, may be coupled to the antibody or the targeting agent, such that one remaining reactive functional group is available for subsequent coupling. The reactive functional

groups may be selected from the group consisting of azides, alkynes, alkenes, dienes, nitrones, cyclooctyne, cyclopropene, trans-cyclooctene, norborene, tetrazine, and any combination thereof.

[0259] The one or more linkers may be the product of a bioorthogonal reaction between the linker already attached to the antibody and the linker already attached to the targeting agent, non-limiting examples of which are reviewed in Kim et al., Curr Opin Chem Bio 17:412-419 (2013).

[0260] The linker already attached to the antibody and the linker already attached to the targeting agent may be reacted to form a linker via cycloaddition, metathesis, metal-mediated cross-coupling reaction, radical polymerization, oxidative coupling, acyl-transfer reaction, and click chemistry. The cycloaddition may be a Huisgen-cycloaddition. The cycloaddition may be a copper-free [3+2] Huisgen-cycloaddition. The cycloaddition may be a Diels-Alder reaction. The cycloaddition may be a hetero Diels-Alder reaction. The linker may be the product of an enzyme-mediated reaction between the linker already attached to the antibody and the linker already attached to the targeting agent. The linker may be a product of a transglutaminase-mediated reaction between the linker already attached to the antibody and the linker already attached to the targeting agent, non-limiting examples of which are described in Lin et al., J. Am. Chem. Soc. 128:4542-4543 (2006) and WO 2013/093809.

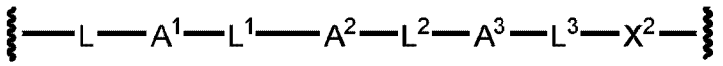
[0261] The one or more linkers may comprise a disulfide bridge that connects two cysteine residues, such as ThioBridge™ technology by PolyTherics. The one or more linkers may comprise a maleimide bridge that connects two amino acid residues. The one or more linkers may comprise a maleimide bridge that connects two cysteine residues.

[0262] The one or more linkers may comprise a cleavable linker. The one or more linkers may comprise a non-cleavable linker. The one or more linkers may comprise a flexible linker. The one or more linkers may comprise an inflexible linker.

[0263] Targeting agent antibody conjugates may be optimized by adjusting linker length. The one or more linkers may be relatively short. The one or more linkers may be relatively long. The one or more linkers may be between about 1 angstroms (Å) to about 120 angstroms (Å) in length. The one or more linkers may be between about 5 angstroms (Å) to about 105 angstroms (Å) in length. The one or more linkers may be between about 10 angstroms (Å) to about 100 angstroms (Å) in length. The one or more linkers may be between about 10 angstroms (Å) to about 90 angstroms (Å) in length. The one or more linkers may be between about 10 angstroms (Å) to about 80 angstroms (Å) in length. The one or more linkers may be between about 10 angstroms (Å) to about 70 angstroms (Å) in length. The one or more linkers may be between about 15 angstroms (Å) to about 45 angstroms (Å) in length. The one or more linkers may be equal to or greater than about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 27, 30, or more angstroms in length. The one or more linkers may be equal to or greater than about 10 angstroms in length. The one or more linkers may be

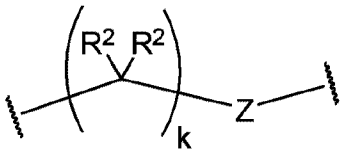
equal to or greater than about 15 angstroms in length. The one or more linkers may be equal to or greater than about 20 angstroms in length. The one or more linkers may be equal to or less than about 110, 100, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, or fewer angstroms in length. The one or more linkers may be equal to or less than about 100 angstroms in length. The one or more linkers may be equal to or less than about 80 angstroms in length. The one or more linkers may be equal to or less than about 60 angstroms in length. The one or more linkers may be equal to or less than about 40 angstroms in length.

[0264] The one or more linkers disclosed herein may comprise a compound of Formula (II):

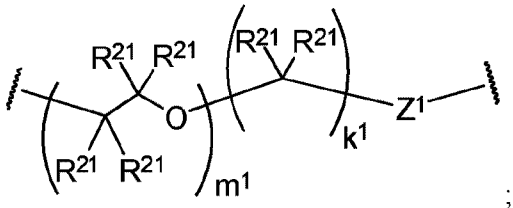


Formula (II)

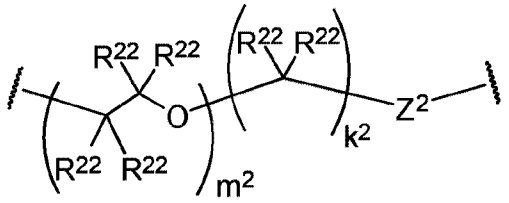
wherein:

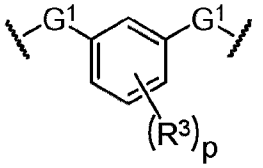
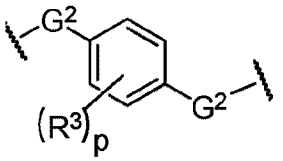
L is  ;

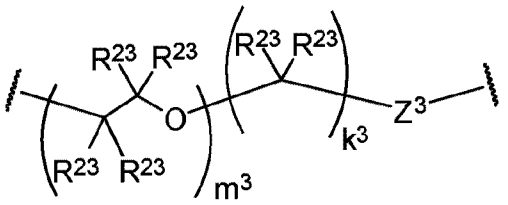
A^1 is selected from the group consisting of an aryl, a 5- to 6-membered heteroaryl, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

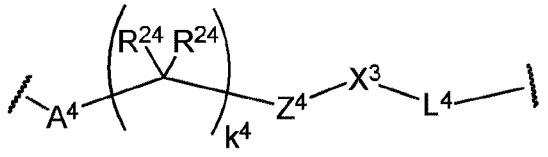
L^1 is  ;

A^2 is selected from the group consisting of a bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

L^2 is 

A^3 is a bond,  , or  ;

L^3 is  ;

X^2 is  ;

A^4 is selected from the group consisting of a
bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-,
and -N(R^1)S(O)$_{1,2}$-;

each R^1 is independently selected from H, alkyl, and haloalkyl;

each R^2, R^{21}, R^{22}, R^{23} and R^{24} is independently selected from H, halo, -OR^1, -CN, -SR^1, alkyl, cycloalkyl, haloalkyl, arylalkyl, and heteroarylalkyl;

each R^3 is independently selected from halo, -OR^1, -CN, -SR^1, alkyl, cycloalkyl, haloalkyl, arylalkyl, heteroarylalkyl, -NO_2, and NR^1R^1;

each G^1 and G^2 is independently selected from the group consisting of a
bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-,
and -N(R^1)S(O)$_{1,2}$-;

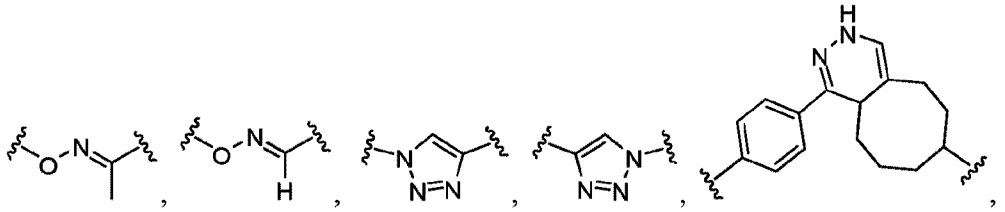
each Z, Z^1, Z^2, and Z^3 is independently selected from the group consisting of a bond, -O-, and -N(R^1)-;

Z^4 is selected from a bond, aryl, and a 5- to 6-membered heteroaryl;

k, k^1, k^2, k^3, and k^4 are each independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

m^1, m^2 and m^3 are each independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

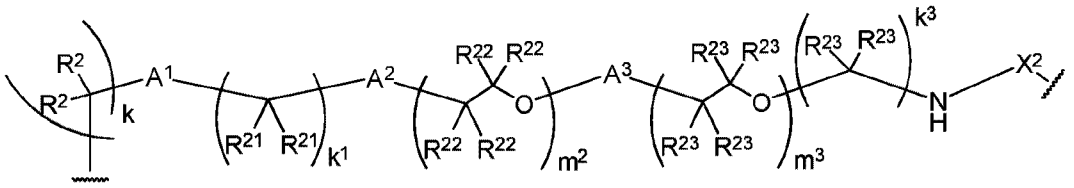
p is 0, 1, 2, 3 or 4;

X^3 is  , or -S-; and

L^4 is a bond directly attached to a modified amino acid; a linker bound to a modified amino acid, wherein the modified amino acid is part of the antibody;

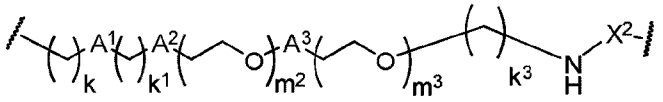
or a stereoisomer thereof.

[0265] The one or more linkers disclosed herein may comprise a compound of Formula (IIa):



Formula (IIa).

[0266] The one or more linkers disclosed herein may comprise a compound of Formula (IIb):



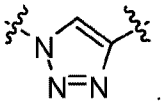
Formula (IIb).

[0267] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), k is 1, 2, 3, or 4; and Z is a bond.

[0268] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), k is 4; and Z is a bond.

[0269] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), A^1 is -C(O)N(R^1)-, a 6-membered aryl, or a 5-membered heteroaryl.

[0270] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), A^1 is -C(O)N(H)-.

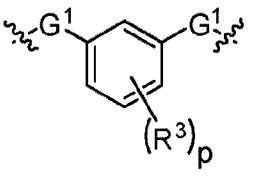
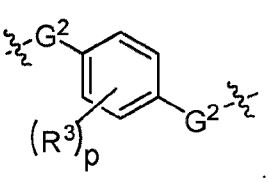
[0271] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), A^1 is  .

[0272] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), m^1 is 0; k^1 is 6 or 7; and Z^1 is a bond.

[0273] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), A^2 is -C(O)N(H)-; m^2 is 2; k^2 is 2; and Z^2 is a bond.

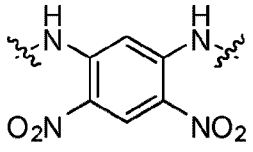
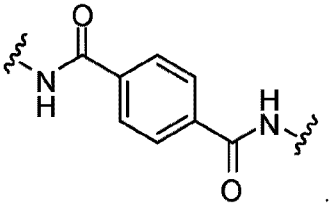
[0274] In some embodiments described above or below of a compound of (II), (IIa), or (IIb), A^2 is -C(O)N(H)-; m^2 is 3; k^2 is 2; and Z^2 is a bond.

[0275] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), A^2 is -C(O)N(H)-; m^2 is 10; k^2 is 2; and Z^2 is a bond.

[0276] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), A^3 is  or  .

[0277] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), R^3 is $-NO_2$; and p is 2.

[0278] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), each G^1 and G^2 are independently selected from the group consisting of –C(O)-, -N(H)-, -C(O)N(H)-, and -N(H)C(O)-.

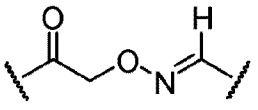
[0279] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), A^3 is  or  .

[0280] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), A^3 is a bond.

[0281] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), m^3 is 0, 1, 2, or 3; k^3 is 2; and Z^3 is -NH-.

[0282] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), each R^2, R^{21}, R^{22}, R^{23}, and R^{24} is independently selected from H, F, $-CH_3$, or $-CF_3$.

[0283] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), each R^2, R^{21}, R^{22}, R^{23}, and R^{24} is H.

[0284] In some embodiments described above or below of a compound of Formula (II), (IIa), or (IIb), X^2 is  .

IV. Targeting Agent Antibody Conjugate Production Methods

[0285] In another aspect, provided herein are methods of producing targeting agent antibody conjugates. The method may comprise conjugating an antibody or antibody fragment disclosed herein to a targeting agent disclosed herein. The method may comprise conjugating the targeting agent to an unnatural amino acid of the antibody or antibody fragment. The method may comprise incorporating one or more unnatural amino acids into the antibody or antibody fragment.

Incorporation of unnatural amino acids

[0286] Incorporating one or more unnatural amino acids into the antibody or antibody fragment may comprise modifying one or more amino acid residues in the antibody or antibody fragment. Modifying the one or more amino acid residues in the antibody or antibody fragment may comprise mutating one or more nucleotides in the nucleotide sequence encoding the targeting agent. Mutating the one or more nucleotides in the nucleotide sequence encoding the targeting agent may comprise altering a codon encoding an amino acid to a nonsense codon.

[0287] Incorporating one or more unnatural amino acids into the antibody or antibody fragment may comprise modifying one or more amino acid residues in the antibody or antibody fragment to produce one or more amber codons in the antibody or antibody fragment.

[0288] The one or more unnatural amino acids may be incorporated into the antibody or antibody fragment in response to an amber codon. The one or more unnatural amino acids may be site-specifically incorporated into the antibody or antibody fragment.

[0289] Incorporating one or more unnatural amino acids into the antibody or antibody fragment may comprise use of one or more genetically encoded unnatural amino acids with orthogonal chemical reactivity relative to the canonical twenty amino acids to site-specifically modify the targeting agent. Incorporating the one or more unnatural amino acids may comprise use of an evolved tRNA/aminoacyl-tRNA synthetase pair to site-specifically incorporate one or more unnatural amino acids at defined sites in the targeting agent in response to one or more amber nonsense codon.

[0290] Incorporating one or more unnatural amino acids into a targeting agent may comprise modifying one or more amino acid residues in a targeting agent. Modifying the one or more amino acid residues in a targeting agent may comprise mutating one or more nucleotides in the nucleotide sequence encoding the targeting agent. Mutating the one or more nucleotides in the nucleotide sequence encoding the targeting agent may comprise altering a codon encoding an amino acid to a nonsense codon.

[0291] Incorporating one or more unnatural amino acids into a targeting agent may comprise modifying one or more amino acid residues in a targeting agent to produce one or more amber codons in a targeting agent.

[0292] The one or more unnatural amino acids may be incorporated into a targeting agent in response to an amber codon. The one or more unnatural amino acids may be site-specifically incorporated into a targeting agent.

[0293] Incorporating one or more unnatural amino acids into a targeting agent may comprise use of one or more genetically encoded unnatural amino acids with orthogonal chemical reactivity relative to the canonical twenty amino acids to site-specifically modify the targeting agent. Incorporating the one or more unnatural amino acids may comprise use of an evolved tRNA/aminoacyl-tRNA synthetase pair to site-specifically incorporate one or more unnatural amino acids at defined sites in the targeting agent in response to one or more amber nonsense codon.

[0294] Additional methods for incorporating unnatural amino acids include, but are not limited to, methods disclosed in Chatterjee et al. (A Versatile Platform for Single- and Multiple-Unnatural Amino Acid Mutagenesis in Escherichia coli, *Biochemistry*, 2013), Kazane et al. (*J Am Chem Soc*, 135(1):340-6, 2013), Kim et al. (*J Am Chem Soc*, 134(24):9918-21, 2012), Johnson et al. (*Nat Chem Biol*, 7(11):779-86, 2011), and Hutchins et al. (*J Mol Biol*, 406(4):595-603, 2011).

Linking antibodies, antibody fragments, and/or targeting agents

[0295] The methods may comprise linking the antibody, antibody fragment, targeting agent or intermediates thereof to produce a targeting agent antibody conjugate comprising (a) an antibody or antibody fragment; (b) one or more linkers; and (c) a targeting agent, wherein the one or more linkers link the first antibody or antibody fragment to the targeting agent. The method may further comprise conjugating the one or more linkers to a targeting agent to produce a targeting agent-linker intermediate and coupling the targeting agent-linker intermediate to the antibody or antibody fragment. The method may further comprise conjugating the one or more linkers to the antibody or antibody fragment to produce an antibody-linker intermediate or antibody fragment-linker intermediate and coupling the antibody-linker intermediate or antibody-fragment-linker intermediate to the targeting agent. Coupling an intermediate to an antibody, antibody fragment or targeting agent may comprise formation of an oxime. Coupling an intermediate to an antibody, antibody fragment or targeting agent may comprise formation of the oxime in an acidic solution. Coupling an intermediate to an antibody, antibody fragment or targeting agent may comprise formation of the oxime in a slightly acidic solution. Coupling an intermediate to an antibody, antibody fragment or targeting agent may comprise formation of the oxime in a slightly neutral solution. The antibody or antibody fragment may comprise an unnatural amino acid. Linking the antibody or antibody fragment to the targeting agent-linker intermediate may comprise forming an oxime between the unnatural amino acid and the targeting agent-linker intermediate. The targeting agent may comprise an unnatural amino acid. Linking the targeting agent to the antibody-linker intermediate or antibody fragment-linker intermediate may comprise forming an oxime between the unnatural amino acid and the antibody-linker intermediate or the antibody fragment-linker intermediate.

[0296] The method of producing a targeting agent antibody conjugate may comprise (a) conjugating a first linker to the antibody or antibody fragment to produce an antibody-linker intermediate or antibody fragment-linker intermediate; (b) conjugating a second linker to the targeting agent to produce a targeting agent-linker intermediate; and (c) linking the two intermediates together to produce the targeting agent antibody conjugate. Conjugating the linker to the antibody, antibody fragment, or targeting agent may comprise production of an ionic bond, a covalent bond, a non-covalent bond, or a combination thereof between the linker and the antibody, antibody fragment, or targeting agent. Conjugating the linker to the antibody, antibody fragment or targeting agent may be performed as described in Roberts et al., Advanced Drug Delivery Reviews 54:459-476 (2002).

[0297] The methods disclosed herein may comprise coupling one or more linkers to one or more antibodies, antibody fragments, targeting agents, or combinations thereof to produce one or more intermediates such as an antibody-linker intermediate, an antibody fragment-linker intermediate and/or a targeting agent antibody conjugate-linker intermediate. The methods may comprise coupling

a first linker to an antibody or antibody fragment to produce an antibody-linker intermediate or antibody fragment-linker intermediate. The methods may comprise coupling a linker to a targeting agent to produce a targeting agent-linker intermediate.

[0298] Coupling of the one or more linkers to the antibody, antibody fragment, or targeting agent may occur simultaneously. Coupling of the one or more linkers to the antibody, antibody fragment, or targeting molecule may occur sequentially. Coupling of the one or more linkers to the antibody, antibody fragment, or targeting molecule may occur in a single reaction volume. Coupling of the one or more linkers to the antibody, antibody fragment, or targeting molecule may occur in two or more reaction volumes.

[0299] Coupling one or more linkers to the antibody, antibody fragment and/or targeting molecule may comprise forming one or more oximes between the linker and the antibody, antibody fragment or targeting molecule. Coupling one or more linkers to the antibody, antibody fragment and/or targeting agent may comprise forming one or more stable bonds between linker and the antibody, antibody fragment or targeting agent. Coupling one or more linkers to the antibody, antibody fragment and/or targeting agent may comprise forming one or more covalent bonds between linker and the antibody, antibody fragment or targeting agent. Coupling one or more linkers to the antibody, antibody fragment and/or targeting agent may comprise forming one or more non-covalent bonds between linker and the antibody, antibody fragment or targeting agent. Coupling one or more linkers to the antibody, antibody fragment and/or ligand may comprise forming one or more ionic bonds between linker and the antibody, antibody fragment or targeting agent.

[0300] Coupling one or more linkers to the antibody or antibody fragment may comprise site specifically coupling one or more linkers to the antibody or antibody fragment. Site-specific coupling may comprise linking the one or more linkers to the unnatural amino acid of the antibody or antibody fragment. Linking the one or more linkers to the unnatural amino acid of the antibody or antibody fragment may comprise formation of an oxime. Linking the one or more linkers to the unnatural amino acid of the antibody or antibody fragment may comprise formation of a sulfide. Linking the one or more linkers to the unnatural amino acid of the antibody or antibody fragment may comprise, by way of non-limiting example, reacting a hydroxylamine of the one or more linkers with an aldehyde or ketone of an amino acid. The amino acid may be an unnatural amino acid. Linking the one or more linkers to the unnatural amino acid of the antibody or antibody fragment may comprise, by way of non-limiting example, reacting a bromo derivative of the one or more linkers with thiol of an amino acid. The amino acid may be an unnatural amino acid.

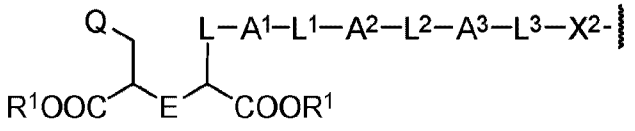
[0301] The targeting agent antibody conjugate may be of Formula (I): X-L-Y or Formula (IA): Y-L-X, wherein:

a. X comprises the antibody or antibody fragment;

b. L comprises the one or more linkers; and

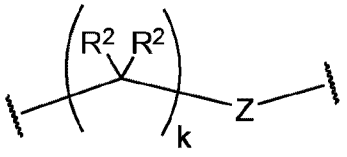
c. Y comprises one or more DUPA molecules.

[0302] The targeting agent antibody conjugate may comprise a compound of Formula (III):

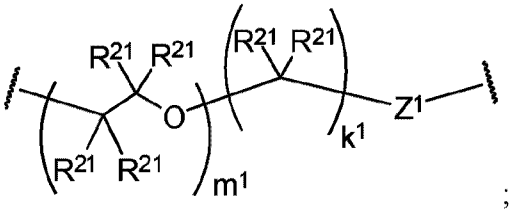


Formula (III)

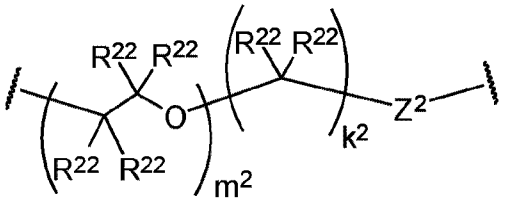
wherein:

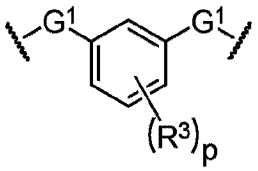
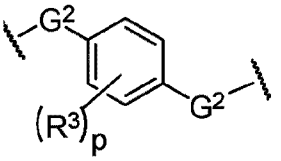
L is  ;

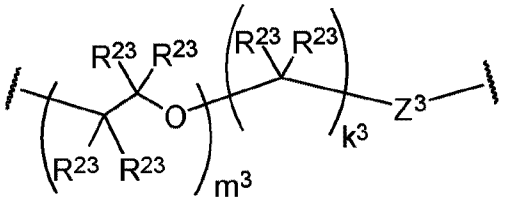
A^1 is selected from the group consisting of an aryl, a 5- to 6-membered heteroaryl, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

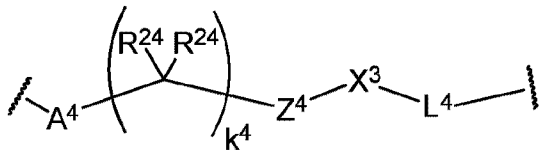
L^1 is  ;

A^2 is selected from the group consisting of a bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

L^2 is 

A^3 is a bond,  , or  ;

L^3 is  ;

X^2 is  ;

A^4 is selected from the group consisting of a bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

each R^1 is independently selected from H, alkyl, and haloalkyl;

each R^2, R^{21}, R^{22}, R^{23}, and R^{24} is independently selected from H, halo, -OR^1, -CN, -SR^1, alkyl, cycloalkyl, haloalkyl, arylalkyl, and heteroarylalkyl;

each R^3 is independently selected from halo, -OR^1, -CN, -SR^1, alkyl, cycloalkyl, haloalkyl, arylalkyl, heteroarylalkyl, -NO_2, and NR^1R^1;

each G^1 and G^2 is independently selected from the group consisting of a bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

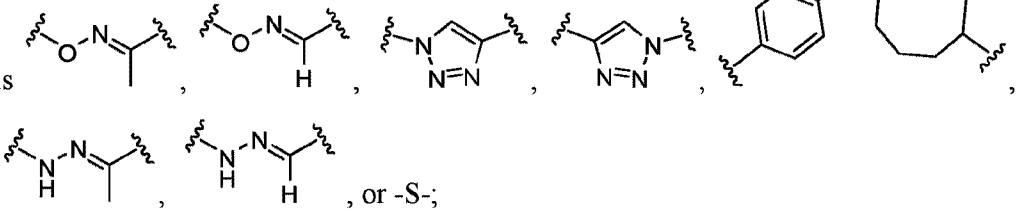
each Z, Z^1, Z^2, and Z^3 is independently selected from the group consisting of a bond, -O-, and -N(R^1)-;

Z^4 is selected from a bond, aryl, and a 5- to 6-membered heteroaryl;

k, k^1, k^2, k^3, and k^4 are each independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

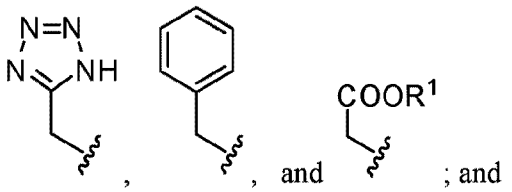
m^1, m^2 and m^3 are each independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

p is 0, 1, 2, 3 or 4;

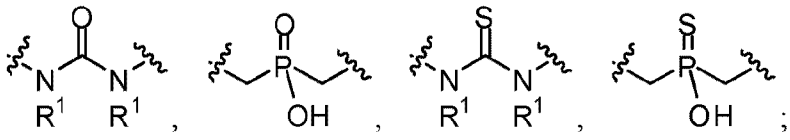
X^3 is  , or -S-;

L^4 is a bond directly attached to a modified amino acid, or a linker bound to a modified amino acid, wherein the modified amino acid is part of the antibody;

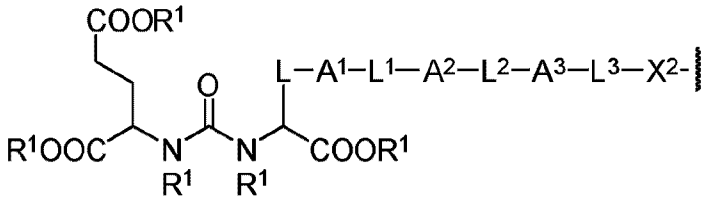
Q is selected from the group consisting of:

 ; and

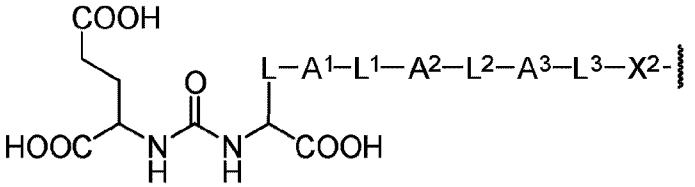
E is selected from the group consisting of:


; and a stereoisomer thereof.

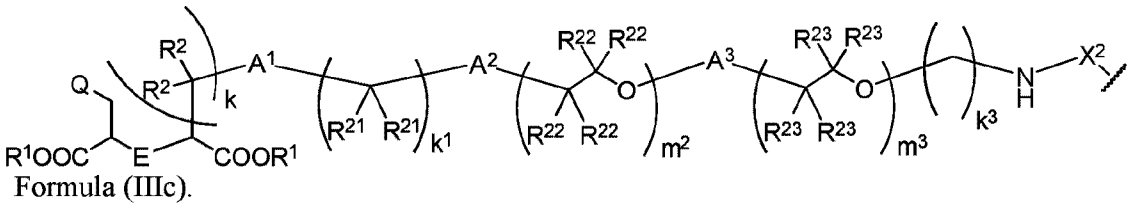
[0303] The targeting agent antibody conjugate may comprise a compound of Formula (IIIa):


Formula (IIIa).

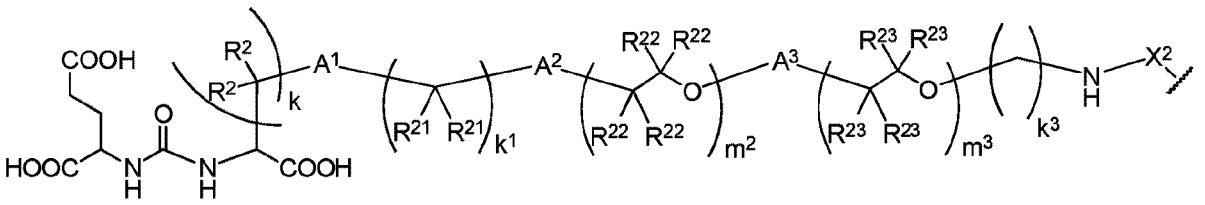
[0304] The targeting agent antibody conjugate may comprise a compound of Formula (IIIb):


Formula (IIIb).

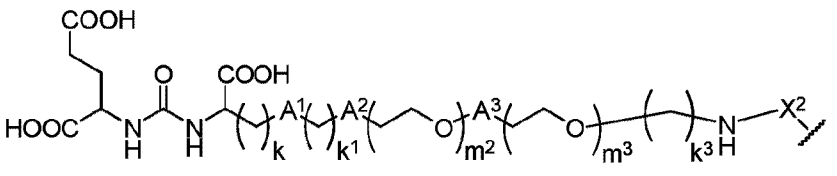
[0305] The targeting agent antibody conjugate may comprise a compound of Formula (IIIc):


Formula (IIIc).

[0306] The targeting agent antibody conjugate may comprise a compound of Formula (IIId):


Formula (IIId).

[0307] The targeting agent antibody conjugate may comprise a compound of Formula (IIIe):

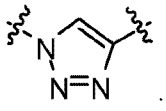

Formula (IIIe).

[0308] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), k is 1, 2, 3, or 4; and Z is a bond.

[0309] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), k is 4; and Z is a bond.

[0310] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), A^1 is -C(O)N(R^1)-, a 6-membered aryl, or a 5-membered heteroaryl.

[0311] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), A^1 is -C(O)N(H)-.

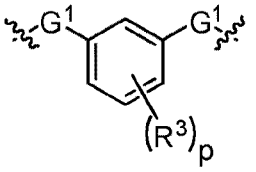
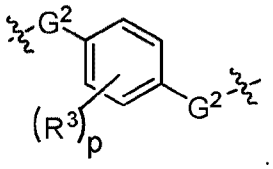
[0312] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), A^1 is  .

[0313] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), m^1 is 0; k^1 is 6 or 7; and Z^1 is a bond.

[0314] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), A^2 is -C(O)N(H)-; m^2 is 2; k^2 is 2; and Z^2 is a bond.

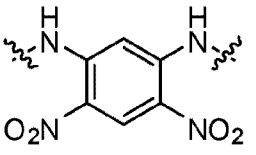
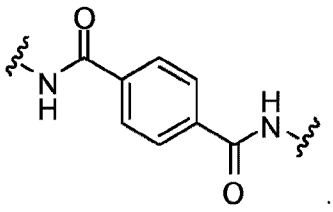
[0315] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), A^2 is -C(O)N(H)-; m^2 is 3; k^2 is 2; and Z^2 is a bond.

[0316] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), A^2 is -C(O)N(H)-; m^2 is 10; k^2 is 2; and Z^2 is a bond.

[0317] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), A^3 is  or  .

[0318] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), R^3 is -NO_2; and p is 2.

[0319] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), each G^1 and G^2 are independently selected from the group consisting of –C(O)-, -N(H)-, -C(O)N(H)-, and -N(H)C(O)-.

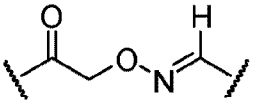
[0320] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), A^3 is  or  .

[0321] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), A^3 is a bond.

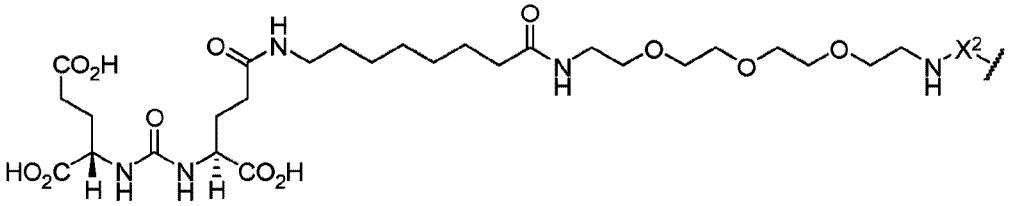
[0322] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), m^3 is 0, 1, 2, or 3; k^3 is 2; and Z^3 is -NH-.

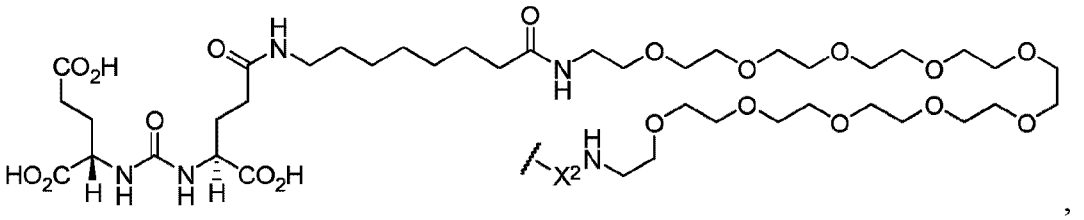
[0323] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), each R^2, R^{21}, R^{22}, R^{23}, and R^{24} is independently selected from H, F, $-CH_3$, and $-CF_3$.

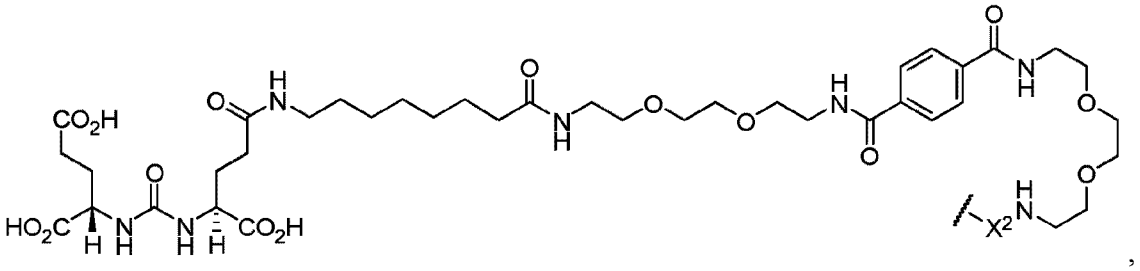
[0324] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), each R^2, R^{21}, R^{22}, R^{23}, and R^{24} is H.

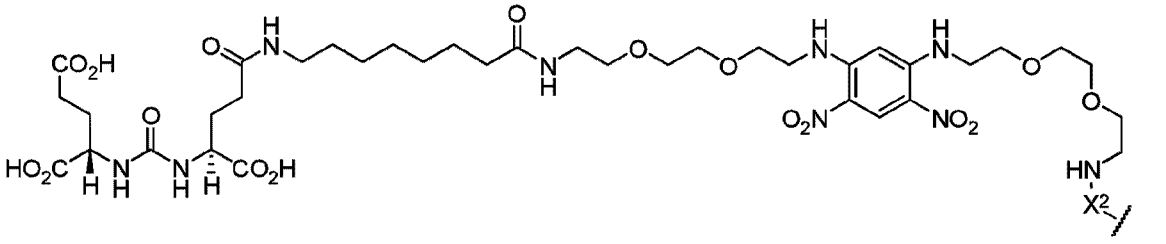
[0325] In some embodiments described above or below of a compound of Formula (III), (IIIa), (IIIb), (IIIc), (IIId), or (IIIe), X^2 is  .

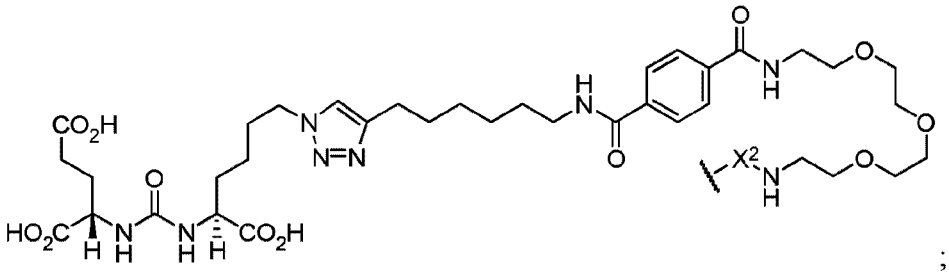
[0326] In some embodiments described above or below of a compound of Formula (III), the compound is selected from:

 ,

 ,

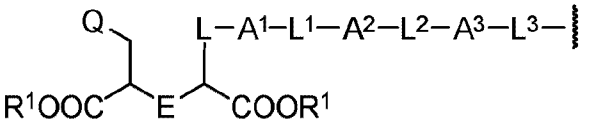
 ,

 ,



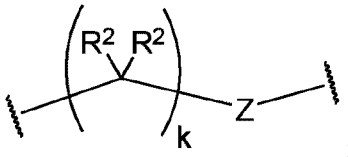
; and a stereoisomer thereof.

[0327] The targeting agent antibody conjugate may comprise a compound of Formula (IV):

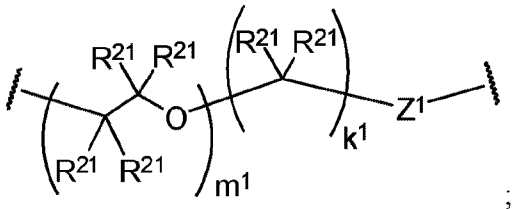


Formula (IV)

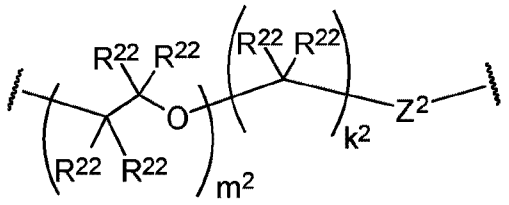
wherein:

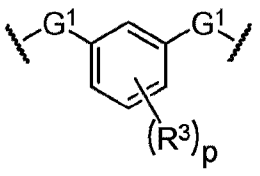
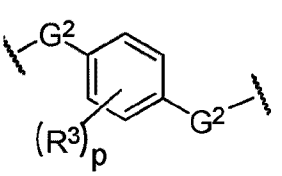
L is  ;

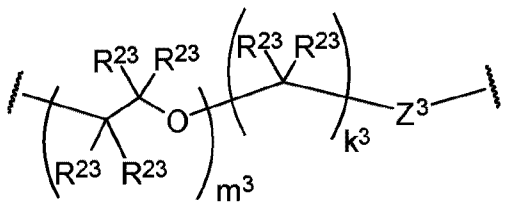
A^1 is selected from the group consisting of an aryl, a 5- to 6-membered heteroaryl, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

L^1 is  ;

A^2 is selected from the group consisting of a bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

L^2 is 

A^3 is a bond,  , or  ;

L^3 is  ;

each R^1 is independently selected from H, alkyl, and haloalkyl;

each R^2, R^{21}, R^{22}, and R^{23} is independently selected from H, halo, $-OR^1$, -CN, $-SR^1$, alkyl, cycloalkyl, haloalkyl, arylalkyl, and heteroarylalkyl;

each R^3 is independently selected from halo, $-OR^1$, -CN, $-SR^1$, alkyl, cycloalkyl, haloalkyl, arylalkyl, heteroarylalkyl, $-NO_2$, and NR^1R^1;

each G^1 and G^2 is independently selected from the group consisting of a bond, -C(O)-, $-N(R^1)-$, -O-, $-C(O)N(R^1)-$, $-N(R^1)C(O)-$, $-S(O)_{1,2}N(R^1)-$, and $-N(R^1)S(O)_{1,2}-$;

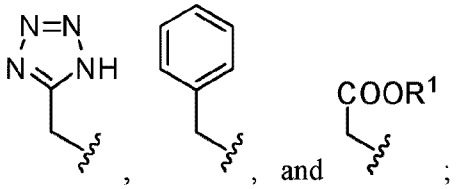
each Z, Z^1, Z^2, and Z^3 is independently selected from the group consisting of a bond, -O-, and $-N(R^1)-$;

k, k^1, k^2, and k^3 are each independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

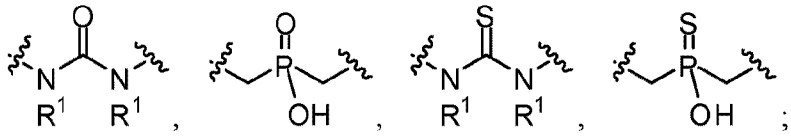
m^1, m^2 and m^3 are each independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

p is 0, 1, 2, 3 or 4;

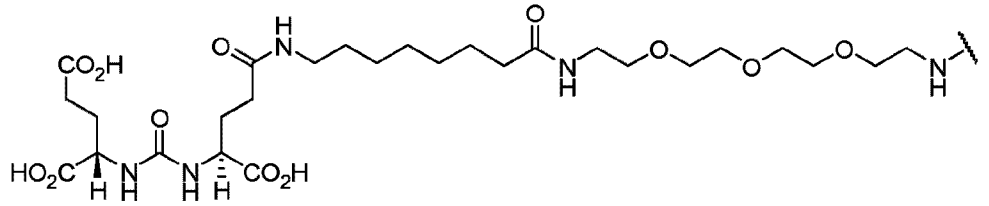
Q is selected from the group consisting of:

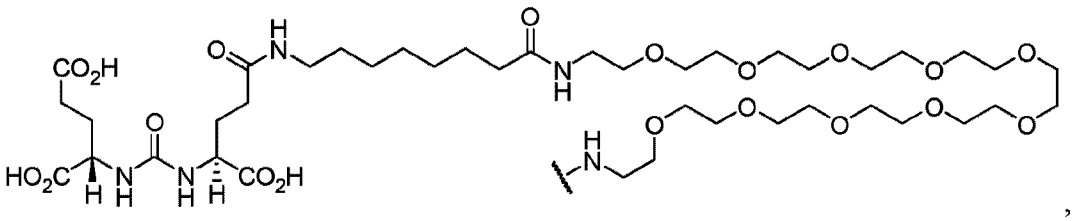
 ;

E is selected from the group consisting of:

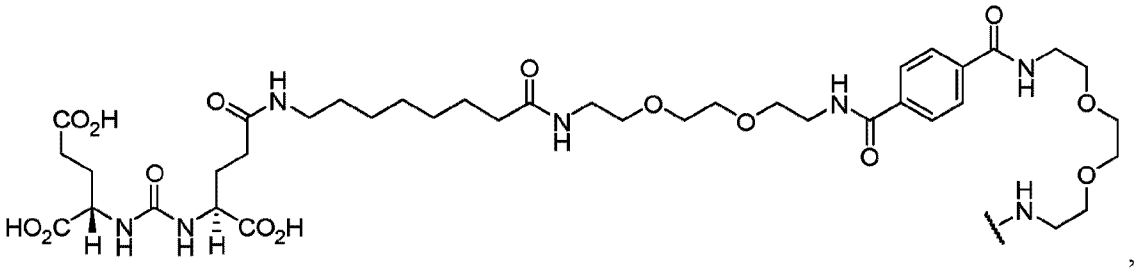
 ; and a stereoisomer thereof.

[0328] In some embodiments described above or below of a compound of Formula (IV), the compound is selected from:

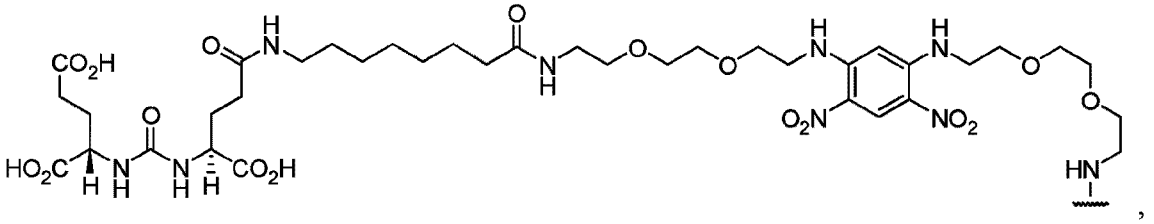
 ,



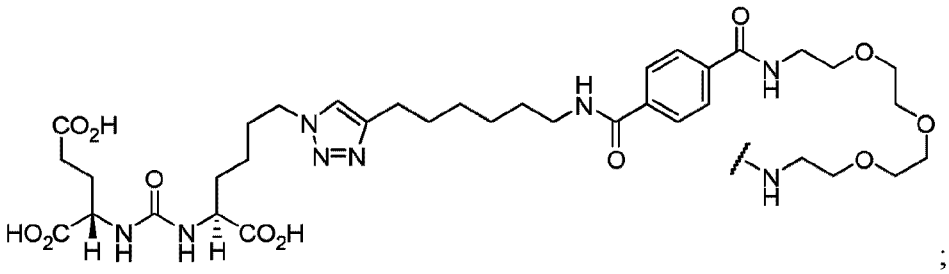
,



,

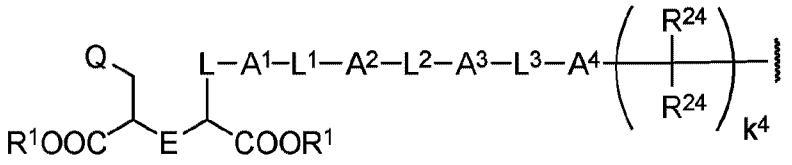


,



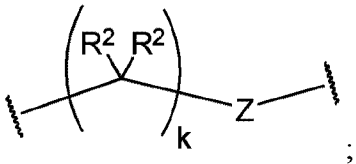
; and a stereoisomer thereof.

[0329] The targeting agent antibody conjugate may comprise a compound of Formula (V):

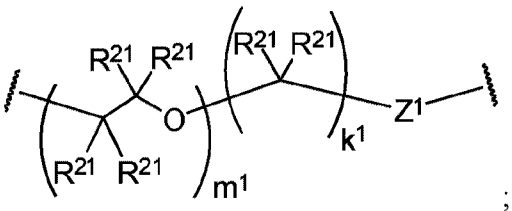


Formula (V)

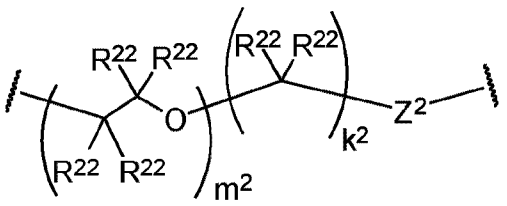
wherein:

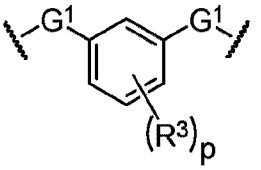
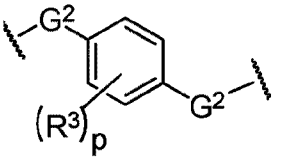
L is  ;

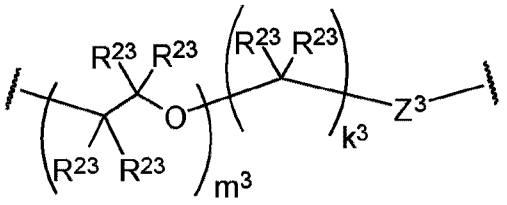
A^1 is selected from the group consisting of an aryl, a 5- to 6-membered heteroaryl, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-, and -N(R^1)S(O)$_{1,2}$-;

L^1 is  ;

A^2 is selected from the group consisting of a
bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-,
and -N(R^1)S(O)$_{1,2}$-;

L^2 is 

A^3 is a bond,  , or  ;

L^3 is  ;

A^4 is selected from the group consisting of a
bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-,
and -N(R^1)S(O)$_{1,2}$-;

each R^1 is independently selected from H, alkyl, and haloalkyl;

each R^2, R^{21}, R^{22}, R^{23}, and R^{24} is independently selected from H, halo, -OR^1, -CN, -SR^1, alkyl, cycloalkyl, haloalkyl, arylalkyl, and heteroarylalkyl;

each R^3 is independently selected from halo, -OR^1, -CN, -SR^1, alkyl, cycloalkyl, haloalkyl, arylalkyl, heteroarylalkyl, -NO_2, and NR^1R^1;

each G^1 and G^2 is independently selected from the group consisting of a
bond, -C(O)-, -N(R^1)-, -O-, -C(O)N(R^1)-, -N(R^1)C(O)-, -S(O)$_{1,2}$N(R^1)-,
and -N(R^1)S(O)$_{1,2}$-;

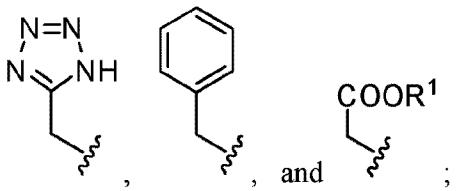
each Z, Z^1, Z^2, and Z^3 is independently selected from the group consisting of a bond, -O-, and -N(R^1)-;

k, k^1, k^2, k^3, and k^4 are each independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

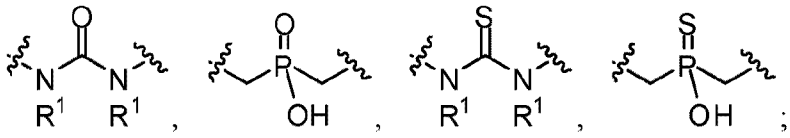
m^1, m^2 and m^3 are each independently selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

p is 0, 1, 2, 3 or 4;

Q is selected from the group consisting of:

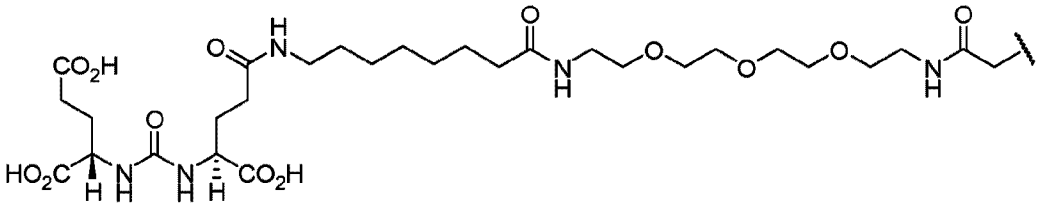


E is selected from the group consisting of:

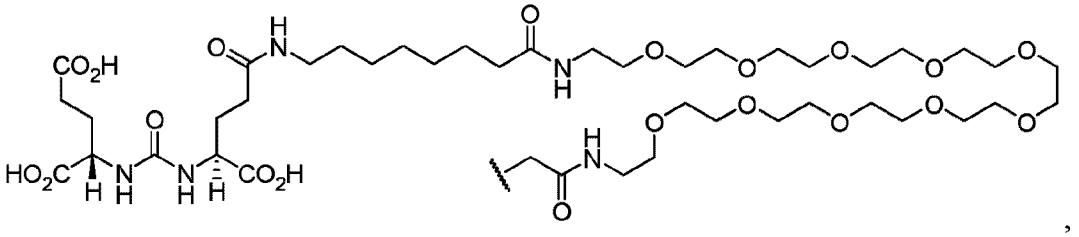


; and a stereoisomer thereof.

[0330] In some embodiments described above or below of a compound of Formula (V), the compound is selected from:



,



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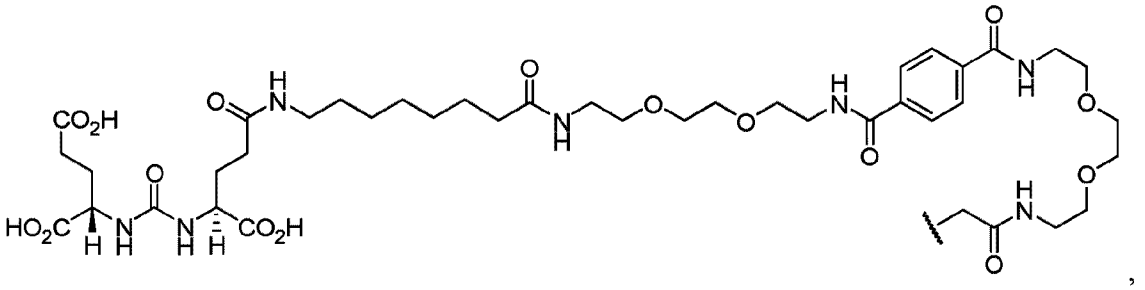
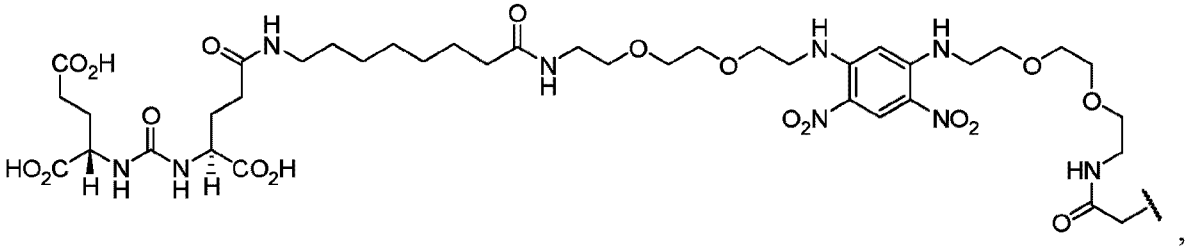
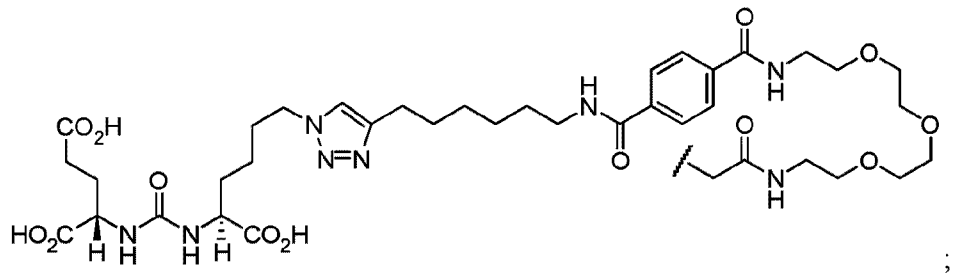


,

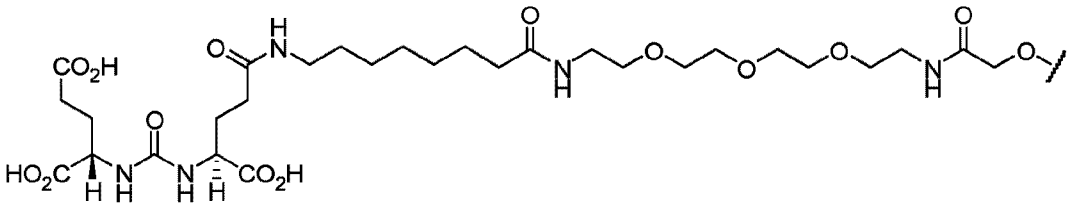


,

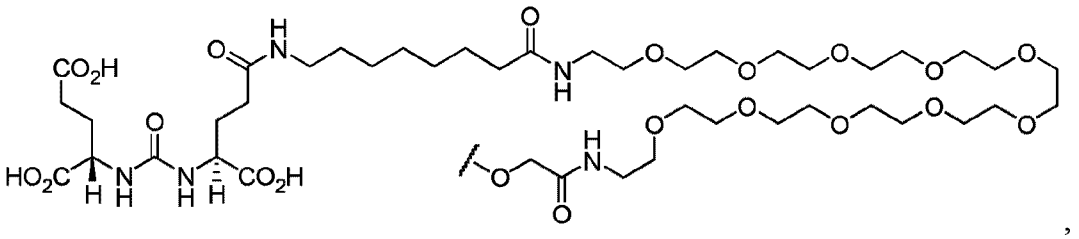


; and a stereoisomer thereof.

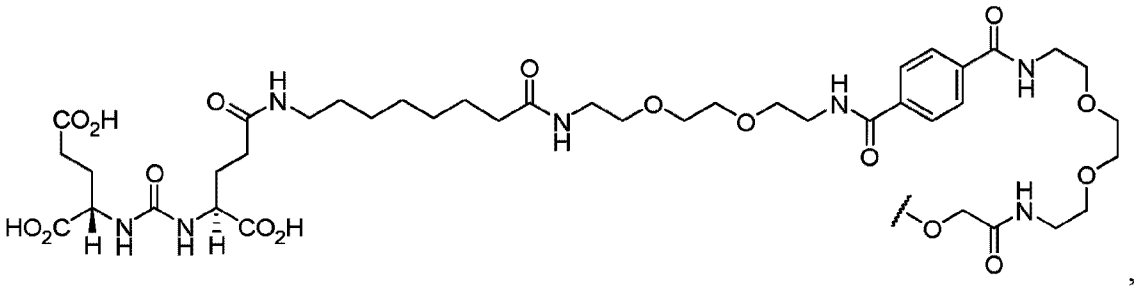
[0331] In some embodiments, the targeting agent-linker comprises a compound selected from:



,



,



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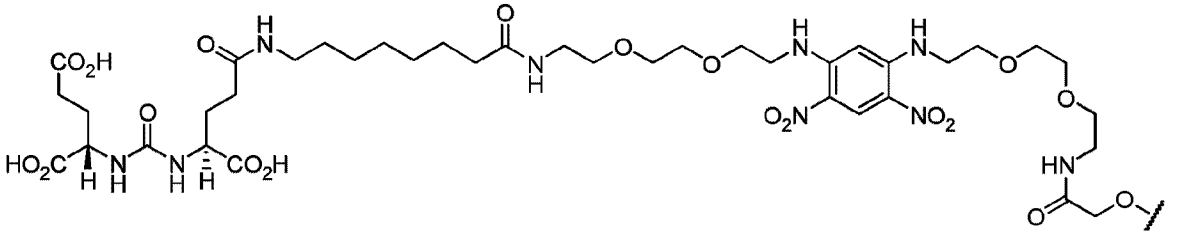
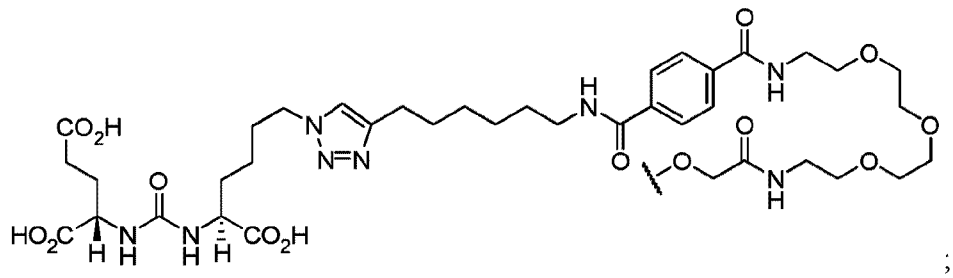
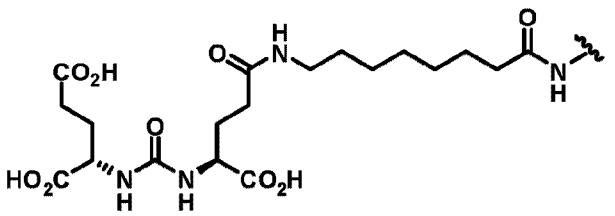


,



; and a stereoisomer thereof.

[0332] In some embodiments, the targeting agent-linker comprises a compound selected from:



, and a stereoisomer thereof.

IV. Pharmaceutical Compositions

[0333] Disclosed herein are pharmaceutical compositions that comprise an antibody and/or targeting agent antibody conjugates disclosed herein and a pharmaceutically acceptable carrier or excipient. The term "pharmaceutically acceptable" as used herein, refers to a material that does not abrogate the biological activity or properties of the agents described herein, and is relatively nontoxic (i.e., the toxicity of the material significantly outweighs the benefit of the material). In some instances, a pharmaceutically acceptable material may be administered to an individual without causing significant undesirable biological effects or significantly interacting in a deleterious manner with any of the components of the composition in which it is contained.

[0334] Pharmaceutical compositions herein may be formulated using one or more physiologically acceptable carriers including excipients and auxiliaries which facilitate processing of the active agents into preparations which are used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. A summary of pharmaceutical compositions is found, for example, in Remington: The Science and Practice of Pharmacy, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980; and Pharmaceutical Dosage Forms and Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins, 1999).

[0335] A pharmaceutical composition disclosed herein may further comprise a pharmaceutically acceptable diluent(s), excipient(s), or carrier(s). The pharmaceutical compositions may include other medicinal or pharmaceutical agents; carriers; adjuvants; preserving, stabilizing, wetting or emulsifying agents; solution promoters; salts for regulating the osmotic pressure; and/or buffers. In addition, the pharmaceutical compositions also contain other therapeutically valuable substances.

[0336] A pharmaceutical composition disclosed herein may be administered to a subject by any suitable administration route, including but not limited to, parenteral (intravenous, subcutaneous, intraperitoneal, intramuscular, intravascular, intrathecal, intravitreal, infusion, or local), topical, oral, or nasal administration. A suitable administration route may comprise a microneedle device.

[0337] Formulations suitable for intramuscular, subcutaneous, peritumoral, or intravenous injection may include physiologically acceptable sterile aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and non-aqueous carriers, diluents, solvents, or vehicles including water, ethanol, polyols (propyleneglycol, polyethylene-glycol, glycerol, cremophor, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity is maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. Formulations suitable for subcutaneous injection also contain optional additives such as preserving, wetting, emulsifying, and dispensing agents.

[0338] For intravenous injections, an active agent may be optionally formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer.

[0339] Parenteral injections optionally involve bolus injection or continuous infusion. Formulations for injection are optionally presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The pharmaceutical compositions described herein may be in a form suitable for parenteral injection as sterile suspension, solution or emulsion in oily or aqueous vehicle, and contain formulatory agents such as suspending, stabilizing, and/or dispersing agents. Pharmaceutical formulations for parenteral administration include aqueous solutions of an active agent in water soluble form. Additionally, suspensions are optionally prepared as appropriate oily injection suspensions.

[0340] The pharmaceutical composition described herein may be in unit dosage forms suitable for single administration of precise dosages. In unit dosage form, the formulation may be divided into unit doses containing appropriate quantities of an active agent disclosed herein. The unit dosage may be in the form of a package containing discrete quantities of the formulation. Non-limiting examples are packaged tablets or capsules, and powders in vials or ampoules. Aqueous suspension compositions may be packaged in single-dose non-reclosable containers. Alternatively, multiple-dose reclosable containers are used, in which case it is typical to include a preservative in the composition. By way of example only, formulations for parenteral injection are presented in unit dosage form, which include, but are not limited to ampoules, or in multi dose containers, with an added preservative.

[0341] The pharmaceutical composition may be administered once daily, twice daily, three times daily or more. The pharmaceutical composition may be administered once weekly, twice weekly, three times weekly or more. The pharmaceutical composition may be administered bi-weekly. The

pharmaceutical composition may be administered monthly. The pharmaceutical composition may be administered as needed.

[0342] The pharmaceutical composition may be co-administered with a therapeutic treatment (e.g., anti-inflammatory treatment, antibiotic, anti-viral drug, chemotherapy, radiation). The therapeutic treatment may comprise an additional targeting agent antibody conjugate.

V. Therapeutic Uses

[0343] Disclosed herein are methods of treating a subject for a condition with a targeting agent antibody conjugate or pharmaceutical composition disclosed herein.

[0344] The condition, by way of non-limiting example, may be a cancer. The cancer, by way of non-limiting example, may be selected from prostate cancer, breast cancer, brain cancer, pancreatic cancer, skin cancer, lung cancer, liver cancer, colon cancer, bladder cancer, ovarian cancer, uterine cancer, leukemia, lymphoma, and testicular cancer. The cancer may be a prostate cancer. The cancer may comprise a recurrent and/or refractory cancer. Examples of cancers include, but are not limited to, sarcomas, carcinomas, lymphomas, or leukemias.

[0345] The cancer may comprise a neuroendocrine cancer. The cancer may comprise a pancreatic cancer. The cancer may comprise an exocrine pancreatic cancer. The cancer may comprise a thyroid cancer. The thyroid cancer may comprise a medullary thyroid cancer.

[0346] The cancer may comprise a prostate cancer. The prostate cancer may be a PSMA-positive prostate cancer. PSMA expression may be highly upregulated and restricted to cancer cells in some or all stages of the prostate cancer. The cancer may be hormone-refractory prostate cancer.

[0347] The cancer may comprise an epithelial cancer. The cancer may comprise a breast cancer. The cancer may comprise an endometrial cancer. The cancer may comprise an ovarian cancer. The ovarian cancer may comprise a stromal ovarian cancer. The cancer may comprise a cervical cancer.

[0348] The cancer may comprise a skin cancer. The skin cancer may comprise a neo-angiogenic skin cancer. The skin cancer may comprise a melanoma.

[0349] The cancer may comprise a kidney cancer.

[0350] The cancer may comprise a lung cancer. The lung cancer may comprise a small cell lung cancer. The lung cancer may comprise a non-small cell lung cancer.

[0351] The cancer may comprise a colorectal cancer. The cancer may comprise a gastric cancer. The cancer may comprise a colon cancer.

[0352] The cancer may comprise a brain cancer. The brain cancer may comprise a brain tumor. The cancer may comprise a glioblastoma. The cancer may comprise an astrocytoma.

[0353] The cancer may comprise a blood cancer. The blood cancer may comprise a leukemia. The leukemia may comprise a myeloid leukemia. The cancer may comprise a lymphoma. The lymphoma may comprise a non-Hodgkin's lymphoma.

[0354] The cancer may comprise a sarcoma. The sarcoma may comprise an Ewing's sarcoma.

[0355] Sarcomas are cancers of the bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue. Sarcomas include, but are not limited to, bone cancer, fibrosarcoma, chondrosarcoma, Ewing's sarcoma, malignant hemangioendothelioma, malignant schwannoma, bilateral vestibular schwannoma, osteosarcoma, soft tissue sarcomas (e.g. alveolar soft part sarcoma, angiosarcoma, cystosarcoma phylloides, dermatofibrosarcoma, desmoid tumor, epithelioid sarcoma, extraskeletal osteosarcoma, fibrosarcoma, hemangiopericytoma, hemangiosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, lymphosarcoma, malignant fibrous histiocytoma, neurofibrosarcoma, rhabdomyosarcoma, and synovial sarcoma).

[0356] Carcinomas are cancers that begin in the epithelial cells, which are cells that cover the surface of the body, produce hormones, and make up glands. By way of non-limiting example, carcinomas include breast cancer, pancreatic cancer, lung cancer, colon cancer, colorectal cancer, rectal cancer, kidney cancer, bladder cancer, stomach cancer, prostate cancer, liver cancer, ovarian cancer, brain cancer, vaginal cancer, vulvar cancer, uterine cancer, oral cancer, penile cancer, testicular cancer, esophageal cancer, skin cancer, cancer of the fallopian tubes, head and neck cancer, gastrointestinal stromal cancer, adenocarcinoma, cutaneous or intraocular melanoma, cancer of the anal region, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, cancer of the urethra, cancer of the renal pelvis, cancer of the ureter, cancer of the endometrium, cancer of the cervix, cancer of the pituitary gland, neoplasms of the central nervous system (CNS), primary CNS lymphoma, brain stem glioma, and spinal axis tumors. In some instances, the cancer is a skin cancer, such as a basal cell carcinoma, squamous, melanoma, nonmelanoma, or actinic (solar) keratosis.

[0357] In some instances, the cancer is a lung cancer. Lung cancer may start in the airways that branch off the trachea to supply the lungs (bronchi) or the small air sacs of the lung (the alveoli). Lung cancers include non-small cell lung carcinoma (NSCLC), small cell lung carcinoma, and mesotheliomia. Examples of NSCLC include squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. The mesothelioma may be a cancerous tumor of the lining of the lung and chest cavitity (pleura) or lining of the abdomen (peritoneum). The mesothelioma may be due to asbestos exposure. The cancer may be a brain cancer, such as a glioblastoma.

[0358] Alternatively, the cancer may be a central nervous system (CNS) tumor. CNS tumors may be classified as gliomas or nongliomas. The glioma may be malignant glioma, high grade glioma, diffuse intrinsic pontine glioma. Examples of gliomas include astrocytomas, oligodendrogliomas (or mixtures of oligodendroglioma and astocytoma elements), and ependymomas. Astrocytomas include, but are not limited to, low-grade astrocytomas, anaplastic astrocytomas, glioblastoma multiforme, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and subependymal giant cell astrocytoma.

Oligodendrogliomas include low-grade oligodendrogliomas (or oligoastrocytomas) and anaplastic oligodendriogliomas. Nongliomas include meningiomas, pituitary adenomas, primary CNS lymphomas, and medulloblastomas. In some instances, the cancer is a meningioma.

[0359] The leukemia may be an acute lymphocytic leukemia, acute myelocytic leukemia, chronic lymphocytic leukemia, or chronic myelocytic leukemia. Additional types of leukemias include hairy cell leukemia, chronic myelomonocytic leukemia, and juvenile myelomonocytic leukemia.

[0360] Lymphomas are cancers of the lymphocytes and may develop from either B or T lymphocytes. The two major types of lymphoma are Hodgkin's lymphoma, previously known as Hodgkin's disease, and non-Hodgkin's lymphoma. Hodgkin's lymphoma is marked by the presence of the Reed-Sternberg cell. Non-Hodgkin's lymphomas are all lymphomas which are not Hodgkin's lymphoma. Non-Hodgkin lymphomas may be indolent lymphomas and aggressive lymphomas. Non-Hodgkin's lymphomas include, but are not limited to, diffuse large B cell lymphoma, follicular lymphoma, mucosa-associated lymphatic tissue lymphoma (MALT), small cell lymphocytic lymphoma, mantle cell lymphoma, Burkitt's lymphoma, mediastinal large B cell lymphoma, Waldenström macroglobulinemia, nodal marginal zone B cell lymphoma (NMZL), splenic marginal zone lymphoma (SMZL), extranodal marginal zone B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, and lymphomatoid granulomatosis.

[0361] The one or more diseases or conditions may be a pathogenic infection. The targeting agent may interact with a cell surface molecule on an infected cell. The targeting agent may interact with a molecule on a bacterium, a virus, or a parasite. Pathogenic infections may be caused by one or more pathogens. In some instances, the pathogen is a bacterium, fungi, virus, or protozoan.

[0362] Exemplary pathogens include but are not limited to: *Bordetella, Borrelia, Brucella, Campylobacter, Chlamydia, Chlamydophila, Clostridium, Corynebacterium, Enterococcus, Escherichia, Francisella, Haemophilus, Helicobacter, Legionella, Leptospira, Listeria, Mycobacterium, Mycoplasma, Neisseria, Pseudomonas, Rickettsia, Salmonella, Shigella, Staphylococcus, Streptococcus, Treponema, Vibrio,* or *Yersinia.* In some cases, the disease or condition caused by the pathogen is tuberculosis and the heterogeneous sample comprises foreign molecules derived from the bacterium *Mycobacterium tuberculosis* and molecules derived from the subject. In some instances, the disease or condition caused by a bacterium is tuberculosis; pneumonia, which may be caused by bacteria such as *Streptococcus* and *Pseudomonas*; a foodborne illness, which may be caused by bacteria such as *Shigella, Campylobacter* and *Salmonella*; or an infection such as tetanus, typhoid fever, diphtheria, syphilis and leprosy. The disease or condition may be bacterial vaginosis, a disease of the vagina caused by an imbalance of naturally occurring bacterial flora. Alternatively, the disease or condition is a bacterial meningitis, a bacterial inflammation of the meninges (e.g., the protective membranes covering the brain and spinal cord).

Other diseases or conditions caused by bacteria include, but are not limited to, bacterial pneumonia, a urinary tract infection, bacterial gastroenteritis, and bacterial skin infection. Examples of bacterial skin infections include, but are not limited to, impetigo which may be caused by *Staphylococcus aureus* or *Streptococcus pyogenes*; erysipelas which may be caused by a streptococcus bacterial infection of the deep epidermis with lymphatic spread; and cellulitis which may be caused by normal skin flora or by exogenous bacteria.

[0363] The pathogen may be a fungus, such as, but not limited to, *Candida, Aspergillus, Cryptococcus, Histoplasma, Pneumocystis,* and *Stachybotrys.* Examples of diseases or conditions caused by a fungus include, but are not limited to, jock itch, yeast infection, ringworm, and athlete's foot.

[0364] The pathogen may be a virus. Examples of viruses include, but are not limited to, adenovirus, coxsackievirus, Epstein-Barr virus, Hepatitis virus (e.g., Hepatitis A, B, and C), herpes simplex virus (type 1 and 2), cytomegalovirus, herpes virus, HIV, influenza virus, measles virus, mumps virus, papillomavirus, parainfluenza virus, poliovirus, respiratory syncytial virus, rubella virus, and varicella-zoster virus. Examples of diseases or conditions caused by viruses include, but are not limited to, cold, flu, hepatitis, AIDS, chicken pox, rubella, mumps, measles, warts, and poliomyelitis.

[0365] The pathogen may be a protozoan, such as, but not limited to *Acanthamoeba (e.g., A. astronyxis, A. castellanii, A. culbertsoni, A. hatchetti, A. polyphaga, A. rhysodes, A. healyi, A. divionensis), Brachiola (e.g., B connori, B. vesicularum), Cryptosporidium (e.g., C. parvum), Cyclospora (e.g., C. cayetanensis), Encephalitozoon (e.g., E. cuniculi, E. hellem, E. intestinalis), Entamoeba (e.g., E. histolytica), Enterocytozoon (e.g., E. bieneusi), Giardia (e.g., G. lamblia), Isospora (e.g, I. belli), Microsporidium (e.g., M. africanum, M. ceylonensis), Naegleria (e.g., N. fowleri), Nosema (e.g., N. algerae, N. ocularum), Pleistophora, Trachipleistophora (e.g., T. anthropophthera, T. hominis),* and *Vittaforma (e.g., V. corneae).*

[0366] The disease or condition may be an autoimmune disease or autoimmune related disease. An autoimmune disorder may be a malfunction of the body's immune system that causes the body to attack its own tissues. Examples of autoimmune diseases and autoimmune related diseases include, but are not limited to, Addison's disease, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome (APS), autoimmune aplastic anemia, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune myocarditis, Behcet's disease, celiac sprue, Crohn's disease, dermatomyositis, eosinophilic fasciitis, erythema nodosum, giant cell arteritis (temporal arteritis), Goodpasture's syndrome, Graves' disease, Hashimoto's disease, idiopathic thrombocytopenic purpura (ITP), IgA nephropathy, juvenile arthritis, diabetes, juvenile diabetes, Kawasaki syndrome, Lambert-Eaton syndrome, lupus (SLE), mixed connective tissue disease (MCTD), multiple sclerosis, myasthenia gravis, pemphigus, polyarteritis nodosa, type I, II, & III autoimmune polyglandular syndromes,

polymyalgia rheumatica, polymyositis, psoriasis, psoriatic arthritis, Reiter's syndrome, relapsing polychondritis, rheumatoid arthritis, sarcoidosis, scleroderma, Sjogren's syndrome, sperm & testicular autoimmunity, stiff person syndrome, Takayasu's arteritis, temporal arteritis/giant cell arteritis, ulcerative colitis, uveitis, vasculitis, vitiligo, and Wegener's granulomatosis.

[0367] The disease or condition may be an inflammatory disease. Examples of inflammatory diseases include, but are not limited to, alveolitis, amyloidosis, angiitis, ankylosing spondylitis, avascular necrosis, Basedow's disease, Bell's palsy, bursitis, carpal tunnel syndrome, celiac disease, cholangitis, chondromalacia patella, chronic active hepatitis, chronic fatigue syndrome, Cogan's syndrome, congenital hip dysplasia, costochondritis, Crohn's Disease, cystic fibrosis, De Quervain's tendinitis, diabetes associated arthritis, diffuse idiopathic skeletal hyperostosis, discoid lupus, Ehlers-Danlos syndrome, familial mediterranean fever, fascitis, fibrositis/fibromyalgia, frozen shoulder, ganglion cysts, giant cell arteritis, gout, Graves' Disease, HIV-associated rheumatic disease syndromes, hyperparathyroid associated arthritis, infectious arthritis, inflammatory bowel syndrome/ irritable bowel syndrome, juvenile rheumatoid arthritis, lyme disease, Marfan's Syndrome, Mikulicz's Disease, mixed connective tissue disease, multiple sclerosis, myofascial pain syndrome, osteoarthritis, osteomalacia, osteoporosis and corticosteroid-induced osteoporosis, Paget's Disease, palindromic rheumatism, Parkinson's Disease, Plummer's Disease, polymyalgia rheumatica, polymyositis, pseudogout, psoriatic arthritis, Raynaud's Phenomenon/Syndrome, Reiter's Syndrome, rheumatic fever, rheumatoid arthritis, sarcoidosis, sciatica (lumbar radiculopathy), scleroderma, scurvy, sickle cell arthritis, Sjogren's Syndrome, spinal stenosis, spondyloisthesis, Still's Disease, systemic lupus erythematosis, Takayasu's (Pulseless) Disease, Tendinitis, tennis elbow/golf elbow, thyroid associated arthritis, trigger finger, ulcerative colitis, Wegener's Granulomatosis, and Whipple's Disease.

EXAMPLES

Example 1. Expression and test of humanized CD3 antibody candidates

[0368] To express humanized Fabs in mammalian cells, VH genes (VH1 and VH2) and VL genes (VL1 ~ VL10) shown in **FIG. 2** were individually cloned into the pFUSE vector under the IL2 signal peptide sequence. Light and heavy chains expression vectors were used to co-transfect Expi293F cells according to manufacturer's protocol. On day 3 or 4, cultured media was harvested and secreted Fabs were purified by Protein G chromatography. Binding affinity of humanized candidates for human and cynomolgus T cells were evaluated by flow cytometry. Briefly, cells were incubated with humanized Fabs at 4°C for 30 min and washed twice with staining buffer (1% BSA in PBS). Bound antibodies were revealed with R-phycoethrin (PE)-conjugated anti-human kappa secondary antibodies (Southern Biotech). After several washes, samples were acquired on a BD LSR II or BD Accuri™ C6 and analyzed using FlowJo 7.6.2 software. In each study, cells were incubated with

Date Reçue/Date Received 2023-06-13

secondary antibody alone and the observed mean fluorescence intensity (MFI) was used to subtract for background and non-specific staining. As shown in **FIG. 3** and **Tables 1** and **2**, huL5H2 demonstrated comparable binding affinity to human CD3 as murine anti-CD3 on human T cells (Jurkat). Moreover, huL5H2 also demonstrated good binding to cynomolgus T cells (HSC-F, **FIG. 4, Table 3**).

Table 1

Antibody	IC50 (nM)
Murine (SEQ ID NOS: 23, 24)	7.732
HuL1H1 (SEQ ID NOS: 25, 28)	~9.227 e -009
HuL2H2 (SEQ ID NOS: 26, 29)	~9.227 e -009
HuL3H2 (SEQ ID NOS: 26, 30)	17.68
Hul4H2 (SEQ ID NOS: 26, 31)	~1.294e+010
HuL5H2 (SEQ ID NOS: 26, 32)	4.462
HuL6H2 (SEQ ID NOS: 26, 33)	676.7
HuL7H2 (SEQ ID NOS: 26, 34)	70.42
HuL8H2 (SEQ ID NOS: 26, 35)	77.99
HuL9H2 (SEQ ID NOS: 26, 36)	118.8
HuL10H2 (SEQ ID NOS: 26, 37)	138.9

Table 2

Concentration (nM)	Murine	HuL1H1	HuL2H2	HuL3H2	HuL4H2	HuL5H2	HuL6H2	HuL7H2	HuL8H2	HuL9H2	HuL10H2
1000.000	54400	1470	1470	666	650	51800	2203	35400	54600	39400	46300
200.000	50600	1460	1460	643	620	48000	835	28900	42000	27900	31300
40.000	41400	1450	1450	650	620	41600	598	13600	22000	9946	12700
8.000	28500	1450	1450	610	620	31700	573	4241	7673	2756	3274
1.600	10500	1440	1440	610	615	13400	597	2040	2117	986	1608
0.320	3563	1400	1400	600	599	4432	572	1574	837	637	688
0.000	1397	1397	1397	559	559	1397	559	1252	559	559	559

Table 3

Concentration (nM)	Murine	HuL5H2
1000.000	109000	106000
200.000	94400	96900
40.000	84800	84000
8.000	60700	63200
1.600	21100	26300
0.320	5328	7074
0.000	1209	1209

Example 2. Expression of HuL5H2 Fab in E.coli (comparison of kappa vs lambda light chain)

[0369] huL5H2 Fab with kappa or lambda light chain constant region was cloned into the pBAD vector and expressed using TOP10 *Escherichia coli (E.coli)* competent cells. Briefly, colonies were picked, inoculated into Terrific Broth (TB, 12.00 g Casein Peptone, 4.00 (mL) Glyerol, 2.31 g K_2HPO_4, 12.54 g K_2HPO_4, 24.00 g Yeastolate), and grown overnight at 37°C (200 rpm). The next

day, the cells were used to inoculate 500 mL TB expression medium in 2 L flasks and was further cultured at 37°C (200 rpm). At an O.D. of 0.8~1, arabinose was supplemented to the growth medium (final: 0.2% m/v) and the cells were grown at 26°C (130 rpm) for 48 hours. The cells were then pelleted, suspended in lysis buffer (30 mM Tris-HCl pH 8.0, 1mM EDTA, 20% sucrose, lysozyme 4 mg/g of cell pellet) at 10 mL/g of cell pellet, and lysed at 37°C (200 rpm). After 30 minutes, the lysate was removed of debris by centrifugation (15000 x g, 20 min) and by filtration (0.22 um). Fabs were purified from the lysate by Protein G chromatography, and confirmed by SDS-PAGE. Using this approach, the Fab consisting of a kappa constant region yielded approximately 4-fold higher expression levels than the Fab composed of a lambda constant region (**FIG. 5**).

Example 3. Expression and generation of cynomolgus cross-reactive anti-CD3-double-p-TriA antibody

[0370] Heavy and light chains of CD3-binding Fabs (clone UCHT-1, SEQ ID NOS: 84,85; or huL5H2, (SEQ ID NOS: 4, 10) including kappa constant regions on the respective light chains (SEQ ID NO: 17) and heavy chain Fab (SEQ ID NO: 19) were cloned into a bicistronic pBAD vector, and site-specific mutations to introduce TAG amber nonsense codon at two different positions (resulting in light chain S205TAG (SEQ ID NO: 18) and heavy chain K141TAG (SEQ ID NO: 20)) were performed using the Quikchange Site-directed Mutagenesis Kit (Stratagene). Antibodies were expressed in *Esherichia coli* (*E.coli*) with an orthogonal *Methanococcus jannaschii* tRNA/aminoacyl-tRNA synthetase specific for *p*-acetyl phenylalanine (pAcF) and purified. Purity and incorporation of pAcF was confirmed by SDS-PAGE and mass spectrometry using a quadrupole time of flight (QTOF) mass spectrometer. The mutant antibody with the pAcF residue incorporated (SEQ ID NOS: 40, 42) was conjugated with 30-fold molar excess of p-TriA in NaOAc (pH 4.5) buffer at 37°C for ≥ 14 days. Completion of the conjugation reaction was confirmed by QTOF. Excess unreacted p-TriA was removed by size filtration using an Amicon filter having a 10K and 30K cut-of (**FIG. 6A**), and the size and purity of the final products were confirmed by SDS-PAGE (**FIG. 6B**).

[0371] Flow cytometry was used to test binding of conjugates to cell surface CD3 on human (Jurkat) and cynomolgus (HSC-F) T cells or PSMA on C4-2 cells. Briefly, cells were incubated with UCHT-1 or huL5H2 antibodies (or corresponding p-TriA conjugates), and bound antibodies were revealed with PE-conjugated anti-human kappa secondary antibodies (Southern Biotech). In each study, cells were incubated with secondary antibody alone and the observed mean fluorescence intensity (MFI) was used to subtract for background and non-specific staining. As shown in **FIG.** 7, huL5H2 and UCHT-1 antibodies and conjugates demonstrated comparable binding to Jurkat (human) T cells and C4-2 (PSMA-positive) cells, respectively, with minimal non-specific binding to DU145 (PSMA-

negative) cells. Notably, only antibodies consisting of huL5H2 Fab bound to cynomogous T cells, HSC-F.

Example 4. *In vitro* studies

[0372] *Cytotoxicity assay*

[0373] *In vitro* cytotoxicity was next performed to determine whether the anti-CD3-double-p-TriA antibody conjugates induced antigen-specific target cell killing. In brief, 1 x 10^5 PBMCs (human) and 1 x 10^4 target cells (C4-2 or DU145) were co-cultured with indicated concentrations of antibody conjugates for 24 hours. Cytotoxicity was measured using the Cytotox-96 Nonradioactive cytotoxicity assay kit (Promega), which quantifies the amount of lactate dehydrogenase (LDH) released from lysed cells into the supernatant. The percent lytic activity was calculated with the following formula: (values used represent absorbance at 490nM) % Cytotoxicity = 100 x [((Target cells + Effector cells + Switch) - (Target cells + Effector cells only))/((Maximum target cell lysis) – (Target cells only))]. As shown in **FIGS. 8A** and **8B**, and respective **Tables 4** and **5**, huL5H2- and UCHT-1-p-TriA antibody conjugates selectively redirected human PBMCs against C4-2 (PSMA-positive) cells with comparable potency (huL5H2-p-TriA, EC_{50} = 18.1 pM; UCHT-1-p-TriA, EC_{50} = 22.9 pM). Minimal non-specific killing of DU145 (PSMA-negative) cells was observed. Only huL5H2-p-TriA induced lysis of C4-2 cells with cynomolgus PBMCs (huL5H2-p-TriA, EC_{50} = 60.5 pM) (**FIG. 9, Table 6**).

Table 4

	Percent Cytotoxicity			
Concentration (pM)	huL5H2-p-TriA		UCHT-1-p-TriA	
25000	69.61369	66.99644	63.16478	55.97257
5000	65.71922	64.40013	58.80967	58.71545
1000	67.31051	64.8817	60.06595	59.33312
200	59.74142	59.96126	56.67399	57.48011
40	46.33061	44.43572	39.51528	38.61495
8	26.75356	28.85783	17.81302	23.59192
1.6	9.375	9.563442	9.06093	10.09736
0.32	7.354481	6.977596	6.642588	6.736809
0.064	6.527429	6.370394	6.778685	5.501466
0	5.606156	7.459171	8.903894	5.888819

Table 5

	Percent Cytotoxicity			
Concentration (pM)	huL5H2-p-TriA		UCHT-1-p-TriA	
25000	1.181365	0.476798	12.01105	11.36723
5000	0.094145	0.476798	12.28438	10.92383
1000	0.46465	0.009111	9.350705	8.797983

200	0.397838	-0.11844	3.319363	2.523688
40	0.1974	0.1974	0.871599	0.57398
8	0.009111	0.112366	0.689383	0.391764
1.6	0.282434	-0.082	0.318878	0.762269
0.32	0.11844	0.264213	0.586127	0.476798
0.064	0.173105	-0.08807	0.555758	0.045554
0	0.094145	-0.14881	0.482872	0.1974

Table 6

	Percent Cytotoxicity against C4-2 ($PSMA^{pos}$)			
Concentration (pM)	huL5H2-p-TriA		UCHT-1-p-TriA	
25000	37.00233	34.55063	-2.59718	-2.443147
5000	33.51732	33.94091	-2.69345	-1.987463
1000	28.60107	28.89631	-0.29309	-2.192842
200	23.08795	21.28447	-1.57671	-2.513745
40	11.97185	13.49293	-1.17237	-3.059282
8	2.184284	2.100849	-1.03117	-3.527801
1.6	-0.90281	-1.98105	-1.7885	-3.579145
0.32	-5.69711	-5.74204	-0.67818	-3.989902

[0374] *Activation markers upregulation and proliferation assay*

[0375] Upregulation of activation markers on human T cells by the anti-CD3-double-p-TriA antibody conjugates was assessed in the presence of target cells. In these studies, equal number (1×10^5) of human PBMC and C4-2 (PSMA-positive) or human PBMC and DU145 (PSMA-negative) cells were co-cultured in the presence of 1, 0.1, 0.01, or 0 nM antibody conjugates in 96 well round bottom plates at 37°C for 24 hours. The next day, cultures were labeled with PE-conjugated anti-CD3 (OKT3), AlexaFluor 488-conjugated CD25 (BC96) and allophycocyanin (APC)-conjugated CD69 (FN50) antibodies (all purchased from Biolegend). Appropriate isotype controls were included in each study to determine background and exclude non-specific staining. Unstained and single color controls were acquired and used for compensation. Data is shown in **FIGS. 10A** and **10B,** and **Tables 7** and **8**.

[0376] The effect of anti-CD3-double-p-TriA antibody conjugates on T cell proliferation was also assessed. 1×10^5 carboxyfluorescein succinimidyl ester (CFSE)-labeled human PBMC and 1×10^5 target cells were co-cultured in the presence 1 nM antibody conjugates for 72 hours. All experiments were acquired on a BD Accuri C6 and analyzed using FlowJo 7.6.2 software. As shown in **FIG. 11A and FIG. 11B**, both p-TriA conjugates induced similar capacity of T cell activation and proliferation, respectively, in a PSMA-dependent manner.

Table 7

	Percent CD69- and CD25-positive					
Target Cell	C4-2 (PSMA-positive)					
Concentration (nM)	huL5H2-p-TriA			UCHT-1-p-TriA		
1	27.2	25.2	25	30.6	28.2	25.8
0.1	12.4	12.2	9.99	15.4	14.8	15.8
0.01	0.266	0.438	0.375	0.456	0.531	0.511
0	0.02	0.02	0.079	0.079	0.079	0.02

Table 8

	Percent CD69- and CD25-positive					
Target Cell	DU145 (PSMA-negative)					
Concentration (nM)	huL5H2-p-TriA			UCHT-1-p-TriA		
1	0.16	0.08	0.06	2.09	1.43	1.85
0.1	0.06	0.08	0.16	0.12	0.16	0.06
0.01	0.14	0.12	0.179	0.16	0.139	0.1
0	0.1	0.06	0.08	0.119	0.159	0.14

[0377] *Cytokine release assay*

[0378] Cytokines in cultured media from activation studies described above were quantified using BD CBA Human Th1/Th2 Kit II (BD Biosciences). Samples were acquired on a BD Accuri C6 and analyzed using FCAP Array software. As shown in **FIGS. 12A** and **12B,** and **Tables 9** and **10**, both p-TriA conjugates induced comparable levels of inflammatory cytokines from human T cells in the presence of PSMA-positive C4-2 cells.

Table 9

	C4-2 (PSMA-positive)							
	huL5H2-p-TriA				UCHT-1-p-TriA			
Concentration (nM)	1	0.1	0.01	0	1	0.1	0.01	0
Cytokines								
IL-2	425.33	71.31	6.51	0	470.16	27.81	4.03	0
IFN-gamma	2524.56	1372.34	51.99	0	3408.74	1146.9	21.3	0
TNF	1212.52	805.51	150.64	0	1427.27	630	73.93	0
IL-4	12.75	8.82	2.37	2.14	15.67	6.97	2.74	1.7
IL-10	289.48	206.09	0	0	372.16	101.46	0	0

Table 10

	DU145 (PSMA-negative)							
	huL5H2-p-TriA				UCHT-1-p-TriA			
Concentration (nM)	1	0.1	0.01	0	1	0.1	0.01	0
Cytokines								
IL-2	18.32	0	7.57	9.65	0	0	0	5.84

IFN-gamma	0	0	0	0	310.48	0	0	0
TNF	0	0	0	0	124.6	0	0	0
IL-4	2.41	2.24	2.52	2.15	1.92	2.02	2.41	2.02
IL-10	0	0	0	0	9.16	0	0	0

Example 5. *In vivo* studies

[0379] *Xenograft*

[0380] The *in vivo* efficacy of huL5H2-p-TriA was established in a C4-2 xenograft model. Six to eight weeks old male NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice were implanted subcutaneously with 1 x 10^6 C4-2 cells in Corning® Matrigel®. Once tumors were approximately 150-200mm^3 in size, 20 x 10^6 activated T cells were infused via intraperitoneal and on the next day, daily intravenous treatment with indicated dose of huL5H2-p-TriA was carried out for 10 days. In parallel, a control group (tumor only) consisting of mice injected daily with PBS were included. Tumor growth was monitored biweekly using external calipers and calculated using the formula: (l x w x h)/2. As shown in **FIGS. 13A** and **13B,** and **Tables 11-16**, huL5H2-p-TriA demonstrated dose-dependent *in vivo* anti-tumor activity in the NSG mouse model reconstituted with human T cells. Moreover, daily and every other day treatments were observed to be equally effective in eradicating C4-2 tumors.

Table 11

	Tumor Volume (mm3)				
Treatment	Vehicle				
Days					
0					
4	92.383	125.126	162.17	176.448	148.903
7	129.386	126.511	143.868	139.514	164.488
11	132.9602	149.7841	190.1222	152.8505	194.4304
14	171.77	163.085	195.765	197.452	266.497
18	262.926	329.476	282.933	232.695	364.021
22	357.874	453.907	514.18	265.011	587.627
25	456.548	683.349	666.83	340.556	782.714
28	704.318	931.33	774.096	357.961	1048.348
32	800.775	1198.76	955.653	376.124	1140.723
35	932.385	1238.736	887.041	439.676	1239.037
39	1268.09	1561.976	1154.103	431.296	1546.235
42	1257.107	1464.272	981.121	441.02	1631.606
46	1175.51	1612.388	1267.491	487.822	1699.22
50	1404.576	1969.644	1097.821	600.271	1738.931

Date Reçue/Date Received 2023-06-13

Table 12

	Tumor Volume (mm3)				
Treatment	huL5H2_DI-2xDUPA 1 mg/kg, no PBMC				
Days					
0					
4	146.311	175.579	138.154	130.337	152.875
7	127.092	141.343	128.933	142.711	175.271
11	125.961	187.973	134.537	151.132	176.831
14	174.67	226.785	175.471	173.263	190.23
18	263.621	260.446	157.4	229.606	288.194
22	347.527	409.022	193.643	484.903	397.567
25	654.71	605.685	279.441	601.698	459.315
28	696.459	731.026	385.881	777.722	562.675
32	769.484	824.674	467.48	935.881	638.843
35	871.458	889.715	635.774	1068.018	762.314
39	885.889	914.236	726.983	1080.212	1073.046
42	995.221	957.272	788.294	1228.208	1162.943
46	866.77	1073.114	731.529	1165.54	1220.265
50	920.34	1036.848	951.114	1215.678	1360.944

Table 13

	Tumor Volume (mm3)				
Treatment	huL5H2_DI-2xDUPA 1 mg/kg				
Days					
0					
4	119.745	151.946	129.252	122.107	134.167
7	138.711	143.926	121.815	129.486	159.216
11	142.1008	143.7369	148.0319	164.6003	151.8278
14	135.943	172.439	152.596	190.116	152.417
18	69.732	75.611	75.24	93.575	67.709
22	53.724	54.004	78.817	61.947	59.459
25	54.76	52.362	83.641	68.407	47.685
28	44.341	51.414	90.731	59.844	45.328
32	39.236	47.24	77.336	61.814	54.213
35	33.123	48.011	56.683	53.165	39.249
39	48.399	44.63	77.121	64.964	42.305
42	50.017	46.581	71.445	67.944	48.805
46	47.685	49.317	76.261	67.46	49.359
50	82.591	54.341	84.279	82.953	65.75

Table 14

	Tumor Volume (mm3)				
Treatment	huL5H2_DI-2xDUPA 0.1 mg/kg				
Days					
0					
4	155.309	113.827	138.675	138.761	130.847
7	145.413	121.06	154.499	138.481	130.569
11	185.5239	159.4957	164.9136	125.4246	181.5284

14	230.52	149.2	188.607	144.861	193.634
18	219.892	129.965	129.517	95.282	117.032
22	167.636	103.128	105.863	99.383	101.899
25	166.383	126.692	93.621	89.752	133.334
28	173.912	153.353	102.111	101.125	102.174
32	166.445	270.662	108.015	104	119.2
35	223.386	311.363	141.26	111.124	89.05
39	184.142	374.221	281.788	103.964	122.728
42	300.629	501.91	538.11	129.219	181.543
46	458.553	611.132	870.141	118.985	278.419
50	638.688	674.049	1382.693	125.465	457.966

Table 15

	Tumor Volume (mm3)				
Treatment	huL5H2_DI-2xDUPA 0.01mg/kg				
Days					
0					
4	128.359	136.876	125.019	153.676	145.595
7	146.067	137.62	130.654	119.173	154.354
11	164.2142	147.8484	142.4045	159.3286	166.5676
14	168.815	233.591	185.52	221.558	218.653
18	208.076	350.684	323.69	334.596	193.371
22	284.866	532.256	529.668	503.61	280.309
25	313.219	731.491	743.735	710.57	393.878
28	465.763	958.391	1026.034	821.939	555.945
32	583.188	1061.614	1038.919	914.388	769.382
35	624.773	1105.795	980.013	973.353	914.341
39	568.327	1152.805	891.729	815.426	1100.677
42	646.524	1158.8	997.195	933.189	1212.134
46	581.986	1168.856	1047.78	969.494	1243.452
50	574.358	1245.395	975.512	1029.648	1562.498

Table 16

	Tumor Volume (mm3)				
Treatment	huL5H2_DI-2xDUPA 1 mg/kg (qod)				
Days					
0					
4	117.598	129.579	157.769	129.106	149.626
7	132.124	151.005	136.636	116.148	118.534
11	152.7356	143.4742	189.7295	166.1541	181.2077
14	172.411	156.371	207.959	208.469	151.423
18	70.619	89.479	112.859	95.388	85.421
22	54.138	61.084	87.967	78.081	58.076
25	48.889	45.474	76.68	66.582	55.171
28	52.419	36.023	68.693	58.217	48.691
32	57.584	44.727	93.237	70.926	70.737
35	46.818	51.959	89.682	39.326	54.995
39	76.485	60.434	161.469	73.506	71.292

	Tumor Volume (mm3)				
Treatment	huL5H2_DI-2xDUPA 1 mg/kg (qod)				
Days					
42	77.774	53.135	138.62	77.48	47.641
46	104.46	55.969	145.498	94.773	44.032
50	129.085	67.366	193.971	86.057	62.449

Example 6. Patient-derived xenograft

[0381] Results in the C4-2 xenograft model were next validated in a patient-derived xenograft (PDX) model using primary cells from a human prostate cancer femoral metastasis, PCSD1 (2). Six to eight weeks old male NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice were implanted subcutaneously with 2 x 10^6 PCSD1 tumor cells. Once a palpable tumor is established, approximately 500mm^3, 20 x 10^6 activated T cells were infused via the intraperitoneal and on the next day, daily intravenous treatment with 1 mg/kg of huL5H2-p-TriA was carried out for 10 days. In parallel, a control group (tumor only) where mice were injected daily with PBS were included. Tumor growth was monitored biweekly using external calipers and calculated using the formula: (l x w2)/2. As shown in **FIGS. 14A** and **14B**, and **Tables 17-19**, huL5H2-p-TriA demonstrated promising efficacy in the PDX model.

Table 17

	Tumor Volume (mm3)								
Treatment	Vehicle								
Days									
28.	650.4750	247.0090	729.0000	*0.0000**	677.6000	789.5680	786.5000	686.8160	104.4300
32.	734.8320	652.8640	1420.0940	*0.0000**	703.8690	1125.0000	571.2560	997.2480	1232.0070
35.	1310.7880	905.5935	1223.1140	*0.0000**	1372.8800	1766.2500	1130.1360	1818.6620	1255.7070
39.	1835.4800	1550.7360	2152.0080	0.0000	2407.1920	2898.9410	1990.0980	1392.6400	2321.8240
42.	2645.3760	1273.7670	2419.2000	0.0000	2683.4690	3444.8960	2573.2080	2094.8400	1909.7000
46.	1993.2640	2179.4480	3495.6160	0.0000	3610.0000	2844.1130	4206.5520	3550.0800	2995.2000
49.	3285.6000	3170.2710	3927.2960	0.0000	4243.6160	3604.0640	5013.2750	4458.5480	3507.1080
51.	3999.7010	4231.2490	4620.8000		5285.4880	6394.0320	4704.4800	4371.1250	4750.8930
54.	5272.1280	4855.0440	5191.5280		6231.2720	6650.4020	6597.9250	3927.2960	6067.4400
57.									
59.									
61.									
28.	1257.107				1464.272	981.121	441.02	1631.606	
32.	1175.51				1612.388	1267.491	487.822	1699.22	
35.	1404.576				1969.644	1097.821	600.271	1738.931	

*These values were not used for calculating the final average tumor volume. Each column represents the data for an individual mouse.

Table 18

	Tumor Volume (mm3)					
Treatment	PBMC + Vehicle					
Days						
28.	744.8760	509.9125	563.5575	826.8750	0.0000	506.250
32.	1636.8960	609.7545	777.7280	443.7600	243.6820	1173.600
35.	1460.7200	567.2480	730.0800	722.1375	103.9680	722.000
39.	2721.7050	1500.0000	1861.8400	1399.3620	278.6630	0.000
42.	2928.3450	898.5600	2681.6630	1314.5140	309.3040	0.000
46.	5049.4500	2598.4000	3146.7800	1141.6680	1302.5280	0.000
49.	5163.3050	2861.5260	4240.0000	2513.8960	3325.2420	0.000
51.	6171.6480	3772.0890	4180.0000	2872.2380	3142.8000	0.000
54.	2967.0480	4808.1250	4863.3610	4686.7520	2936.7720	
57.						693.668
59.						443.576
61.						419.813
28.	995.221	957.272	788.294	1228.208	1162.943	
32.	866.77	1073.114	731.529	1165.54	1220.265	
35.	920.34	1036.848	951.114	1215.678	1360.944	
*These values were not used for calculating the final average tumor volume. Each column represents the data for an individual mouse.						

Table 19

	Tumor Volume (mm3)					
Treatment	PBMC + huL5H2_DI-2xDUPA (1mg/kg, qd)					
Days						
28.	711.504	437.1125	592.012	402.040	337.561	*774.400**
32.	1238.400	425.250	587.250	306.5605	252.6523	*1381.203**
35.	1137.150	207.1035	235.468	514.425	107.648	*208.088**
39.	105.966	0.000	0.000	295.074	0.000	
42.	112.0905	0.000	0.000	271.472	0.000	
46.	184.049	0.000	0.000	332.838	0.000	
49.	725.000	0.000	107.217	473.984	0.000	
51.	1609.699	0.000	0.000	188.356	718.8005	
54.						
57.	404.9415	0.000	337.500	898.128	0.000	
59.	173.400	0.000	321.408	681.462	0.000	
61.	105.5925	0.000	369.820	816.480	0.000	
28.	50.017	46.581	71.445	67.944	48.805	
32.	47.685	49.317	76.261	67.46	49.359	
35.	82.591	54.341	84.279	82.953	65.75	
*These values were not used for calculating the final average tumor volume.						

Example 7. Expression, generation, and characterization of de-immunized huL5H2_DI-2xDUPA antibody conjugate

[0382] In silico immunogenicity analysis (EpiVax) of HuL5H2 antibody predicted the antibody to be potentially immunogenic (immunogenicity score = 27.74), due to the potential T cell epitopes found

within the heavy chain sequence. Four de-immunizing mutations (K19R, S41P, K89R, and T90A) were introduced to generate a de-immunized (DI) version of HuL5H2 antibody (DI-HuL5H2) with a significantly reduced immunogenicity score (-51.19) as shown in **FIG. 15**. Site-specific point mutations at positions K19, S41, K89, and T90 on the heavy chain were introduced in the HuL5H2 using the Quikchange Site-directed Mutagenesis Kit (Stratagene) and expressed as described above. The pAcF mutant DI-HuL5H2 was further conjugated with 30-fold molar excess of p-TriA in NaOAc (pH 4.5) buffer at 37°C for ≥ 14 days. Excess p-TriA was removed by size filtration (Amicon, 10K and 30K) and completion of conjugation reaction was confirmed by QTOF (**FIG. 16**).

[0383] Flow cytometry was used to assess for potential differential binding to cell surface CD3 on human (Jurkat) and cynomolgus T cells as a result of the "de-immunization" process. As described above, cells were incubated with huL5H2 or DI-huL5H2 antibodies for 30 min and bound antibodies were revealed with R-phycoethrin (PE)-conjugated anti-human kappa secondary antibodies (Southern Biotech). After several washes, samples were acquired on a BD LSRII or BD Accuri C6 and analyzed using FlowJo software. In these studies, similar binding profiles to human and cynomolgus T cells were observed with both antibodies, which suggest that cross-reactivity to human CD3 was retained even after introducing de-immunizing mutations (**FIGS. 17A** and **17B**, and **Tables 20-21**) (Jurkat: huL5H2 IC_{50} = 5.9 nM and DI-huL5H2 IC_{50} = 4.6 nM; HSC-F: huL5H2 IC_{50} = 3.8 nM and DI-huL5H2 IC_{50} = 3.4 nM).

Table 20

	Mean Fluorescence Intensity			
	Jurkat (huCD3+)			
CD3 antibody	huL5H2		DI-huL5H2	
Concentration (nM)				
1000	29858.33	29858.33	30058.33	30658.33
200	27858.33	27958.33	28958.33	28158.33
40	24558.33	24158.33	25658.33	25058.33
8	16858.33	16458.33	18358.33	17858.33
1.6	6402.333	5426.333	7748.333	7030.333
0.32	1293.333	1189.333	1530.333	1558.333

Table 21

	Mean Fluorescence Intensity			
	HSC-F(cyCD3+)			
CD3 antibody	huL5H2		DI-huL5H2	
Concentration (nM)				
1000	71317.33	62217.33	73017.33	73917.33
200	69317.33	69417.33	70317.33	70217.33

40	63917.33	63417.33	64717.33	63917.33
8	49717.33	48617.33	51417.33	51317.33
1.6	18417.33	18017.33	21117.33	20617.33
0.32	4049.333	3968.333	4790.333	4614.333

Example 8. *In vitro* studies

[0384] *Cytotoxicity assay*

[0385] The *in vitro* activity of the huL5H2_DI-2xDUPA conjugate was assessed in cytotoxicity assays. As described previously in Example 4, 1 x 10^5 PBMCs (human) and 1 x 10^4 target cells (C4-2) were co-cultured with indicated concentrations of antibody conjugates for 24 hours. Cytotoxicity was determined by the amount of lactate dehydrogenase (LDH) released from lysed cells. As shown in **FIG. 18**, and **Tables 22-23**, huL5H2- and huL5H2_DI-2xDUPA conjugates selectively redirected human PBMCs against C4-2 (PSMA-positive) cells with comparable potency (huL5H2-p-TriA, EC_{50} = 3.2 pM; DI-huL5H2-p-TriA, EC_{50} = 3.1 pM) and induced minimal non-specific killing of DU145 (PSMA-negative) cells.

Table 22

	Percent Cytotoxicity					
Target cells	C4-2 (PSMA-positive)					
CD3 antibody conjugates	huL5H2-p-TriA			DI-huL5H2-p-TriA		
Concentration (pM)						
5000	72.30169	70.27051	75.91704	74.08192	69.01573	69.66665
1000	75.47786	74.30935	70.78027	74.6152	67.82368	69.92544
200	72.14484	70.19993	73.33689	68.26286	68.20796	68.92162
40	71.07044	66.02777	70.05092	60.45967	64.84357	59.9107
8	54.00537	53.54267	54.02106	52.63295	55.45622	47.84125
1.6	21.89075	23.37296	20.05563	22.1966	22.88673	18.72242
0.32	3.516009	6.911768	3.139574	4.637472	5.21781	3.915971
0.064	1.767154	1.367192	-0.83652	0.865278	-0.88358	-1.07179
0	3.1646695	2.5451199	2.2471088	3.1646695	2.5451199	2.2471088

Table 23

	Percent Cytotoxicity					
Target cells	DU145 (PSMA-negative)					
CD3 antibody conjugates	huL5H2-p-TriA			DI-huL5H2-p-TriA		
Concentration (pM)						
5000	0.500375	-0.10841	0.825619	0.867317	0.20849	1.000751
1000	1.334334	0.783921	0.708865	0.508715	0.20849	-0.34192

200	0.225169	0.333584	0.60045	0.075056	-0.15845	0.550413
40	0.792261	0.366942	0.567092	0.341923	-0.58377	-0.64215
8	0.016679	-0.4003	0.20015	0.083396	-0.27521	-0.51705
1.6	0.333584	-0.14177	0.575432	-0.4003	0.083396	-1.04245
0.32	0.016679	-0.47536	0.583771	-0.36694	-0.17513	-0.58377
0.064	0.241848	-0.28355	0.150113	-0.53373	-0.52539	-0.98407
0	0.4264309	0.1595641	-0.0072276	0.4264309	0.1595641	-0.0072276

Example 9. Synthesis and comparison of huL5H2_DI-2xDUPA and huL5H2_DI-1xDUPA

[0386] *In vitro binding comparison assay*

[0387] Binding affinity was measured by Octet (ForteBio) using an in-house instrument; the interaction was tested using the DUPA-CD3 as the ligand and reversed interaction as the analyte Each ligand was prepared at 20 μg/mL, 15 ug/ml, 10 μg/mL, and 5 μg/mL in PBS. Analytes were serially diluted 1.2 X from 6 μg/mL to 1 μg/mL in the 1X PBS. 100 mM glycine (pH 2.87) regeneration solution, and 1x PBS for baseline stabilization was also prepared. Biosensor, ligand and analytes pair is following: (1) CH-1 – antibodies – recombinant human or cynomolgus monkey CD3 delta/epsilon complex, (2) Fc – recombinant human or cynomolgus monkey CD3 delta/epsilon complex – antibodies (3) CH-1 – antibodies – recombinant human or cynomolgus monkey PSMA; and (4) Ni-NTA – recombinant human or cynomolgus monkey PSMA – antibodies. Prior to the binding measurements, the sensor tips were pre-hydrated in 1 × PBS for 30 min, followed by 1 cycles of pre-conditioning with 60-sec dips in glycine (pH 2.87). The sensor tips were then transferred to the ligand-containing wells for a 180-sec loading step. After a 120-sec baseline dip in 1 × PBS, the binding kinetics were measured by dipping the ligand-coated sensors into the wells containing corresponding analyte at varying concentrations. The binding interactions were monitored over a 180-sec association period and followed by a 7.5 to 15-min dissociation period in new wells containing fresh 1 × PBS. Binding was comparable between huL5H2_DI-2xDUPA and huL5H2_DI-1xDUPA to CD3 with an affinity of 10 nM; huL5H2_DI-2xDUPA showed significantly higher binding when PMSA was used as the ligand, due to avidity effects (**Table 24**). Binding affinity of both conjugates to cynoPSMA (cynomolgus PSMA) and cynoCD3 (cynomolgus CD3) was similar to the binding affinity of both conjugates to human PSMA and human CD3, respectively.

Table 24

Binding to	Capture	Ligand	Analyte	Affinity
CD3	CH1	L5H2-DI-1X-DUPA	hu.CD3 d/e-Fc	4.83E-09
CD3	CH1	L5H2-DI-2X-DUPA	hu.CD3 d/e-Fc	5.13E-09
cynoCD3	CH1	L5H2-DI-1X-DUPA	cy.CD3 d/e-Fc	3.29E-09
CD3	Fc	hu.CD3de-Fc	L5H2-DI-1X-DUPA	2.12E-08
CD3	Fc	hu.CD3de-Fc	L5H2-DI-2X-DUPA	1.10E-08
cynoCD3	Fc	cy.CD3 d/e-Fc	L5H2-DI-1X-DUPA	1.13E-08

PSMA	CH1	L5H2-DI-1X-DUPA	hu.PSMA	2.36E-11
PSMA	CH1	L5H2-DI-2X-DUPA	hu.PSMA	9.03E-11
cynoPSMA	CH1	L5H2-DI-1X-DUPA	hu.PSMA	n/a
PSMA	Ni-NTA	hu.PSMA	L5H2-DI-1X-DUPA	2.17E-09
PSMA	Ni-NTA	hu.PSMA	L5H2-DI-2X-DUPA	<1.0E-12
cynoPSMA	Ni-NTA	cyno.PSMA	L5H2-DI-1X-DUPA	5.11E-09

[0388] *Synthesis of huL5H2_DI-1xDUPA*

[0389] Humanized anti-CD3 containing single mutant Fab format antibodies were buffer exchanged into conjugation buffer, consisting of 50 mM NaOAc (pH 4.5), 150 mM NaCl and 10% glycerol using PD-10 disposable column and concentrated to 30 mg/ml using Amicon 10K filter. The oxime ligation was conducted with 24~215 molar excess of prostate-specific membrane antigen (PSMA)-binding small molecule ligands to 10 mg/ml antibodies, and the reaction was completed within 18 hours at room temperature, as monitored by liquid chromatography-mass spectrometer. Excess small molecules were removed by size filtration (Amicon 10K) and the conjugates were buffer exchanged into PBS (pH 7.4) followed by removing potential aggregated by a millex GV 0.22um filter before in vitro and in vivo studies. Formic acid salts of DUPA were found to be optimal for conjugation, with a 99.86% conjugation efficiency (**Table 25 and Table 26**).

Table 25

Calibr ID	Salt	Scale	% conjugation	Recovery	Ratio
CBR-001-623-836-1	Formic acid	2 mg	98.91	90.79	54
CBR-001-623-840-7	Formic acid	2 mg	99.48	0.4	74
CBR-001-625-095-6	Formic acid	2 mg	99.86	96.81	68
CBR-001-625-094-5	Formic acid	2 mg	99.86	92.45	81

Table 26

Protein	Small Molec Batch	Salt	% conjugation	Ratio	Notes
huL5H2_DI-1xpAcF	CBR-001-600-008-1	HCl	N/A	215	aggregation
huL5H2_DI-1xpAcF	CBR-001-620-049-0	Na2CO3	93.48	40	
huL5H2_DI-1xpAcF	CBR-001-620-048-9	Li2CO3	74.33	54	
huL5H2_DI-1xpAcF	WuxiDUPA	Li2CO3	90.29	96	
huL5H2_DI-1xpAcF	CBR-001-623-837-2	Li2CO3	90.34	133	
huL5H2_DI-1xpAcF	CBR-001-597-963-2	TFA	99.56	31	
huL5H2_DI-1xpAcF	CBR-001-593-245-3	TFA	99.68	24	
huL5H2_DI-1xpAcF	TSRI	TFA	91.16	57	aggregation

huL5H2_DI-1xpAcF	CBR-001-623-836-1	Formic acid	98.91	27	
huL5H2_DI-1xpAcF	CBR-001-623-840-7	Formic acid	99.48	37	
huL5H2_DI-1xpAcF	CBR-001-624-015-6	TFA	99.47	37	aggregation
huL5H2_DI-1xpAcF	**CBR-001-625-095-6**	**Formic acid**	**99.86**	**34**	
huL5H2_DI-1xpAcF	**CBR-001-625-094-5**	**Formic acid**	**99.86**	**40**	

[0390] *Comparison of PSMA-mediated internalization*

[0391] Flow cytometry was used to determine the internalization rates of huL5H2_DI-1xDUPA and -2xDUPA conjugates (**FIG. 21A, FIG. 21B, FIG. 21C**). Antibody conjugates were randomly conjugated with Alexa Fluor 488 using Alexa Fluor 488 antibody labeling kit (Thermo Fisher Scientific) as per manufacturer's protocol. Corresponding antibodies that were not conjugated to DUPA (i.e. 1xTAG) served as antigen-specific controls. 25 ug of Alexa Fluor 488-labelled antibodies were incubated with 0.5 x 106 C4-2 (PSMA-positive) cells at 37°C for specified durations or on ice for 30 minutes (control). Internalization was halted and excess conjugates were removed with subsequent washes using ice-cold staining buffer (2% FBS/1mM EDTA/DPBS). For each time point, cells were incubated with or without an anti-Alexa Fluor 488 antibody (Thermo Fisher Scientific) on ice for 30 minutes. The mean fluorescence of quenched (Q) and non-quenched (NonQ) cells were assessed on a BD FACSCanto™ II and used to calculate internalization rates as described previously (Cancer Immunol Immunother. 2008 Dec;57(12):1879-90 and Mol Biol Cell. 2004 Dec;15(12):5268-82). In these studies, both huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA conjugates internalized at comparable rates on PSMA-positive cells (**Table 27**).

Table 27

JSM-6-147	huL5H2_DI-1xDUPA	huL5H2_DI-2xDUPA
K_e = slope	0.007402	0.006595
$T_{1/2} = \ln(2)/K_e$	96.64323 (1.6h)	105.1019 (1.8h)
JSM-6-088	huL5H2_DI-1xDUPA	huL5H2_DI-2xDUPA
K_e = slope	0.007527	0.006439
$T_{1/2} = \ln(2)/K_e$	92.0881 (1.5h)	107.6483 (1.8h)

[0392] *In vitro cytoxicity*

[0393] The in vitro efficacy of huL5H2_DI-1xDUPA and -2xDUPA was assessed in cytotoxicity assays. As described previously in Example 4, 1 x 10^5 PBMCs (human) and 1 x 10^4 target cells (C4-2, PSMA-positive or DU145, PSMA-negative) were co-cultured at a 10:1 (Effector:Target cell) ratio with indicated concentrations of antibody conjugates for 24 hours. Cytotoxicity was determined by calculating the amount of lactate dehydrogenase (LDH) released from lysed target cells. As shown, both conjugates selectively redirected human PBMCs against C4-2 cells, where a slightly increased efficacy was observed with huL5H2_DI-1xDUPA (EC50 = 18.5 pM) in comparison to huL5H2_DI-2xDUPA (EC50 = 39.9 pM) (**FIG. 22**). No off-target killing of DU145 was observed with both conjugates.

[0394] *Cytokine release*

[0395] Cytokines in cultured media from cytotoxicity assays described above were quantified using BD CBA Human Th1/Th2 Kit II (BD Biosciences). Samples were acquired on a BD Accuri C6 and analyzed using the FCAP Array software. As shown, both huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA induced comparable antigen-specific release of human inflammatory cytokines in the presence of PSMA-positive C4-2 cells. huL5H2_DI-1xDUPA and -2xDUPA demonstrated a similar cytokine profile (**FIG. 23**).

[0396] *In vitro activation and proliferation*

[0397] Upregulation of activation markers on human PBMC by huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA antibody conjugates was assessed in the presence of PSMA-positive and PSMA–negative target cells. In these studies, equal number (1 x 10^5) of human PBMC and C4-2 (PSMA-positive) or DU145 (PSMA-negative) cells were co-cultured in the presence of 1, 0.1, 0.01, or 0 nM antibody conjugates in 96 well round bottom plates at 37°C for 24 hours. The next day, cultures were labeled with PE-conjugated anti-CD3 (OKT3), AlexaFluor 488-conjugated CD25 (BC96) and allophycocyanin (APC)-conjugated CD69 (FN50) antibodies (all purchased from Biolegend). Appropriate isotype controls were included in each study to determine background and exclude non-specific staining. Unstained and single color controls were acquired and used for compensation. The effect of huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA antibody conjugates on T-cell proliferation was also assessed. 1 x 10^5 carboxyfluorescein succinimidyl ester (CFSE)-labeled human PBMC and 1 x 10^5 mitomycin-treated target cells were cocultured in the presence 1 nM antibody conjugates. After 72 hours, cultures were labeled with anti-CD3 antibody and 7-AAD dye to assess for live, dividing T-cells on the BD Accuri C6. As shown in **FIG. 24 and FIG. 25**, both L5H2_DI antibody conjugates induced similar capacity of T-cell activation and proliferation, respectively, in a PSMA-dependent manner.

[0398] *PSMA quantification assay*

[0399] To compare the *in vitro* activity of huL5H2_DI-1xDUPA and -2xDUPA antibody conjugates against different cancer cells with varying antigen densities, the relative number of cell surface PSMA per cell found on different prostate cancer cell lines was established (**Table 28**). Cell lines were stained with a PE-conjugated anti-human PSMA antibody (Biolegend) and acquired on a BD FACSCanto ™ II or BD Accuri C6. Antigen densities were determined by extrapolating the signal intensities from a standard curve generated by using the QuantiBRITE PE Fluorescent Quantitation kit (BD Pharmingen).

Table 28

Prostate Cancer Cell Lines	**Rel. No. PSMA/cell**	**±SD**
LNCaP	179620	85951
C4-2	110341	43526
VCap	46738	27521
22Rv-1 (sorted)	12217	2935
22Rv-1 (parent)	3606	1770
DU145	114	86

[0400] The *in vitro* activity of huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA antibody conjugates against different cancer cells with varying cell surface PSMA densities was assessed in cytotoxicity assays. As described previously in Example 4, 1 x 10^5 PBMCs (human) and 1 x 10^4 target cells (C4-2) were cocultured with indicated concentrations of antibody conjugates for 24 hours. Cytotoxicity was determined by the amount of lactate dehydrogenase (LDH) released from lysed cells. In these studies, an insignificant increase in target cell killing was observed with huL5H2_DI-1xDUPA in comparison to huL5H2_DI-2xDUPA (**FIG. 26 and FIG. 27**).

[0401] *PSMA competition assay*

[0402] To determine whether soluble PSMA can negatively impact the activity of huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA antibody conjugates, competition assays were established using human PBMC or Jurkat T-cells that stably express the firefly luciferase gene driven by nuclear factor of activated T-cells (NFAT) response elements (Jurkat NFAT-Luc, Invivogen). In assays using human PBMC, 1 x 10^5 PBMCs and 1 x 10^4 target cells (C4-2) were cocultured in the presence of 20 pM huL5H2_DI antibody conjugates and varying concentrations of human PSMA (huPSMA, R&D systems). After 24 hours, target cell lysis was determined by measuring the amount of lactate dehydrogenase (LDH) released. Similarly, 2 x 10^5 Jurkat NFAT-Luc cells and 2 x 10^4 C4-2 cells were cocultured in the presence of 50 pM huL5H2_DI antibody conjugates and varying concentrations of human PSMA (huPSMA, R&D systems) for 24 hours. Luciferase production was measured using the Gaussia Luciferase Glow Assay kit (Pierce) as per manufacturer's instructions. In these studies, soluble huPSMA (up to 10 nM) did not interfere with both huL5H2_DI antibody

conjugates in redirecting human PBMCs against PSMA-positive cells (**FIG. 28 and FIG. 29)** To the same extent, only higher concentrations of huPSMA (≥ 1 nM) was observed to inhibit T-cell activation in cultures containing either huL5H2_DI-1xDUPA or huL5H2_DI-2xDUPA.

[0403] *Jurkat activation assay*

[0404] To validate the activity of different production batches (DUPA sources listed as P0####; with chemistry indicating the salt state, **FIG. 30**) of either huL5H2_DI-1xDUPA or huL5H2_DI-2xDUPA with minimal assay-to-assay variation, a T-cell activation assay was established using a stable Jurkat T-cell line that expresses the firefly luciferase gene driven by six NFAT response elements (Jurkat NFAT-Luc, Invivogen). As described previously in Example 4, 2 x 10^5 Jurkat NFAT-Luc cells and 2 x 10^4 target cells were cocultured in the presence of varying concentrations of antibody conjugates. Phorbol 12- myristate 13-acetate (PMA) and ionomycin (ION))-treated cells were included as controls. After 24 hours, luciferase production was measured using the Gaussia Luciferase Glow Assay kit (Pierce) as per manufacturer's instructions. Experimental values were normalized to the absorbance collected from PMA and ION treated cells or recombinant luciferase (recombinant Lucia, Invivogen) (**FIG. 31A, and FIG. 31B**). Each bar in **FIG. 31B** represents an average RLU value of two wells containing Recombinant Lucia on each plate containing specific treatment samples. This data demonstrate minimal plate-to-plate variation during read-out.

[0405] *Serum Stability*

[0406] To determine degradation, loss or gain of activity in serum, 0.5 mg/ml conjugates were added to normal CD1 mouse, human **(FIG. 33A)**, rat and cynomolgus monkey **(FIG. 33B)** followed by incubation at 37 °C up to 48 hours in an incubator. As shown in **FIG. 32,** the conjugates were purified by KappaSelect affinity resin and performed high resolution mass spec on LCMS-QTOF and SDS-PAGE. In addition, conjugates exposed with various serum were collected/filtered and tested cytotoxicity using PMSA positive prostate cancer cell C4-2 and PSMA negative DU145 cell in mouse and human serum (**FIG. 33A**), as well as rat and monkey serum (**FIG. 33B**). Cytokine release from T-cells in the presence of prostate cancer cells was measured for mouse and human serum (**FIG. 34A**), as well as rat and monkey serum (**FIG. 34B**). No appreciable change in structure or loss of activity/function within 48 hr was observed for either huL5H2_DI-1xDUPA or huL5H2_DI-2xDUPA antibody conjugates.

[0407] *In vivo: C4-2 xenograft model*

[0408] Six to eight weeks old male NOD.Cg-*Prkdc*^{scid}*IL2rg*$^{tm1Wjl/SzJ}$ (NSG) mice were subcutaneously (SC) implanted with 1 x 10^6 C4-2 cells in Matrigel (Corning). Once tumors reached approximately 150-200mm^3 in size, 20 x 10^6 human activated T-cells or 10 x 10^6 human PBMCs were infused via intraperitoneal (IP) and on the next day, daily intravenous (IV) treatment with indicated dose of huL5H2-DI antibody conjugates was initiated and carried out for 10 days. Tumor

growth was monitored every 3 days using external calipers and calculated using the formula: (1 x w x h)/2. Body weight was measured using an electronic scale every day during treatment and every 3 days post-treatment.

[0409] To compare the efficacy of huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA in an in vivo setting, tumor bearing mice received 20 x 106 human activated T-cells via IP and daily IV injections of antibody conjugates at doses 0.05 mg/kg, 0.2 mg/kg, and 1.0 mg/kg for a total of 10 doses (**FIG. 35A**). Here, tumor regression was comparable in animals treated with either 0.2 mg/kg or 1 mg/kg of huL5H2_DI-1xDUPA or huL5H2_DI-2xDUPA (**FIG. 35B**, arrows denoting 1 mg/kg). However, significant body weight loss was observed in mice dosed with 1 mg/kg of both antibody conjugates (**FIG. 35C**).

[0410] In an attempt to circumvent the significant body weight loss observed above, the anti-tumor activity of huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA was assessed in an alternate treatment regimen that entails every other day dosing. As previously described in Example 4, C4-2 tumor bearing mice received 20 x 10^6 human activated T-cells via IP and daily (QD) or every-other-day (QOD) IV injections of antibody conjugates at 0.2 mg/kg for a total of 10 doses (**FIG. 36A**). In parallel, tumor-free mice also received the same treatment regimen. Plasma was collected after the 5^{th} (QD) or 3^{rd} (QOD) dose to measure *in vivo* cytokines using BD CBA Human Th1/Th2 Kit II (**FIG. 37A**) and BD CBA Mouse Inflammatory kit (BD Biosciences, **FIG. 37B**). Samples were acquired on a BD Accuri C6 and analyzed using the FCAP Array software. Regardless of treatment schedule, tumor regression was comparable with both huL5H2_DI antibody conjugates (**FIG. 36B**). **FIG. 36C** shows a QOD injection schedule of huL5H2_DI-2xDUPA or huL5H2_DI-1xDUPA demonstrated similar dose-dependent *in vivo* anti-tumor activity in the NSG mouse model reconstituted with human T cells. huL5H2_DI-2xDUPA was observed to afford greater weight loss in comparison to huL5H2_DI-1xDUPA (**FIG. 38A** and **FIG. 38B**). **FIG. 38C** shows a control experiment that measured weight loss in the absence of tumor.

[0411] To determine whether huL5H2_DI antibody conjugates alone causes toxicity in NSG mice, tumor-free animals received daily injections of huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA at 0.2 mg/kg for a total of 10 doses (**FIG. 39A**). 24 hours after the last injection, animals were euthanized for sampling. Blood was collected for blood cell analysis using the scil Vet abc (scil) and to determine plasma chemistry profile using the Spotchem EZ (scil) (platelets; **FIG. 40A**; blood cells, **FIG. 40B**, renal function, **FIG. 40C;** liver function **FIG. 40D;** and miscellaneous analytes, **FIG. 40E**), and specified organs were harvested for H&E staining (tissue processing, staining and scoring provided by Histotox) (**Table 29** and **Table 30**). Here, weight loss or aberrant number of blood cells and levels of serum proteins was not observed, which suggests that neither huL5H2_DI-

1xDUPA nor huL5H2_DI-2xDUPA induce overt toxicity in the absence of T-cells (as a function of overall body weight, **FIG. 39B,** and percent body weight loss, **FIG. 39C**).

Table 29

Group	Treatment (IV)	Protein ID	N =
A			3
B	PBS, QD x 10d		3
C	L5H2_DI-2xTAG (0.2mg/kg), QD x 10d	P00791	3
D	L5H2_DI-2xDUPA (0.2mg/kg), QD x 10d	P00793	3
E	L5H2_DI-1xTAG (0.2mg/kg), QD x 10d	P00790	3
F	L5H2_DI-1xDUPA (0.2mg/kg), QD x 10d	P00792	3

Table 30

			Prostate	**Large intestine**	**Small intestine**	**Kidney**			**Brain**
Study Group	**Group**	**Animal**	Examined	Examined	Examined	Basophilic tubules	Inflammation, cortical	Dilatation, tubular, cortical	Examined
No Therapeutic	**A**	1	0	0	0	0	0	0	0
		2	0	0	0	1	0	1	0
		3	0	0	0	0	0	0	0
		AVERAGE				*0.33*	*0.00*	*0.33*	
		SD				*0.58*	*0.00*	*0.58*	
Vehicle	**B**	4	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0
		6	0	0	0	0	0	0	0
		AVERAGE				*0.00*	*0.00*	*0.00*	
		SD				*0.00*	*0.00*	*0.00*	
L5H2_DI-2xTAG (0.2mg/kg, QD x 10d)	**C**	7	0	0	0	0	0	0	0
		8	0	0	0	1	0	0	0
		9	0	0	0	1	0	0	0
		AVERAGE				*0.67*	*0.00*	*0.00*	
		SD				*0.58*	*0.00*	*0.00*	
L5H2_DI-2xDU0A (0.2mg/kg, QD x 10d)	**D**	10	0	0	0	0	0	0	0
		11	0	0	0	1	1	0	0
		12	0	0	0	0	0	0	0
		AVERAGE				*0.33*	*0.33*	*0.00*	
		SD				*0.58*	*0.58*	*0.00*	
L5H2_DI-1xTAG (0.2mg/kg, QD x 10d)	**E**	13	0	0	0	1	0	0	0
		14	0	0	0	0	0	0	0
		15	0	0	0	1	0	1	0

		AVERAGE				*0.67*	*0.00*	*0.33*	
		SD				*0.58*	*0.00*	*0.58*	
L5H2_DI-1xDU0A (0.2mg/kg, QD x 10d)	F	16	0	0	0	0	0	0	0
		17	0	0	0	0	0	0	0
		18	0	0	0	0	0	0	0
		AVERAGE				*0.00*	*0.00*	*0.00*	
		SD				*0.00*	*0.00*	*0.00*	
		Toxicity:	0 none	1.00 minimal	2.00 slight	3.00 moderate	4.00 marked	5.00 severe	

[0412] To compare the efficacy of huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA in an *in* vivo setting using human PBMC instead of activated T-cells, tumor bearing mice received 10 x 10^6 human PBMC via IP and daily IV injections of antibody conjugates at 0.2 mg/kg for a total of 23 doses (**FIG. 41** and **Table 31**). After the 20^{th} dose, blood was collected and stained for CD3, CD4 and CD8 T-cells. 24 hours after the last dose, blood was collected for blood cell analysis using the scil Vet abc (scil) and for plasma chemistry analysis using the Spotchem EZ (scil) (**FIG. 44A-C, FIG. 45A-E**), and indicated organs were harvested for H&E staining (tissue processing, staining and scoring provided by Histotox) (**Table 32** and **Table 33**, AVG = average). Here, the use of human PBMC instead of expanded T-cells in a C4-2 xenograft model resulted in delayed anti-tumor activity of huL5H2_DI-1xDUPA and huL5H2_DI-2xDUPA, where 1xDUPA provided a marginal advantage (**FIG. 42**). Weight loss was observed in mice receiving huL5H2_DI-2xDUPA only, which corresponded with beginning stages of tumor regression (each line = one mouse, **FIG. 43**). Indication of graft-versus-host disease (GvHD) was observed in mice that received PBMC alone, independent of huL5H2-DI treatment. However, no overt toxicity (i.e. body weight loss, aberrant blood cell analysis and chemistry, and tissue damage) was associated with huL5H2_DI-1xDUPA treatment.

Table 31

Group	Target cells (SC)	Effector cells (IP)	Treatment (IV)	Protein ID	N =
A					3
B	C4-2 (1.5x10^6)	1x DPBS	DPBS, QD		5
C	C4-2 (1.5x10^6)	PBL (10x10^6)	DPBS, QD		5
D	C4-2 (1.5x10^6)	PBL (10x10^6)	L5H2_DI-1xDUPA (1mg/kg), QD	P00816, P00792	5
E	C4-2 (1.5x10^6)	PBL (10x10^6)	L5H2_DI-1xDUPA (0.2mg/kg), QD	P00816, P00792	5
F	C4-2 (1.5x10^6)	PBL (10x10^6)	L5H2_DI-1xDUPA (0.01g/kg), QD	P00816, P00792	5

G	C4-2 (1.5x10^6)	PBL (10x10^6)	L5H2_DI-2xDUPA (0.2mg/kg), QD	P00813, P00793	5
H		PBL (10x10^6)	DPBS, QD		3
I		PBL (10x10^6)	L5H2_DI-2xDUPA (0.2mg/kg), QD	P00813, P00793	3
J		PBL (10x10^6)	L5H2_DI-1xDUPA (0.2mg/kg), QD	P00816, P00792	3
K		PBS	DPBS, QD		3
L		PBS	L5H2_DI-2xDUPA (0.2mg/kg), QD	P00813, P00793	3
M		PBS	L5H2_DI-1xDUPA (0.2mg/kg), QD	P00816, P00792	3
N					3

Table 32

Group	Tumor	Effector cells (IP)	Treatment (IV)	Protein ID	N =
H	None	PBL (10x10^6)	DPBS, QD	n/a	3
I	None	PBL (10x10^6)	L5H2_DI-2xDUPA (0.2mg/kg), QD	P00813, P00793	3
J	None	PBL (10x10^6)	L5H2_DI-1xDUPA (0.2mg/kg), QD	P00816, P00792	3
K	None	1x DPBS	DPBS, QD	n/a	3
L	None	1x DPBS	L5H2_DI-2xDUPA (0.2mg/kg), QD	P00813, P00793	3
M	None	1x DPBS	L5H2_DI-1xDUPA (0.2mg/kg), QD	P00816, P00792	3
N	none	PBS	PBS	n/a	3

Table 33

		Brain	**Kidney**			**Small intestine**	**Large intestine**		**Prostate**	**Urinary Bladder**
Group	**Animal**	**Dilatation, ventricular**	**Basophilic tubules**	**Inflammation, subacute, cortex/pelvis**	**Dilatation, tubular, cortical**	**Inflammation, subacute, pancreas**	**Adhesion, serosa**	**Inflammation, subacute, mucosa**	**Inflammation, subacute**	**Inflammation, subacute**
	22	0	0	3	0	1	1	0	3	3
H	23	0	0	3	0	4	0	2	3	3
	24	0	0	3	0	3	0	0	3	3
	AVG	*0.00*	*0.00*	*3.00*	*0.00*	*2.67*	*0.33*	*0.67*	*3.00*	*3.00*
	SD	*0.00*	*0.00*	*0.00*	*0.00*	*1.53*	*0.58*	*1.15*	*0.00*	*0.00*
	25	0	0	2	0	0	0	0	2	2
I	26	0	0	3	0	2	0	0	1	1
	27	2	0	2	0	0	0	0	3	3
	AVG	*0.67*	*0.00*	*2.33*	*0.00*	*0.67*	*0.00*	*0.00*	*2.00*	*2.00*

	SD	*1.15*	*0.00*	*0.58*	*0.00*	*1.15*	*0.00*	*0.00*	*1.00*	*1.00*
	28	0	0	3	0	1	0	0	1	1
J	29	0	0	3	0	1	1	0	2	3
	30	0	0	3	0	0	0	0	2	1
	AVG	*0.00*	*0.00*	*3.00*	*0.00*	*0.67*	*0.33*	*0.00*	*1.67*	*1.67*
	SD	*0.00*	*0.00*	*0.00*	*0.00*	*0.58*	*0.58*	*0.00*	*0.58*	*1.15*
	31	0	0	0	0	0	0	0	0	0
K	32	0	0	0	0	0	0	0	0	0
	33	0	0	0	0	0	0	0	0	0
	AVG	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*
	SD	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*
	34	0	0	0	0	0	0	0	0	0
L	35	0	0	0	0	0	0	0	0	0
	36	0	0	0	0	0	0	0	0	0
	AVG	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*
	SD	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*
	37	0	0	0	0	0	0	0	0	0
M	38	0	0	0	0	0	0	0	0	0
	39	0	0	0	0	0	0	0	0	0
	AVG	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*
	SD	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*
	19	0	0	0	0	0	0	0	0	0
N	20	0	0	0	0	0	0	0	0	0
	21	0	0	0	0	0	0	0	0	0
	AVG	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*
	SD	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*
		0	1.00	2.00	*3.00*	*4.00*	*5.00*			

[0413] *Mouse PK assay*

[0414] Male C57BL/6J mouse (Jackson laboratory) were injected i.v. at time 0 with 1 mg/kg conjugates (n = 3 mouse per group). Blood was collected at regular intervals out to 48 h and was processed to plasma. Samples were quantified by electrochemiluminescence technology from Meso Scale Discovery. The capture was recombinant human PSMA (R&D System), and the detection antibody was CaptureSelect biotin anti-IgG-CH1 conjugate (Life Technologies). Pharmacokinetic parameters were determined by noncompartmental analysis using Phoenix WinNonlin 6.3 software (Certara USA, Inc). huL5H2-DI_2xDUPA demonstrated a prolonged exposure compared to huL5H2-DI_1xDUPA (**Table 34** and **FIG. 46**).

Table 34

	$t_{1/2}$ (hrs)	**C_{max} (ng/mL)**	**AUC_{last} (ng*hr/mL)**	**AUC_{inf} (ng*hr/mL)**
huL5H2-DI_1xDUPA	5.87	18348	14228	14244
huL5H2-DI_1xDUPA	9.08	22789	43950	43980

Table 35

Antibody Domain Nucleotide Sequences		
SEQ ID NO.	**Antibody Domain**	**Sequence**
1	Murine anti-CD3 VL	CAAGCAGTTGTGACGCAAGAATCGGCCCTGACCACGAGTCCGGGTGA AACCGTTACGCTGACCTGTCGCTCAAGTACCGGCGCTGTTACCACGAG TAACTATGCGAATTGGGTGCAGGAAAAACCGGATCACCTGTTTACCG GCCTGATTGGCGGTACGAACAAACGTGCGCCGGGTGTTCCGGCACGTT TCTCGGGCAGCCTGATTGGTGATAAAGCAGCACTGACGATCACCGGC GCCCAAACCGAAGACGAAGCAATCTATTTTTGCGCTCTGTGGTACTCT AACCTGTGGGTGTTCGGCGGTGGCACGAAACTGACCGTTCTG
2	Murine anti-CD3 VH	GAAGTCCAGCTGGTTGAATCTGGTGGCGGTCTGGTGCAACCGAAAGG CTCTCTGAAACTGAGTTGCGCAGCTTCCGGTTTTACGTTCAACACCTAT GCGATGAATTGGGTTCGCCAGGCGCCGGGTAAAGGTCTGGAATGGGT CGCGCGTATCCGCAGCAAATATAACAATTACGCAACCTATTACGCTGA TTCAGTGAAAGACCGTTTTACGATTTCGCGCGATGACTCCCAGTCAAT CCTGTACCTGCAAATGAACAATCTGAAAACGGAAGATACCGCCATGT ATTACTGCGTCCGTCACGGCAACTTTGGTAATTCCTATGTGTCATGGTT CGCATACTGGGGCCAGGGTACGCTGGTTACCGTCAGCTCT
3	VH1	GAAGTCCAGCTGGTTGAATCTGGTGGCGGTCTGGTTCAACCGGGCGGT TCGCTGAAACTGAGCTGCGCAGCTTCTGGCTTTACGTTCAACACCTAT GCGATGAATTGGGTTCGCCAGGCCTCAGGCAAAGGTCTGGAATGGGT CGGTCGTATTCGCTCGAAATATAACAATTACGCAACCTATTACGCTGA TAGCGTGAAAGACCGTTTCACCATCAGTCGCGATGACTCCAAAAACA CGCTGTATCTGCAAATGAATAGCCTGAAAACGGAAGATACCGCGGTC TATTACTGCGTGCGTCATGGCAACTTTGGTAATTCTTATGTGAGCTGGT TCGCCTACTGGGGCCAGGGTACGCTGGTTACCGTCAGCTCT
4	VH2	GAAGTCCAGCTGGTTGAATCTGGTGGCGGTCTGGTTCAACCGGGCGGT TCGCTGAAACTGAGCTGCGCAGCTTCTGGCTTTACGTTCAACACCTAT GCGATGAATTGGGTTCGCCAGGCCTCAGGCAAAGGTCTGGAATGGGT CGCTCGTATTCGCTCGAAATATAACAATTACGCAACCTATTACGCTGA TAGCGTGAAAGACCGTTTCACCATCAGTCGCGATGACTCCAAAAACA CGCTGTATCTGCAAATGAATAGCCTGAAAACGGAAGATACCGCGGTC TATTACTGCGTGCGTCATGGCAACTTTGGTAATTCTTATGTGAGCTGGT TCGCCTACTGGGGCCAGGGTACGCTGGTTACCGTCAGCTCT
5	DI-VH2	GAAGTCCAGCTGGTTGAATCTGGTGGCGGTCTGGTTCAACCGGGCGGT TCGCTGAGACTGAGCTGCGCAGCTTCTGGCTTTACGTTCAACACCTAT GCGATGAATTGGGTTCGCCAGGCCCCGGGCAAAGGTCTGGAATGGGT CGCTCGTATTCGCTCGAAATATAACAATTACGCAACCTATTACGCTGA

Antibody Domain Nucleotide Sequences		
SEQ ID NO.	**Antibody Domain**	**Sequence**
		TAGCGTGAAAGACCGTTTCACCATCAGTCGCGATGACTCCAAAAACA CGCTGTATCTGCAAATGAATAGCCTGAGAGCGGAAGATACCGCGGTC TATTACTGCGTGCGTCATGGCAACTTTGGTAATTCTTATGTGAGCTGGT TCGCCTACTGGGGCCAGGGTACGCTGGTTACCGTCAGCTCT
6	VL1	CAAGCTGTTGTGACCCAAGAACCGAGTCTGACCGTGTCTCCGGGCGGC ACCGTTACGCTGACCTGTGGCTCCTCTACCGGCGCAGTCACCACGAGC AACTATGCAAATTGGTTCCAGCAAAAACCGGGTCAGGCTCCGCGTAC CCTGATTTACGGTACGAACAAACGTGCGCCGTGGACCCCGGCACGTTT TTCGGGCAGCCTGCTGGGCGGTAAAGCAGCACTGACCATCAGTGGTG CGCAGCCGGAAGATGAAGCAGAATATTACTGCGCTCTGTGGTATAGC AACCTGTGGGTCTTTGGCGGTGGCACGAAACTGACCGTGCTG
7	VL2	CAAGCTGTTGTGACCCAAGAACCGAGTCTGACCGTGTCTCCGGGCGGC ACCGTTACGCTGACCTGTGGCTCCTCTACCGGCGCAGTCACCACGAGC AACTATGCAAATTGGGTGCAGCAAAAACCGGGTCAGGCTTTTCGTGG CCTGATTTACGGTACGAACAAACGTGCGCCGTGGACCCCGGCACGTTT TTCGGGCAGCCTGCTGGGCGGTAAAGCAGCACTGACCATCAGTGGTG CGCAGCCGGAAGATGAAGCAGAATATTACTGCGCTCTGTGGTATAGC AACCTGTGGGTCTTTGGCGGTGGCACGAAACTGACCGTGCTG
8	VL3	CAAGCTGTTGTGACCCAAGAACCGAGTCTGACCGTGTCTCCGGGCGGC ACCGTTACGCTGACCTGTGGCTCCTCTACCGGCGCAGTCACCACGAGC AACTATGCAAATTGGGTGCAGCAAAAACCGGGTCAGGCTTTTCGTGG CCTGATTGGCGGTACGAACAAACGTGCGCCGTGGACCCCGGCACGTTT TTCGGGCAGCCTGCTGGGCGGTAAAGCAGCACTGACCATCAGTGGTG CGCAGCCGGAAGATGAAGCAGAATATTACTGCGCTCTGTGGTATAGC AACCTGTGGGTCTTTGGCGGTGGCACGAAACTGACCGTGCTG
9	VL4	CAAGCTGTTGTGACCCAAGAACCGAGTCTGACCGTGTCTCCGGGCGGC ACCGTTACGCTGACCTGTGGCTCCTCTACCGGCGCAGTCACCACGAGC AACTATGCAAATTGGGTGCAGCAAAAACCGGGTCAGGCTTTTCGTGG CCTGATTTACGGTACGAACAAACGTGCGCCGTGGACCCCGGCACGTTT TTCGGGCAGCCTGCTGGGCGATAAAGCAGCACTGACCATCAGTGGTG CGCAGCCGGAAGATGAAGCAGAATATTACTGCGCTCTGTGGTATAGC AACCTGTGGGTCTTTGGCGGTGGCACGAAACTGACCGTGCTG
10	VL5	CAAGCTGTTGTGACCCAAGAACCGAGTCTGACCGTGTCTCCGGGCGGC ACCGTTACGCTGACCTGTCGCTCCTCTACCGGCGCAGTCACCACGAGC AACTATGCAAATTGGGTGCAGCAAAAACCGGATCATCTGTTTCGTGGC

Antibody Domain Nucleotide Sequences		
SEQ ID NO.	**Antibody Domain**	**Sequence**
		CTGATTgGCGGTACGAACAAACGTGCGCCGGGGACCCCGGCACGTTTT TCGGGCAGCCTGCTGGGCGATAAAGCAGCACTGACCATCAGTGGTGC GCAGCCGGAAGATGAAGCAGAATATTACTGCGCTCTGTGGTATAGCA ACCTGTGGGTCTTTGGCGGTGGCACGAAACTGACCGTGCTG
11	VL6	CAAGCTGTTGTGACCCAAGAACCGAGTCTGACCGTGTCTCCGGGCGGC ACCGTTACGCTGACCTGTCGCTCCTCTACCGGCGCAGTCACCACGAGC AACTATGCAAATTGGTTCCAGCAAAAACCGGATCATCTGCCGCGTACC CTGATTTACGGTACGAACAAACGTGCGCCGGGGACCCCGGCACGTTTT TCGGGCAGCCTGCTGGGCGATAAAGCAGCACTGACCATCAGTGGTGC GCAGCCGGAAGATGAAGCAGAATATTACTGCGCTCTGTGGTATAGCA ACCTGTGGGTCTTTGGCGGTGGCACGAAACTGACCGTGCTG
12	VL7	CAAGCTGTTGTGACCCAAGAACCGAGTCTGACCGTGTCTCCGGGCGGC ACCGTTACGCTGACCTGTCGCTCCTCTACCGGCGCAGTCACCACGAGC AACTATGCAAATTGGGTGCAGCAAAAACCGGGTCAGGCGTTTCGTGG CCTGATTGGCGGTACGAACAAACGTGCGCCGGGGACCCCGGCACGTT TTTCGGGCAGCCTGCTGGGCGATAAAGCAGCACTGACCATCAGTGGT GCGCAGCCGGAAGATGAAGCAGAATATTACTGCGCTCTGTGGTATAG CAACCTGTGGGTCTTTGGCGGTGGCACGAAACTGACCGTGCTG
13	VL8	CAAGCTGTTGTGACCCAAGAACCGAGTCTGACCGTGTCTCCGGGCGGC ACCGTTACGCTGACCTGTGGCTCCTCTACCGGCGCAGTCACCACGAGC AACTATGCAAATTGGGTGCAGCAAAAACCGGATCATCTGTTTCGTGGC CTGATTGGCGGTACGAACAAACGTGCGCCGGGGACCCCGGCACGTTT TTCGGGCAGCCTGCTGGGCGGTAAAGCAGCACTGACCATCAGTGGTG CGCAGCCGGAAGATGAAGCAGAATATTACTGCGCTCTGTGGTATAGC AACCTGTGGGTCTTTGGCGGTGGCACGAAACTGACCGTGCTG
14	VL9	CAAGCTGTTGTGACCCAAGAACCGAGTCTGACCGTGTCTCCGGGCGGC ACCGTTACGCTGACCTGTGGCTCCTCTACCGGCGCAGTCACCACGAGC AACTATGCAAATTGGGTGCAGCAAAAACCGGGTCAGGCGTTTCGTGG CCTGATTGGCGGTACGAACAAACGTGCGCCGGGGGTCCCGGATCGTTT TTCGGGCAGCCTGCTGGGCGGTAAAGCAGCACTGACCATCAGTGGTG CGCAGCCGGAAGATGAAGCAGAATATTACTGCGCTCTGTGGTATAGC AACCTGTGGGTCTTTGGCGGTGGCACGAAACTGACCGTGCTG
15	VL10	CAAGCTGTTGTGACCCAAGAACCGAGTCTGACCACGTCTCCGGGCGG CACCGTTACGCTGACCTGTCGCTCCTCTACCGGCGCAGTCACCACGAG CAACTATGCAAATTGGGTGCAGCAAAAACCGGATCATCTGTTTACTGG

Antibody Domain Nucleotide Sequences		
SEQ ID NO.	**Antibody Domain**	**Sequence**
		CCTGATTGGCGGTACGAACAAACGTGCGCCGGGGGTCCCGGCACGTT TTTCGGGCAGCCTGATTGGCGATAAAGCAGCACTGACCATCAGTGGTG CGCAGCCGGAAGATGAAGCAGAATATTACTGCGCTCTGTGGTATAGC AACCTGTGGGTCTTTGGCGGTGGCACGAAACTGACCGTGCTG
16	VL5 lambda	CAAGCTGTTGTGACCCAAGAACCGAGTCTGACCGTGTCTCCGGGCGGC ACCGTTACGCTGACCTGTCGCTCCTCTACCGGCGCAGTCACCACGAGC AACTATGCAAATTGGGTGCAGCAAAAACCGGATCATCTGTTTCGTGGC CTGATTgGCGGTACGAACAAACGTGCGCCGGGGACCCCGGCACGTTTT TCGGGCAGCCTGCTGGGCGATAAAGCAGCACTGACCATCAGTGGTGC GCAGCCGGAAGATGAAGCAGAATATTACTGCGCTCTGTGGTATAGCA ACCTGTGGGTCTTTGGCGGTGGCACGAAACTGACCGTGCTGGGTCAGC CGAAAGCAGCTCCGAGCGTCACCCTGTTTCCGCCGAGCAGCGAAGAA CTGCAAGCAAATAAAGCTACCCTGGTTTGTCTGATTAGCGATTTCTAT CCGGGCGCAGTCACGGTGGCATGGAAAGCAGACAGTTCCCCGGTTAA AGCTGGTGTCGAAACCACGACCCCGTCTAAACAGAGTAACAATAAAT ATGCGGCCTCATCGTACCTGAGTCTGACCCCGGAACAGTGGAAATCCC ATCGTTCTTACAGTTGCCAAGTGACCCACGAAGGCAGCACGGTGGAA AAAACCGTTGCGCCGACGGAATGTAGC
17	VL5 kappa	CAAGCTGTTGTGACCCAAGAACCGAGTCTGACCGTGTCTCCGGGCGGC ACCGTTACGCTGACCTGTCGCTCCTCTACCGGCGCAGTCACCACGAGC AACTATGCAAATTGGGTGCAGCAAAAACCGGATCATCTGTTTCGTGGC CTGATTGGCGGTACGAACAAACGTGCGCCGGGGACCCCGGCACGTTT TTCGGGCAGCCTGCTGGGCGATAAAGCAGCACTGACCATCAGTGGTG CGCAGCCGGAAGATGAAGCAGAATATTACTGCGCTCTGTGGTATAGC AACCTGTGGGTCTTTGGCGGTGGCACGAAACTGACCGTTCTGAAACGA ACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGT TGAAATCTGGAACTGCCTCTGTCGTGTGCCTGCTGAATAACTTCTATC CCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCG GGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCAC CTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGA AACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTG**TCT**TCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
18	VL5 kappa-205TAG	CAAGCTGTTGTGACCCAAGAACCGAGTCTGACCGTGTCTCCGGGCGGC ACCGTTACGCTGACCTGTCGCTCCTCTACCGGCGCAGTCACCACGAGC AACTATGCAAATTGGGTGCAGCAAAAACCGGATCATCTGTTTCGTGGC CTGATTGGCGGTACGAACAAACGTGCGCCGGGGACCCCGGCACGTTT TTCGGGCAGCCTGCTGGGCGATAAAGCAGCACTGACCATCAGTGGTG

Antibody Domain Nucleotide Sequences		
SEQ ID NO.	**Antibody Domain**	**Sequence**
		CGCAGCCGGAAGATGAAGCAGAATATTACTGCGCTCTGTGGTATAGC AACCTGTGGGTCTTTGGCGGTGGCACGAAACTGACCGTTCTGAAACGA ACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGT TGAAATCTGGAACTGCCTCTGTCGTGTGCCTGCTGAATAACTTCTATC CCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCG GGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCAC CTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGA AACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTG**TAG**TCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
19	H2	GAAGTCCAGCTGGTTGAATCTGGTGGCGGTCTGGTTCAACCGGGCGGT TCGCTGAAACTGAGCTGCGCAGCTTCTGGCTTTACGTTCAACACCTAT GCGATGAATTGGGTTCGCCAGGCCTCAGGCAAAGGTCTGGAATGGGT CGCTCGTATTCGCTCGAAATATAACAATTACGCAACCTATTACGCTGA TAGCGTGAAAGACCGTTTCACCATCAGTCGCGATGACTCCAAAAACA CGCTGTATCTGCAAATGAATAGCCTGAAAACGGAAGATACCGCGGTC TATTACTGCGTGCGTCATGGCAACTTTGGTAATTCTTATGTGAGCTGGT TCGCCTACTGGGGCCAGGGTACGCTGGTTACCGTCAGCTCTGCCTCCA CCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCC**AAG**AGCACCT CTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCG AACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTG CACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACTGTGCCCTCTAGCAGCTTGGGCACCCAGACCTACATC TGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGT TGAGCCCAAATCTTGTGACAAAACTCACACA
20	H2-141TAG	GAAGTCCAGCTGGTTGAATCTGGTGGCGGTCTGGTTCAACCGGGCGGT TCGCTGAAACTGAGCTGCGCAGCTTCTGGCTTTACGTTCAACACCTAT GCGATGAATTGGGTTCGCCAGGCCTCAGGCAAAGGTCTGGAATGGGT CGCTCGTATTCGCTCGAAATATAACAATTACGCAACCTATTACGCTGA TAGCGTGAAAGACCGTTTCACCATCAGTCGCGATGACTCCAAAAACA CGCTGTATCTGCAAATGAATAGCCTGAAAACGGAAGATACCGCGGTC TATTACTGCGTGCGTCATGGCAACTTTGGTAATTCTTATGTGAGCTGGT TCGCCTACTGGGGCCAGGGTACGCTGGTTACCGTCAGCTCTGCCTCCA CCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCC**TAG**AGCACCT CTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCG AACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTG CACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACTGTGCCCTCTTAGAGCTTGGGCACCCAGACCTACATC TGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGT

Antibody Domain Nucleotide Sequences		
SEQ ID NO.	**Antibody Domain**	**Sequence**
		TGAGCCCAAATCTTGTGACAAAACTCACACA
21	DI-H2	GAAGTCCAGCTGGTTGAATCTGGTGGCGGTCTGGTTCAACCGGGCGGT TCGCTGAGACTGAGCTGCGCAGCTTCTGGCTTTACGTTCAACACCTAT GCGATGAATTGGGTTCGCCAGGCCCCGGGCAAAGGTCTGGAATGGGT CGCTCGTATTCGCTCGAAATATAACAATTACGCAACCTATTACGCTGA TAGCGTGAAAGACCGTTTCACCATCAGTCGCGATGACTCCAAAAACA CGCTGTATCTGCAAATGAATAGCCTGAGAGCGGAAGATACCGCGGTC TATTACTGCGTGCGTCATGGCAACTTTGGTAATTCTTATGTGAGCTGGT TCGCCTACTGGGGCCAGGGTACGCTGGTTACCGTCAGCTCTGCCTCCA CCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCC**AAG**AGCACCT CTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCG AACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTG CACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACTGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATC TGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGT TGAGCCCAAATCTTGTGACAAAACTCACACA
22	DI-H2 141TAG	GAAGTCCAGCTGGTTGAATCTGGTGGCGGTCTGGTTCAACCGGGCGGT TCGCTGAGACTGAGCTGCGCAGCTTCTGGCTTTACGTTCAACACCTAT GCGATGAATTGGGTTCGCCAGGCCCCGGGCAAAGGTCTGGAATGGGT CGCTCGTATTCGCTCGAAATATAACAATTACGCAACCTATTACGCTGA TAGCGTGAAAGACCGTTTCACCATCAGTCGCGATGACTCCAAAAACA CGCTGTATCTGCAAATGAATAGCCTGAGAGCGGAAGATACCGCGGTC TATTACTGCGTGCGTCATGGCAACTTTGGTAATTCTTATGTGAGCTGGT TCGCCTACTGGGGCCAGGGTACGCTGGTTACCGTCAGCTCTGCCTCCA CCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCC**TAG**AGCACCT CTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCG AACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTG CACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACTGTGCCCTCTAGCAGCTTGGGCACCCAGACCTACATC TGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGT TGAGCCCAAATCTTGTGACAAAACTCACACA

Abbreviations: VH= heavy chain variable domain; VL= light chain variable domain; DI= de-immunized; TAG = STOP codon, encodes an unnatural amino acid; H = heavy chain Fab (heavy chain variable + C_H1 domains); Bold/underlined codons are sites for (replacement with) unnatural amino acids.

Table 36

Antibody Domain Amino Acid Sequences		
SEQ ID NO.	**Antibody Domain**	**Sequence**
23	Murine Anti-CD3 VL	QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGL IGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLW VFGGGTKLTVL
24	Murine Anti-CD3 VH	EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEW VARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMY YCVRHGNFGNSYVSWFAYWGQGTLVTVSS
25	VH1	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGLEW VGRIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLKTEDTAV YYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
26	VH2	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGLEW VARIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLKTEDTAV YYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
27	DI-VH2	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEW VARIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAV YYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
28	VL1	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWFQQKPGQAPRTL IYGTNKRAPWTPARFSGSLLGGKAALTISGAQPEDEAEYYCALWYSNL WVFGGGTKLTVL
29	VL2	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAFRG LIYGTNKRAPWTPARFSGSLLGGKAALTISGAQPEDEAEYYCALWYSNL WVFGGGTKLTVL
30	VL3	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAFRG LIGGTNKRAPWTPARFSGSLLGGKAALTISGAQPEDEAEYYCALWYSNL WVFGGGTKLTVL
31	VL4	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAFRG LIYGTNKRAPWTPARFSGSLLGDKAALTISGAQPEDEAEYYCALWYSNL WVFGGGTKLTVL
32	VL5	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPDHLFRG LIGGTNKRAPGTPARFSGSLLGDKAALTISGAQPEDEAEYYCALWYSNL WVFGGGTKLTVL

Antibody Domain Amino Acid Sequences		
SEQ ID NO.	**Antibody Domain**	**Sequence**
33	VL6	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWFQQKPDHLPRTL IYGTNKRAPGTPARFSGSLLGDKAALTISGAQPEDEAEYYCALWYSNLW VFGGGTKLTVL
34	VL7	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRG LIGGTNKRAPGTPARFSGSLLGDKAALTISGAQPEDEAEYYCALWYSNL WVFGGGTKLTVL
35	VL8	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPDHLFRG LIGGTNKRAPGTPARFSGSLLGGKAALTISGAQPEDEAEYYCALWYSNL WVFGGGTKLTVL
36	VL9	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAFRG LIGGTNKRAPGVPDRFSGSLLGGKAALTISGAQPEDEAEYYCALWYSNL WVFGGGTKLTVL
37	VL10	QAVVTQEPSLTTSPGGTVTLTCRSSTGAVTTSNYANWVQQKPDHLFTGL IGGTNKRAPGVPARFSGSLIGDKAALTISGAQPEDEAEYYCALWYSNLW VFGGGTKLTVL
38	VL5 lambda	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPDHLFRG LIGGTNKRAPGTPARFSGSLLGDKAALTISGAQPEDEAEYYCALWYSNL WVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSY SCQVTHEGSTVEKTVAPTECS
39	VL5 kappa	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPDHLFRG LIGGTNKRAPGTPARFSGSLLGDKAALTISGAQPEDEAEYYCALWYSNL WVFGGGTKLTVLKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGL**S**SPVTKSFNRGEC
40	VL5 kappa 205TAG	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPDHLFRG LIGGTNKRAPGTPARFSGSLLGDKAALTISGAQPEDEAEYYCALWYSNL WVFGGGTKLTVLKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGL**pAcF**SPVTKSFNRGEC
41	H2	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGLEW VARIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLKTEDTAV

Antibody Domain Amino Acid Sequences		
SEQ ID NO.	**Antibody Domain**	**Sequence**
		YYCVRHGNFGNSYVSWFAYWGQGTLVTVSSASTKGPSVFPLAPSS**K**STS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
42	H2 141TAG	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGLEW VARIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLKTEDTAV YYCVRHGNFGNSYVSWFAYWGQGTLVTVSSASTKGPSVFPLAPSS**pAcF** STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
43	DI-H2	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEW VARIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAV YYCVRHGNFGNSYVSWFAYWGQGTLVTVSSASTKGPSVFPLAPSS**K**STS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
44	DI-H2 141TAG	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEW VARIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAV YYCVRHGNFGNSYVSWFAYWGQGTLVTVSSASTKGPSVFPLAPSS**pAcF** STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
45	DI-H2 (K19R)	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQASGKGLEW VARIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLKTEDTAV YYCVRHGNFGNSYVSWFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
46	DI-H2 (S41P)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEW VARIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLKTEDTAV YYCVRHGNFGNSYVSWFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
47	DI-H2 (K89R)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGLEW VARIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLRTEDTAV YYCVRHGNFGNSYVSWFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT

Antibody Domain Amino Acid Sequences		
SEQ ID NO.	**Antibody Domain**	**Sequence**
48	DI-H2 (T90A)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGLEW VARIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAV YYCVRHGNFGNSYVSWFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
49	DI-VH2 Minimal	RLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTLYLQMNSLRA
50	DI-VH2 Super Minimal	PGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSL RA
Abbreviations: VH= heavy chain variable domain; VL= light chain variable domain; DI= de-immunized; Bold/underlined amino acids are sites for (replacement with) unnatural amino acids; H = heavy chain Fab (heavy chain variable + C_H1 domains); pAcF = p-acetylphenylalanine.		

Table 37

Inter-CDR Antibody Domain Amino Acid Sequences		
SEQ ID NO.	**Antibody Domain**	**Sequence**
51	LCDR1	RSSTGAVTTSNYAN
52	LCDR2	GTNKRAP
53	LCDR3	ALWYSNLWV
54	HCDR1	GFTFNTYAMN
55	HCDR2	RIRSKYNNYATYYADSVKD
56	HCDR3	HGNFGNSYVSWFAY
57	LC Inter-CDR1/2 Region Option 1	WVQQKPGQAFRGLIY
58	LC Inter-CDR1/2 Region	WVQQKPGQAFRGLIG

Inter-CDR Antibody Domain Amino Acid Sequences		
SEQ ID NO.	**Antibody Domain** Option 2	**Sequence**
59	LC Inter-CDR1/2 Region Option 3	WVQQKPDHLFRGLIG
60	LC Inter-CDR1/2 Region Option 4	WFQQKPDHLFRTLIY
61	LC Inter-CDR1/2 Region Option 5	WVQQKPGQAFRGLIG
62	Super Minimal LC Inter-CDR1/2 Region Option 1	DHLFR
63	Super Minimal LC Inter-CDR1/2 Region Option 2	KPDHLFR
64	Minimal LC Inter-CDR1/2 Region	QKPDHLFR
65	Variable Minimal LC Inter-CDR1/2 Region	Q X_1X_2DHLFR, wherein X_1 and X_2 are selected from any amino acid
66	Variable LC Inter-CDR1/2 Region Arginine	$X_1VX_2X_3X_4X_5$DHLFRGX_6X_7G, wherein X_1 X_2 X_3 X_4 X_5 X_6, and X_7 are selected from any amino acid.
67	Variable LC Inter-CDR1/2 Region Glutamine	X_1VX_2Q X_3X_4DHLFX_5GX_6X_7G, wherein X_1 X_2 X_3 X_4 X_5 X_6, and X_7 are selected from any amino acid.
68	Variable LC Inter-CDR1/2 Region	$X_1VX_2X_3X_4X_5$DHLFX_6GX_7X_8G, wherein X_1 X_2 X_3 X_4 X_5 X_6, and X_7 are selected from any amino acid.
69	HC Pre-CDR1 Region Option 1	EVQLVESGGGLVQPGGSLXLSCAAS, wherein X is selected from Lysine (K) and Arginine (R)
70	HC Inter-CDR1/2 Region Option 1	WVRQASGKGLEWVX, wherein X is selected from Glycine (G) and Alanine (A)
71	HC Inter-CDR1/2 Region Option 2	WVRQAPGKGLEWVX, wherein X is selected from Glycine (G) and Alanine (A)

Inter-CDR Antibody Domain Amino Acid Sequences		
SEQ ID NO.	**Antibody Domain**	**Sequence**
72	LC Inter-CDR2/3 Region Option 1	WTPARFSGSLLGGKAALTISGAQPEDEAEYYC
73	LC Inter-CDR2/3 Region Option 2	WTPARFSGSLLGDKAALTISGAQPEDEAEYYC
74	LC Inter-CDR2/3 Region Option 3	GTPARFSGSLLGDKAALTISGAQPEDEAEYYC
75	LC Inter-CDR2/3 Region Option 4	GTPARFSGSLLGGKAALTISGAQPEDEAEYYC
76	LC Inter-CDR2/3 Region Option 5	GVPDRFSGSLLGGKAALTISGAQPEDEAEYYC
77	LC Inter-CDR2/3 Region Option 6	GVPARFSGSLLGGKAALTISGAQPEDEAEYYC
78	HC Inter-CDR2/3 Region Option 1	RFTISRDDSKNTLYLQMNSLKTEDTAVYYCVR
79	HC Inter-CDR2/3 Region Option 2	RFTISRDDSKNTLYLQMNSL X_1 X_2EDTAVYYCVR, wherein X_1 is selected from Lysine (K) and Arginine (R), and X_2 is selected from Threonine (T) and Alanine (A)
Abbreviations: LC = light chain; HC = heavy chain		

Table 38

Targeting Agent Antibody Conjugate Amino Acid Sequences		
SEQ ID NO.		**Sequence**
80	DI-H2 N terminus	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWV RQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISR DDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVS WFAYWGQGTLVTVSSASTKGPSVFPLAPSS
81	DI-H2 C terminus	STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT

Targeting Agent Antibody Conjugate Amino Acid Sequences		
SEQ ID NO.		**Sequence**
		KVDKKVEPKSCDKTHT
82	LC kappa N terminus	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANW VQQKPDHLFRGLIGGTNKRAPGTPARFSGSLLGDKAAL TISGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLKRT VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGL
83	LC kappa C terminus	SPVTKSFNRGEC
Abbreviations: LC = light chain; DI= de-immunized; H = heavy chain Fab (heavy chain variable + C_H1 domains)		

Table 39

Additional Amino Acid Sequences		
SEQ ID NO.		**Sequence**
84	UCHT-1 HC	DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQ KPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSL QPEDFATYYCQQGNTLPWTFGQGTKVEIKRTVAAPSV FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
85	UCHT-1 LC	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWV RQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDK SKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFD VWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHT
86	HC CH1	ASTKGPSVFPLAPSS**K**STSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
87	HC Pre-CDR1 Region	EVQLVESGGGLVQPGGSLRLSCAAS

Additional Amino Acid Sequences		
SEQ ID NO.		**Sequence**
	Option 2	
88	HC Pre-CDR1 Region Option 3	EVQLVESGGGLVQPGGSLKLSCAAS
89	HC Inter-CDR1/2 Region Option 3	WVRQASGKGLEWVG
90	HC Inter-CDR1/2 Region Option 3	WVRQASGKGLEWVA
91	HC Inter-CDR1/2 Region Option 4	WVRQAPGKGLEWVG
92	HC Inter-CDR1/2 Region Option 5	WVRQAPGKGLEWVA
93	HC Inter-CDR2/3 Region Option 3	RFTISRDDSKNTLYLQMNSLKTEDTAVYYCVR
94	HC Inter-CDR2/3 Region Option 4	RFTISRDDSKNTLYLQMNSLKAEDTAVYYCVR
95	HC Inter-CDR2/3 Region Option 5	RFTISRDDSKNTLYLQMNSLRTEDTAVYYCVR
96	HC Inter-CDR2/3 Region Option 6	RFTISRDDSKNTLYLQMNSLRAEDTAVYYCVR
97	LC Pre-CDR1 Region Option 1	QAVVTQEPSLTVSPGGTVTLTC
98	HC CH1	ASTKGPSVFPLAPSS**pAcF**STSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
99	HC CH1 A	ASTKGPSVFPLAPSS
100	HC CH1 B	STSGGTAALG
101	HC CH1 C	CLVKDYFPEP

Additional Amino Acid Sequences		
SEQ ID NO.		**Sequence**
102	HC CH1 D	VTVSWNSGAL
103	HC CH1 E	TSGVHTFPAV
104	HC CH1 F	LQSSGLYSLS
105	HC CH1 G	SVVTVPSSSL
106	HC CH1 H	GTQTYICNVN
107	HC CH1 I	HKPSNTKVDK
108	HC CH1 J	KVEPKSCDKTHT
109	HC CH1 K	STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHT
110	LC End Region	FGGGTKLTVL
111	VL5 CL1	KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGL**S**SPVTKSFNRGEC
112	VL5 CL2	KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGL**pAcF**SPVTKSFNRGEC
113	VL CLA	KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGL
114	VL CLB	SPVTKSFNRGEC
115	VL CLA1	KRTVAAPSVF
116	VL CLA2	IFPPSDEQLK
117	VL CLA3	SGTASVVCLL
118	VL CLA4	NNFYPREAKV

Additional Amino Acid Sequences		
SEQ ID NO.		**Sequence**
119	VL CLA5	QWKVDNALQS
120	VL CLA6	GNSQESVTEQ
121	VL CLA7	DSKDSTYSLS
122	VL CLA8	STLTLSKADY
123	VL CLA9	EKHKVYACEVTHQGL
124	VH2 end	WGQGTLVTVSS
Abbreviations: LC = light chain; DI= de-immunized; H = heavy chain Fab (heavy chain variable + C_H1 domains)		

CLAIMS

What is claimed is:

1. A CD3 binding antibody comprising a light chain variable region comprising SEQ ID NO: 32 and a heavy chain variable region comprising SEQ ID NO: 27.

2. The antibody of claim 1, comprising SEQ ID NO: 111 or 112.

3. The antibody of claim 1 or claim 2, comprising SEQ ID NO: 86 or 98.

4. The antibody of any one of claims 1-3, comprising an unnatural amino acid.

5. The antibody of claim 4, wherein the unnatural amino acid is located within: a light chain constant domain sequence, a heavy chain constant domain sequence, or the light chain constant domain sequence and the heavy chain constant domain sequence.

6. The antibody of claim 4 or claim 5, wherein the unnatural amino acid is para-acetylphenylalanine.

7. The antibody of claim 1, comprising a heavy chain sequence at least 95% identical to SEQ ID NO: 44.

8. The antibody of claim 1 or claim 7, comprising a light chain sequence at least 95% identical to SEQ ID NO: 39.

9. A composition comprising the antibody of any one of claims 1-8 and a cell-targeting molecule.

10. A composition comprising a cell-targeting molecule connected to the antibody of any one of claims 4-6 via the unnatural amino acid.

11. The composition of claim 9 or claim 10, wherein the cell-targeting molecule interacts with prostate-specific membrane antigen (PSMA) or a folate receptor.

Date Reçue/Date Received 2023-06-13

FIG. 1A

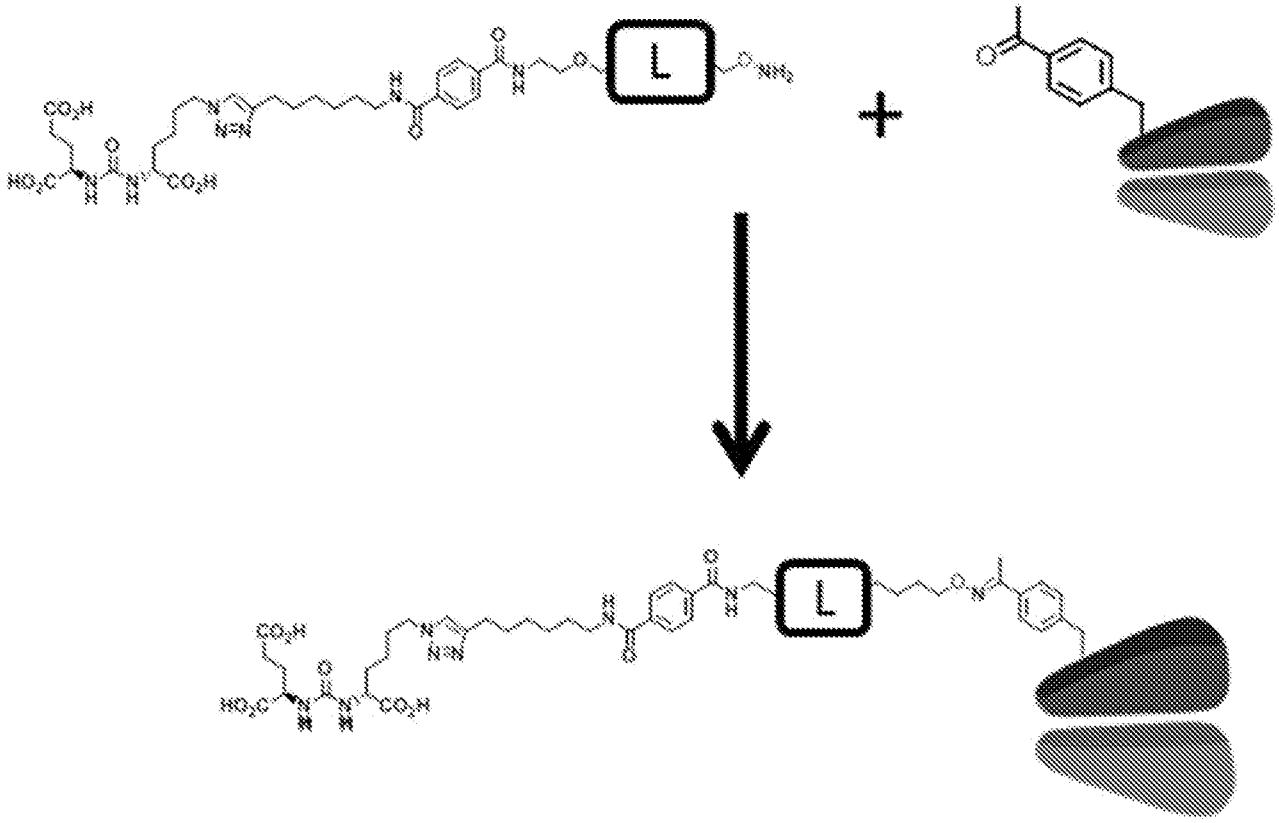


FIG. 1B

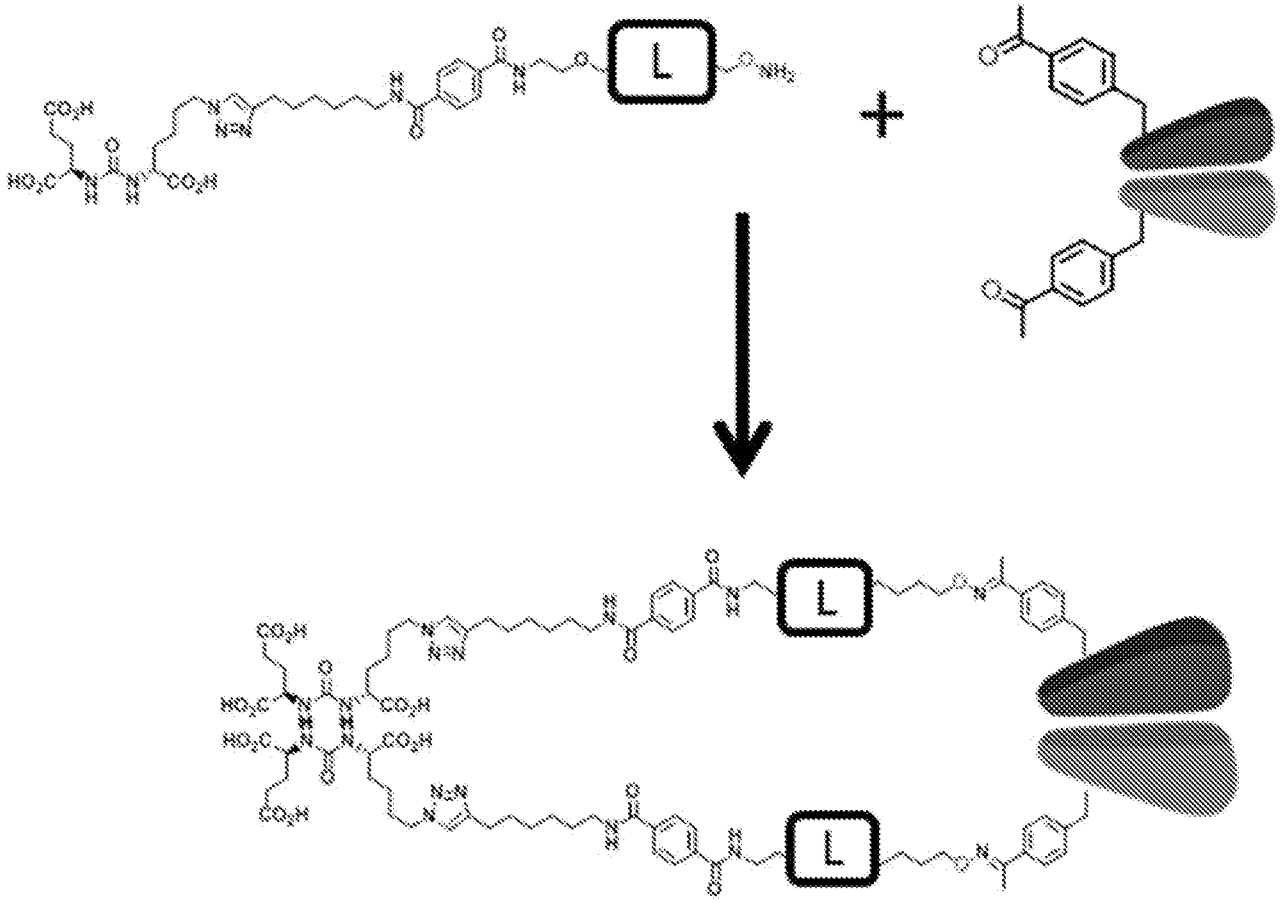


FIG. 1C

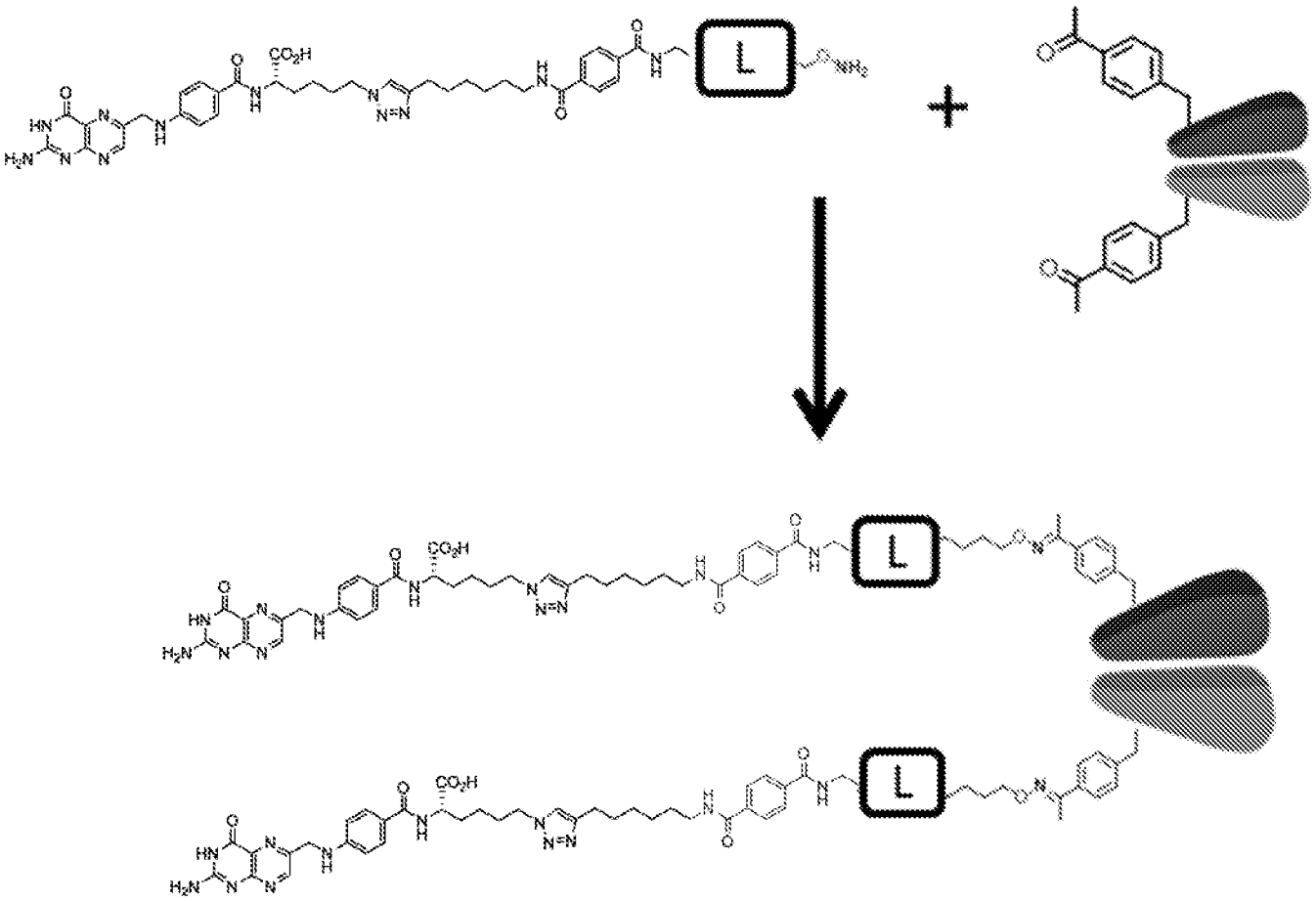
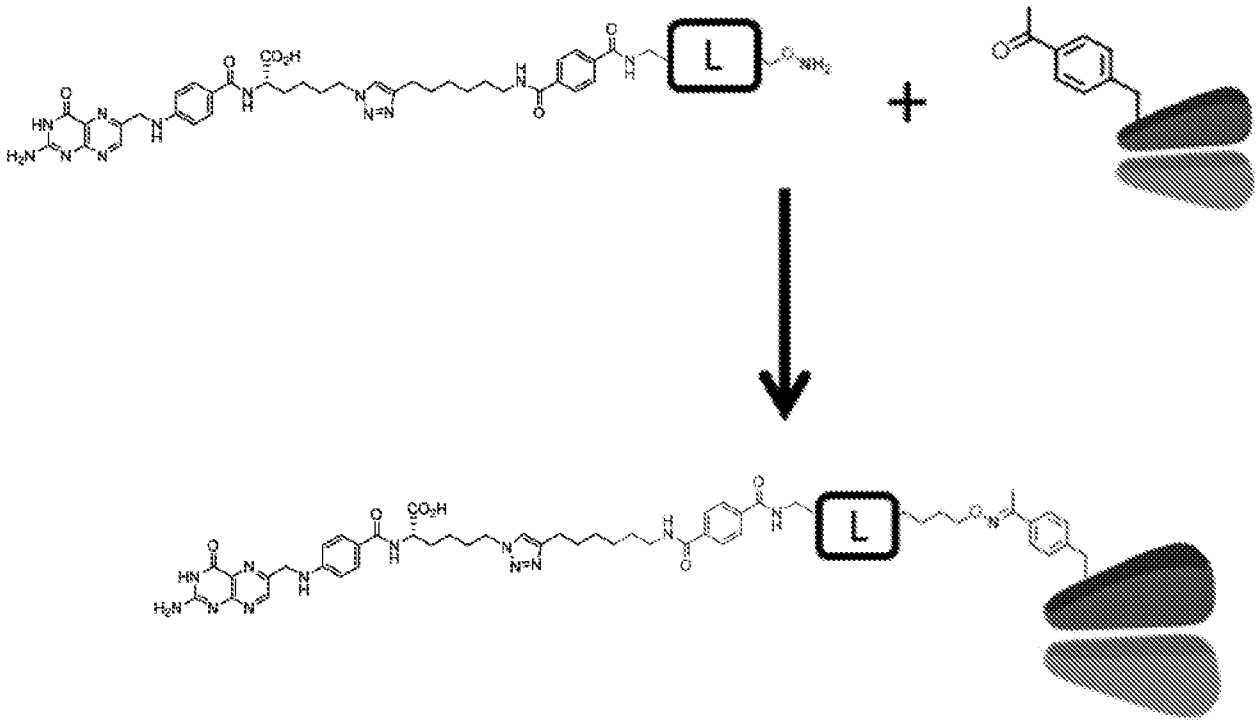


FIG. 1D



VH sequences

```
Mur SP34  EVQLVESGGGLVQPKGSLKLSCAAS HCDR1 WVRQAPGKGLEWVA HCDR2 RFTISRDDSQSILYLQMNNLKTEDTAMYYCVR HCDR3 WGQGTLVTVSS
Murine                  *                     *       *                      ****    *       *

IGHV3-73  EVQLVESGGGLVQPGGSLKLSCAAS       WVRQASGKGLEWVG       RFTISRDDSKNTAYLQMNSLKTEDTAVYYCTR       WGQGTLVTVSS
VH hu1    EVQLVESGGGLVQPGGSLKLSCAAS       WVRQASGKGLEWVG       RFTISRDDSKNTLYLQMNSLKTEDTAVYYCVR       WGQGTLVTVSS
VH hu2    -------------------------       -------------A       --------------------------------       -----------
```

VL sequences

```
Mur SP34  QAVVTQESALTTSPGETVTLTC LCDR1 WVQEKPDHLFTGLIG LCDR2 GVPARFSGSLIGDKAALTITGAQTEDEAIYFC LCDR3 FGGGTKLTVL
Murine          **   *   *              * *  ******  *        **         * *    **   *    * *

IGLV7-46  QAVVTQEPSLTVSPGGTVTLTC       WFQQKPGQAPRTLIY       WTPARFSGSLLGGKAALTLSGAQPEDEAEYYC
VL hu1    QAVVTQEPSLTVSPGGTVTLTC       WFQQKPGQAPRTLIY       WTPARFSGSLLGGKAALTISGAQPEDEAEYYC       FGGGTKLTVL
VL hu2    ----------------------       -V-------F-G--Y       W-----------G-----I---------E-Y-       ----------
VL hu3    ----------------------       -V-------F-G--G       W-----------G-----I---------E-Y-       ----------
VL hu4    ----------------------       -V-------F-G--Y       W-----------D-----I---------E-Y-       ----------
VL hu5    ----------------------       -V----DHLF-G--G       G-----------D-----I---------E-Y-       ----------
VL hu6    ----------------------       -F----DHLP-T--Y       G-----------D-----I---------E-Y-       ----------
VL hu7    ----------------------       -V----GQAF-G--G       G-----------D-----I---------E-Y-       ----------
VL hu8    ----------------------       -V----DHLF-G--G       G-----------G-----I---------E-Y-       ----------
VL hu9    ----------------------       -V----GQAF-G--G       GV-D--------G-----I---------E-Y-       ----------
VL hu10   -----------T----------       -V----DHLF-G--G       GV----------G-----I---------E-Y-       ----------
```

FIG. 2

FIG. 3

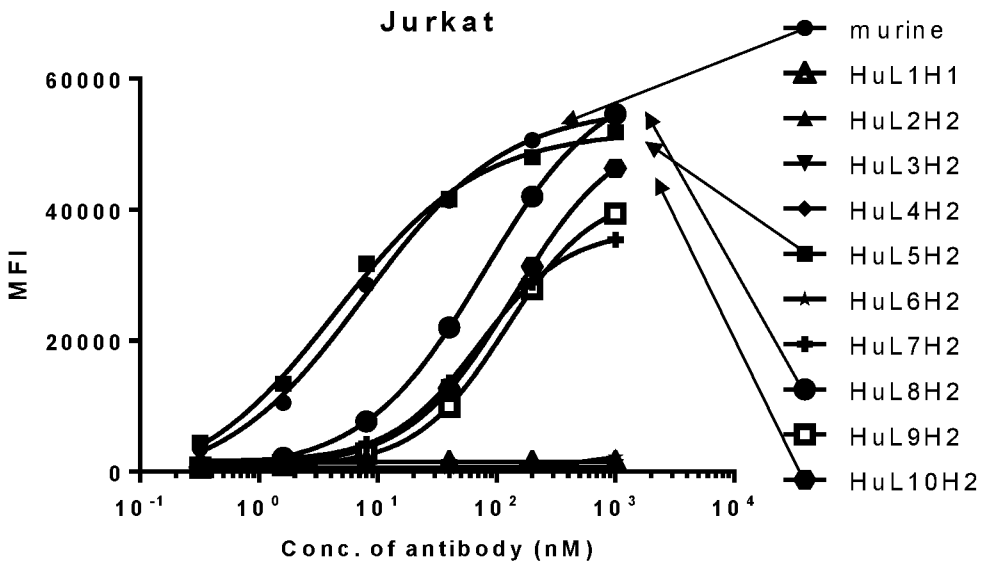


FIG. 4

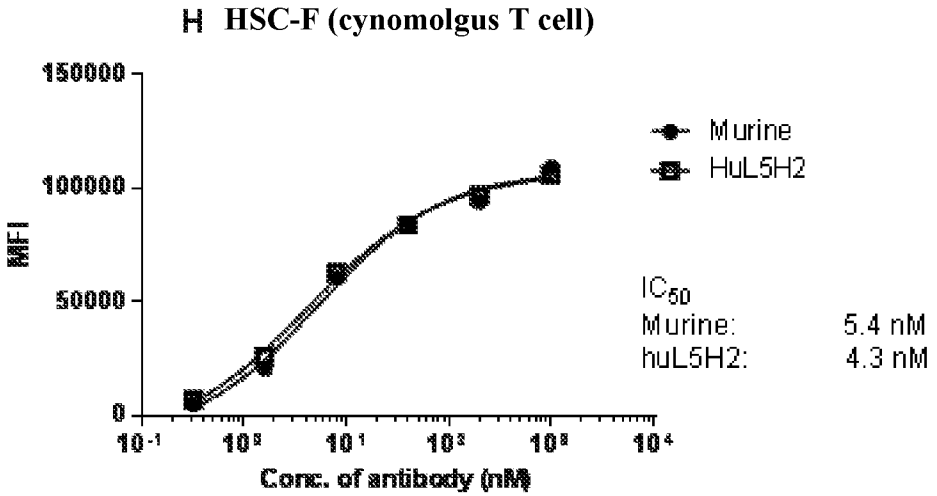


FIG. 5

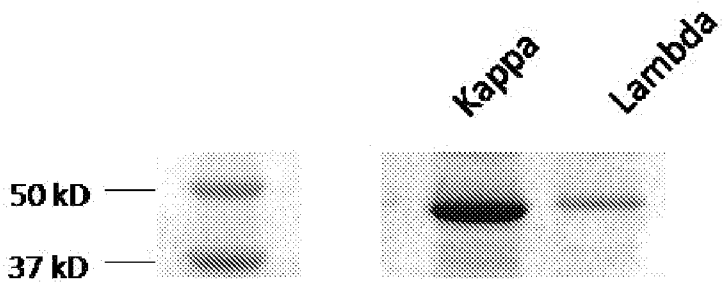


FIG. 6A

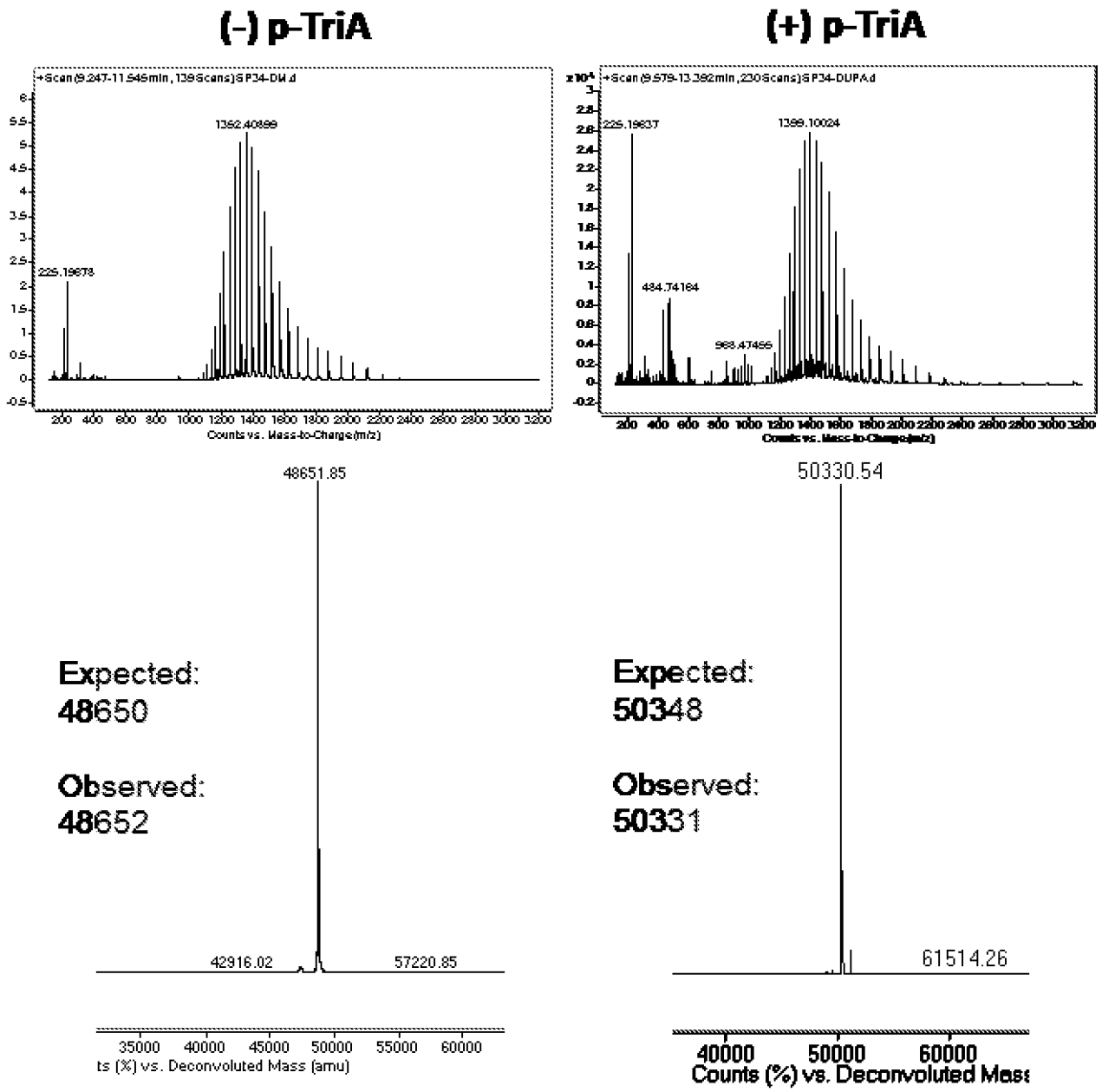

(-) p-TriA
(+) p-TriA
1362.40899
225.19673
1399.10024
225.19837
484.74164
963.47466
48651.85
50330.54
Expected:
48650
Observed:
48652
Expected:
50348
Observed:
50331
42916.02
57220.85
61514.26
35000 40000 45000 50000 55000 60000
ts (%) vs. Deconvoluted Mass (amu)
40000 50000 60000
Counts (%) vs. Deconvoluted Mass

FIG. 6B

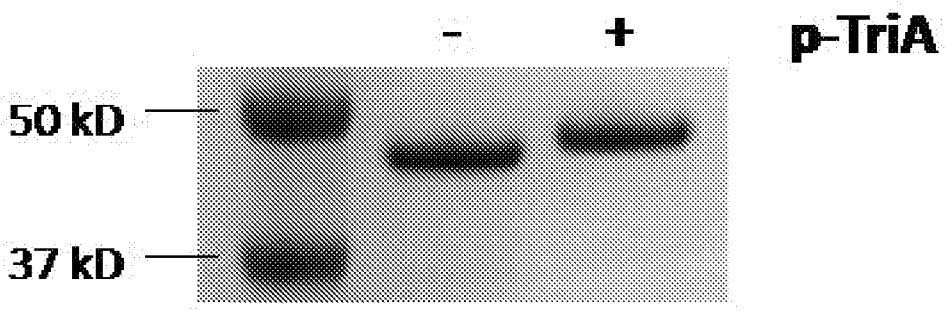

-
+
p-TriA
50 kD
37 kD

FIG. 7

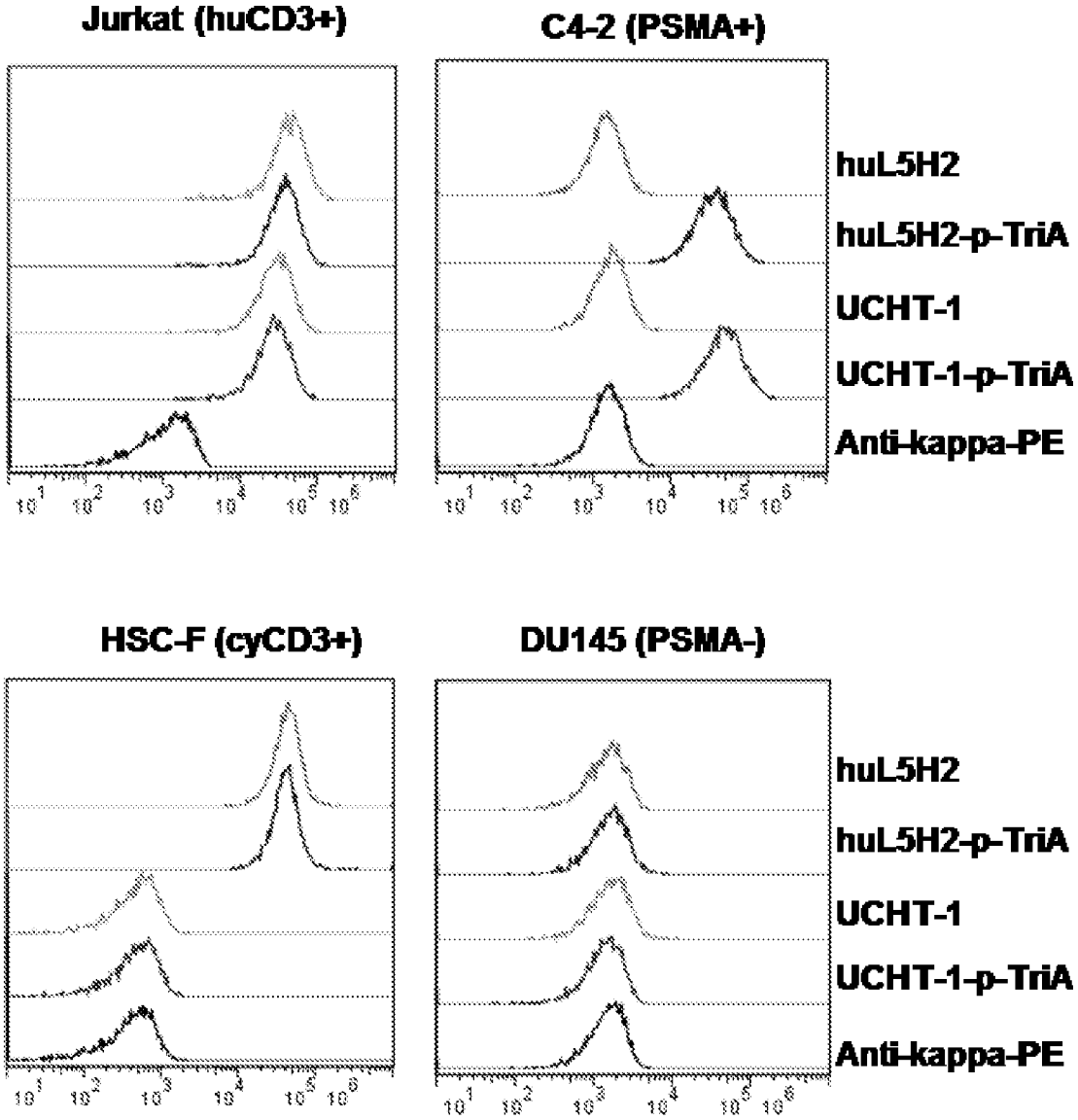

Jurkat (huCD3+)
C4-2 (PSMA+)
HSC-F (cyCD3+)
DU145 (PSMA-)
huL5H2
huL5H2-p-TriA
UCHT-1
UCHT-1-p-TriA
Anti-kappa-PE

FIG. 8A

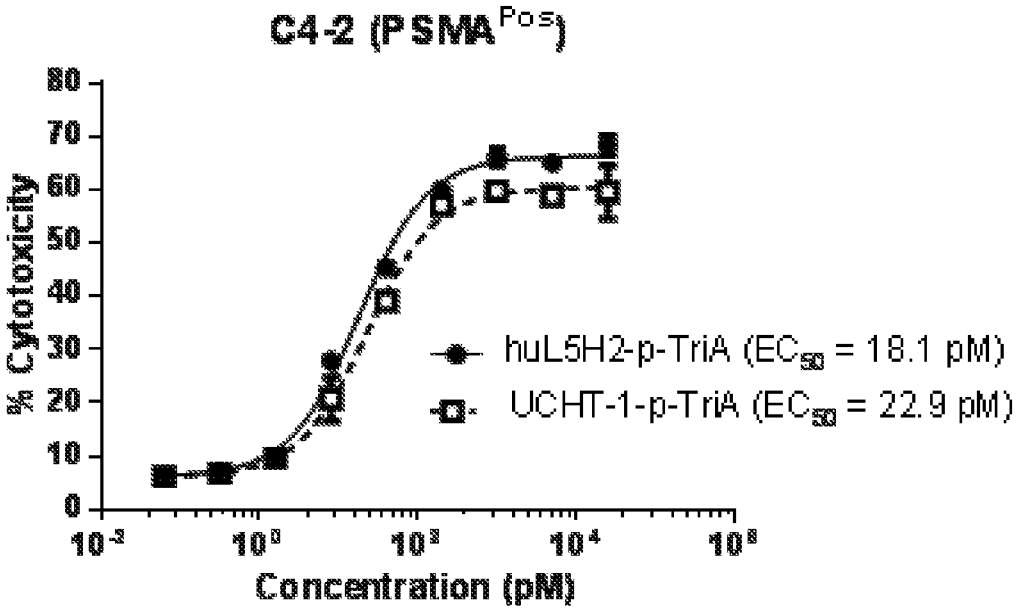

C4-2 (PSMA Pos)
% Cytotoxicity
Concentration (pM)
huL5H2-p-TriA (EC50 = 18.1 pM)
UCHT-1-p-TriA (EC50 = 22.9 pM)

FIG. 8B

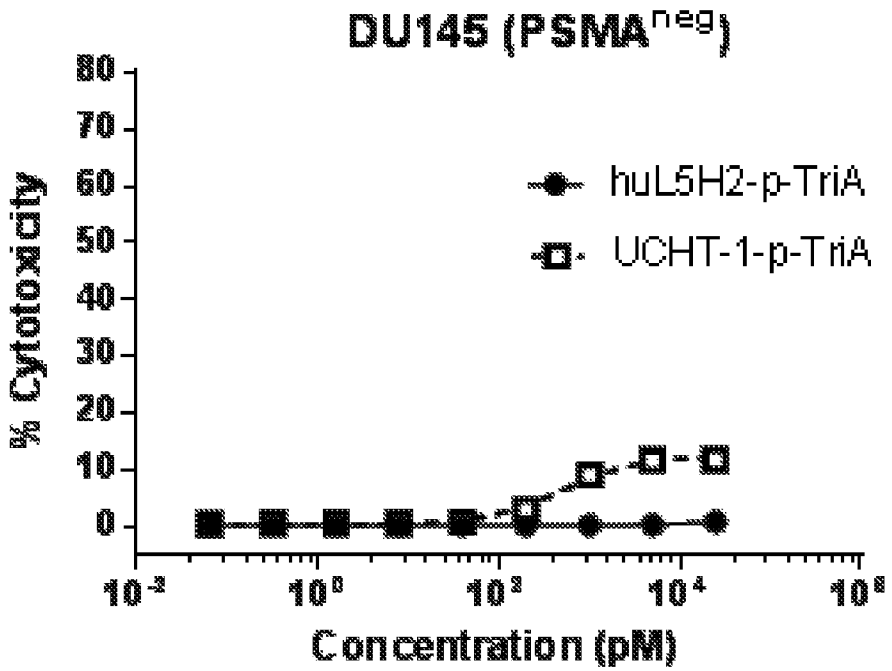

DU145 (PSMA neg)
% Cytotoxicity
Concentration (pM)
huL5H2-p-TriA
UCHT-1-p-TriA

FIG. 9

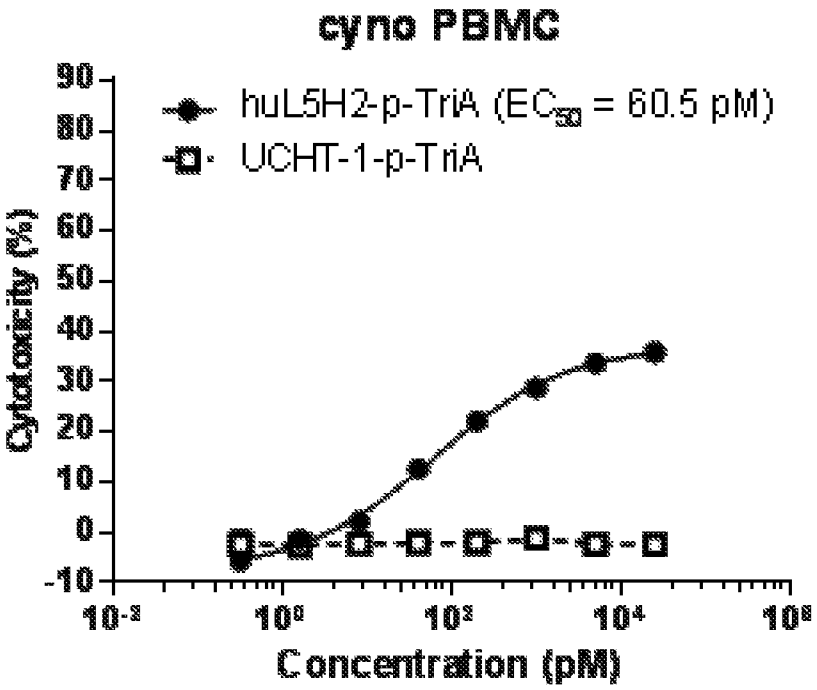

cyno PBMC
huL5H2-p-TriA (EC_{50} = 60.5 pM)
UCHT-1-p-TriA
Cytotoxicity (%)
90
80
70
60
50
40
30
20
10
0
-10
10^{-3}
10^{0}
10^{3}
10^{4}
10^{8}
Concentration (pM)

FIG. 10A

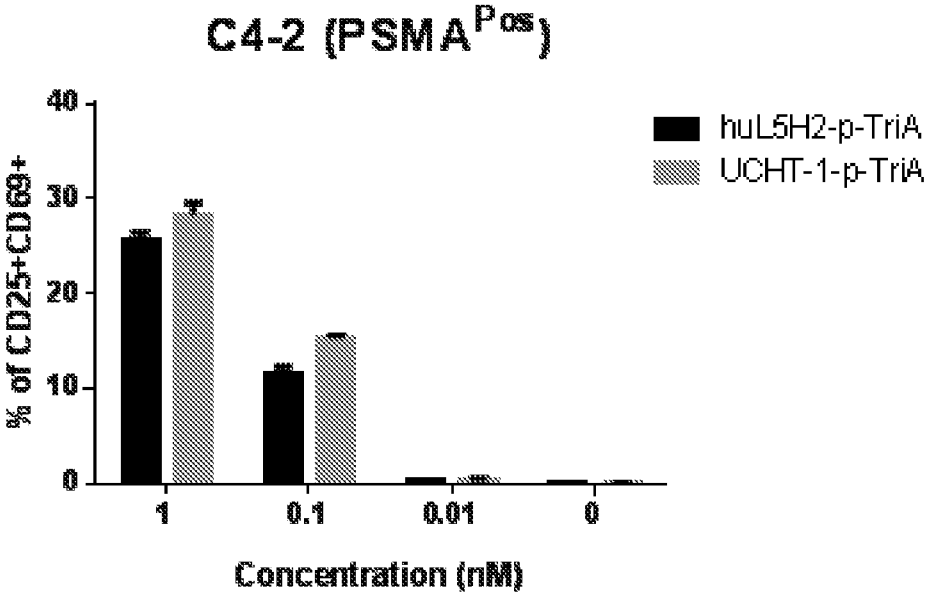


FIG. 10B

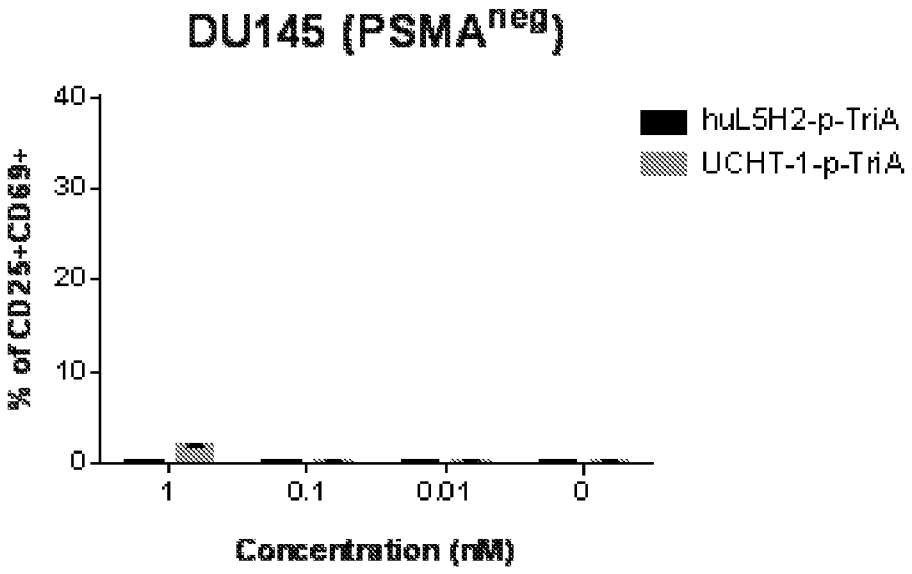


FIG. 11A

C4-2 (PSMA^{pos})

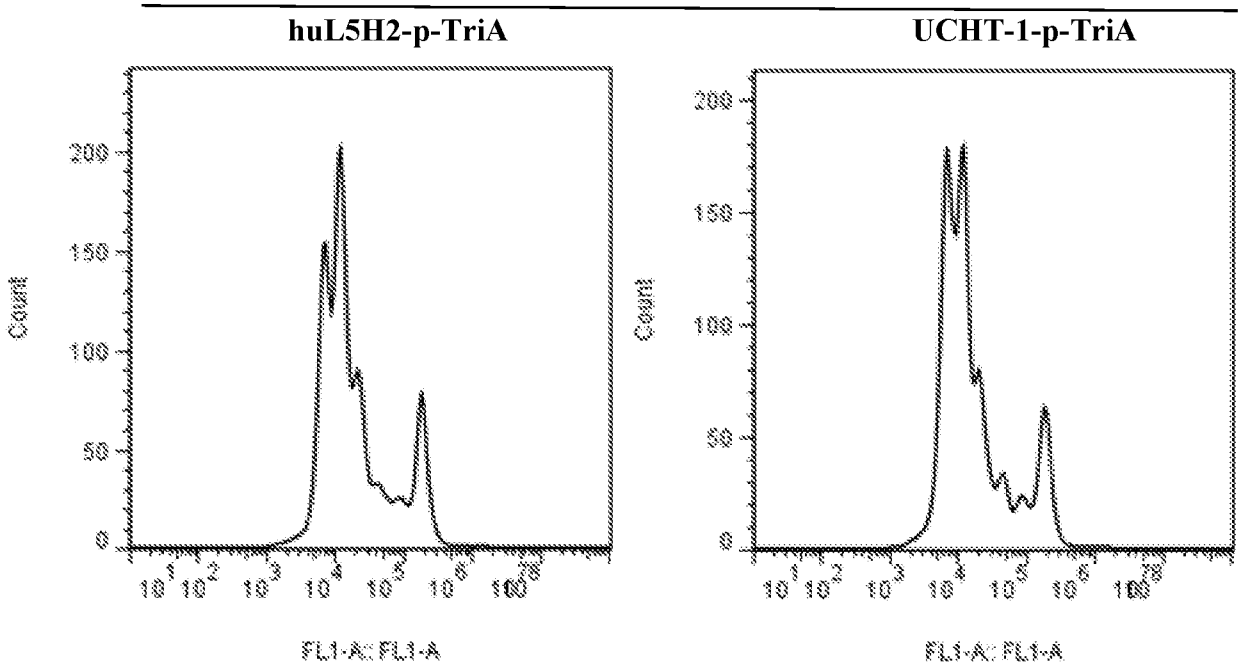


FIG. 11B

DU145 (PSMA^{neg})

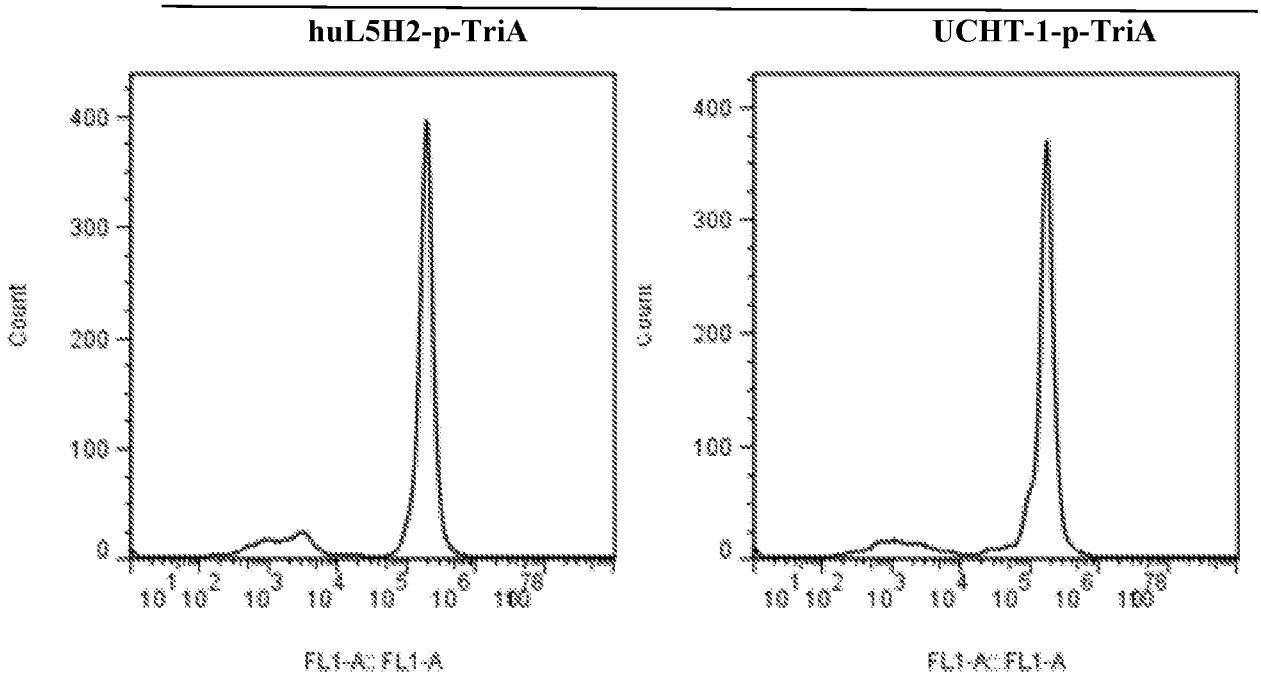


FIG. 12A

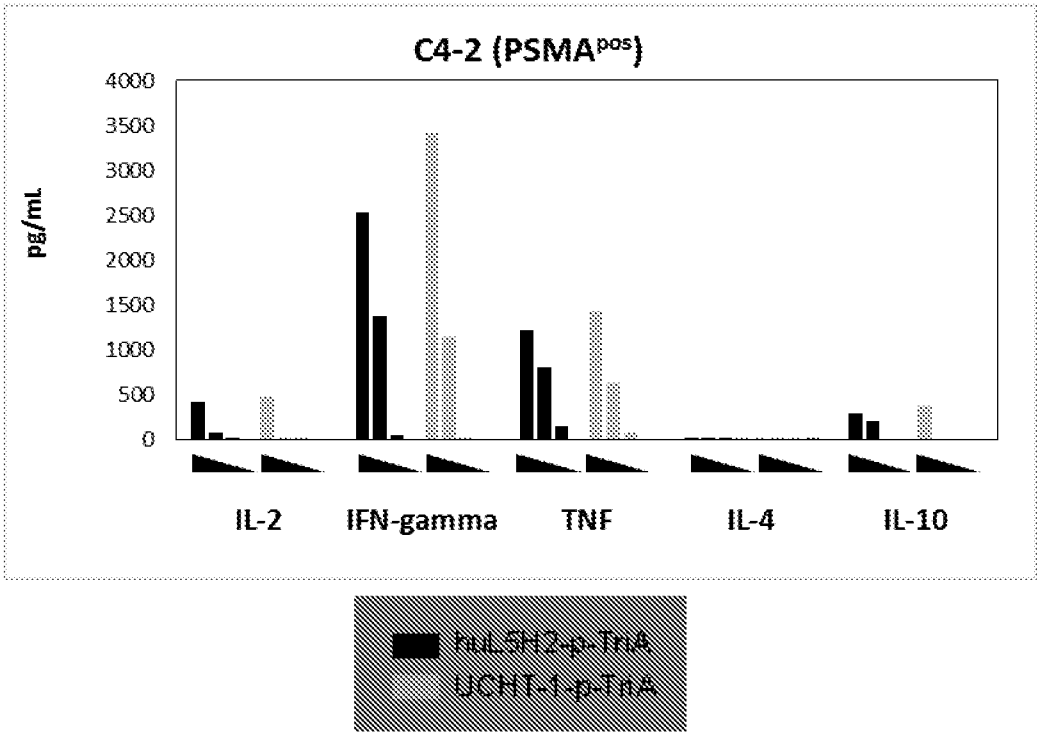
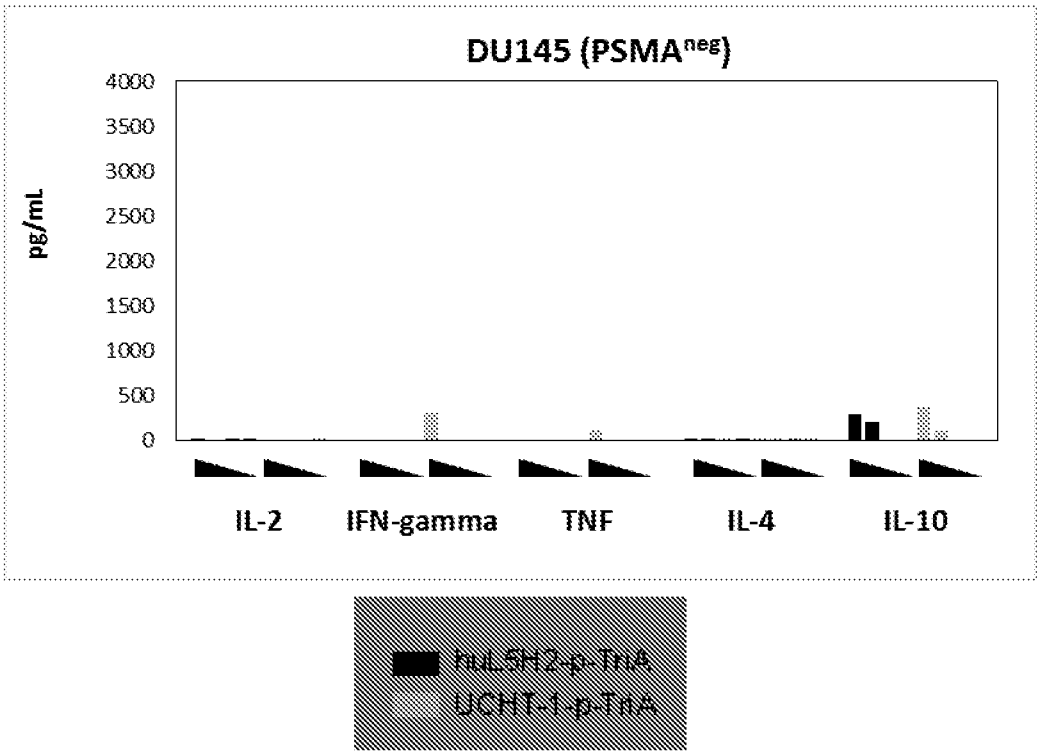


FIG. 12B



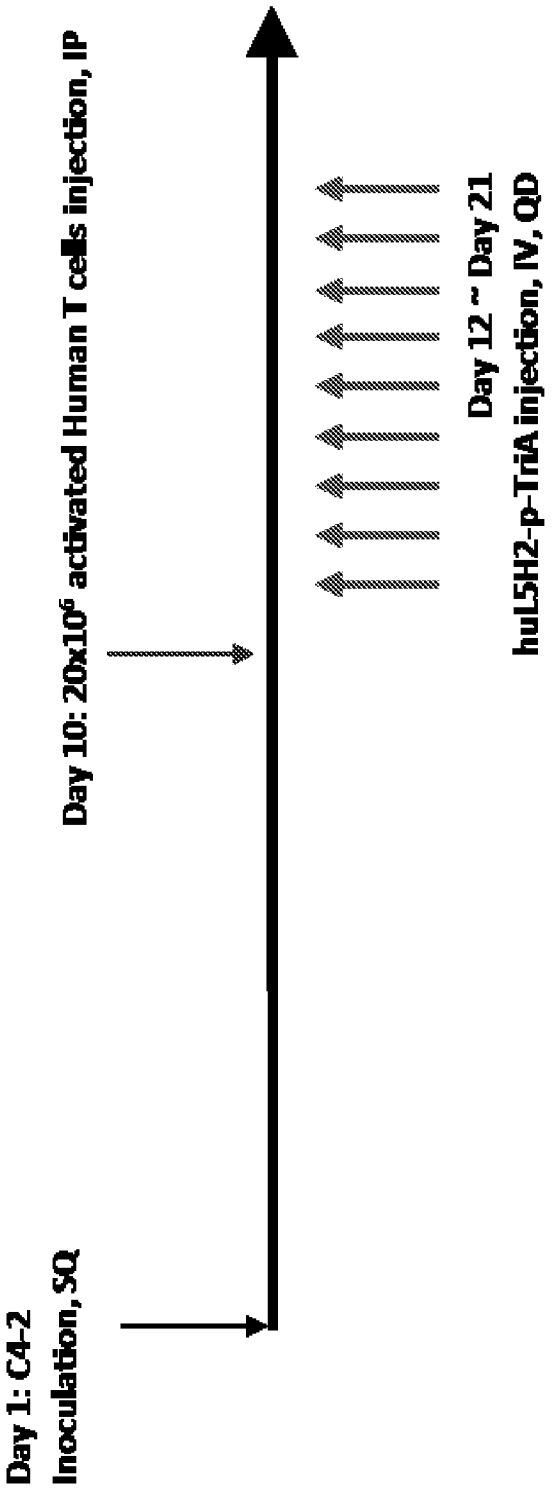

Day 1: C4-2
Inoculation, SQ
Day 10: 20x10^6 activated Human T cells injection, IP
Day 12 ~ Day 21
huL5H2-p-TriA injection, IV, QD

FIG. 13A

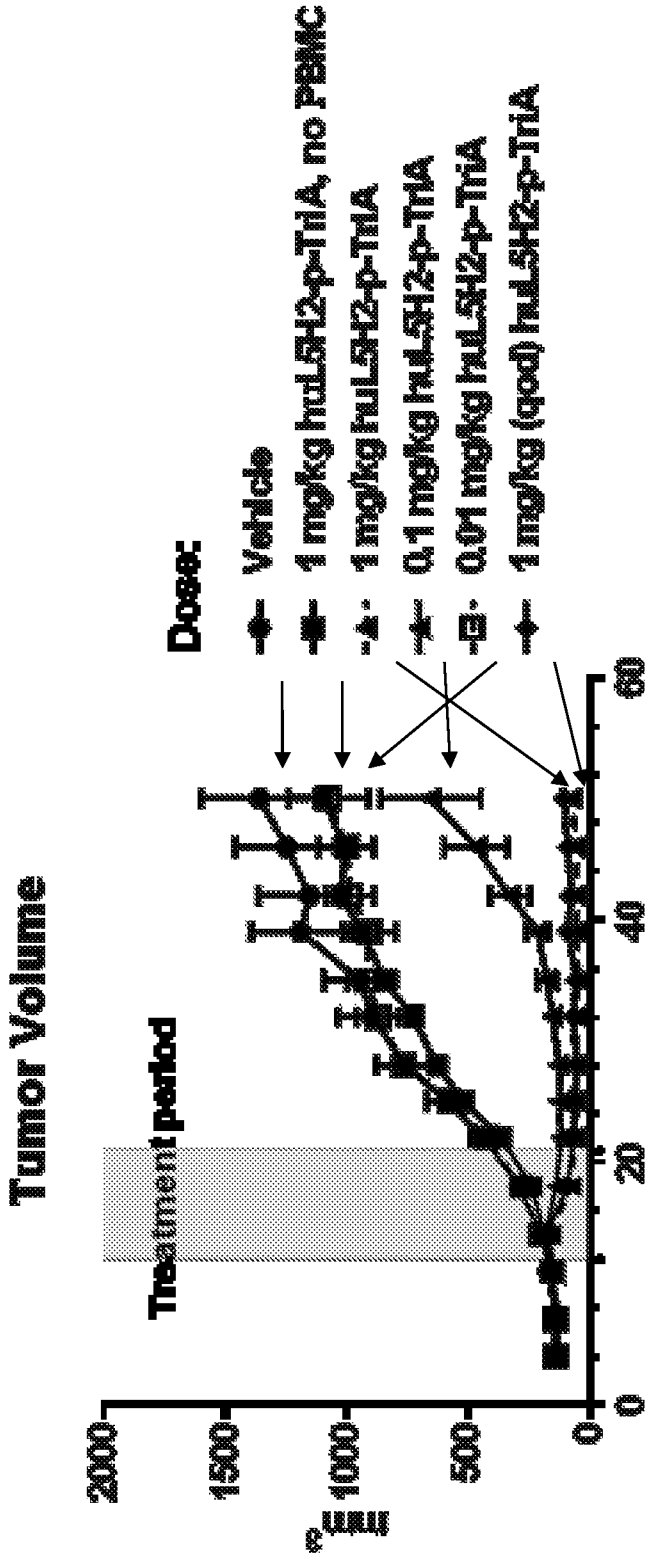

Tumor Volume
Treatment period
Dose:
Vehicle
1 mg/kg huLSH2-p-TriA, no PBMC
1 mg/kg huLSH2-p-TriA
0.1 mg/kg huLSH2-p-TriA
0.01 mg/kg huLSH2-p-TriA
1 mg/kg (qod) huLSH2-p-TriA
mm3
2000
1500
1000
500
0
0
20
40
60
Days

FIG. 13B

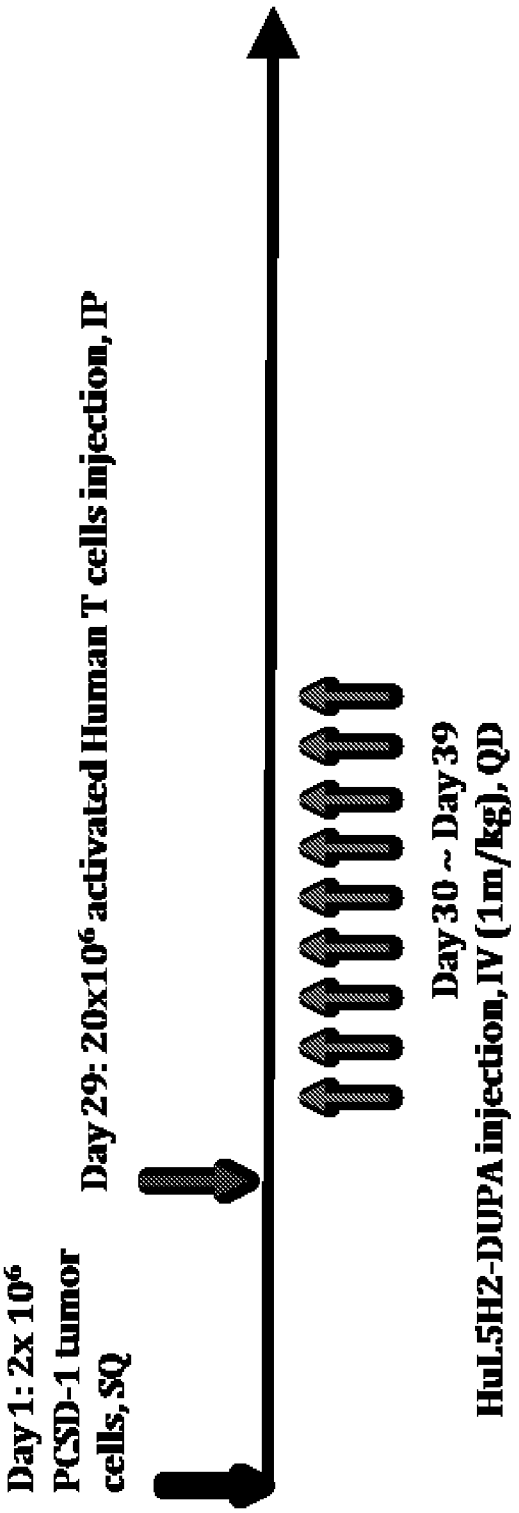

Day 1: 2x 10⁶ PCSD-1 tumor cells, SQ
Day 29: 20x10⁶ activated Human T cells injection, IP
Day 30 ~ Day 39
HuL5H2-DUPA injection, IV (1m/kg), QD

FIG. 14A

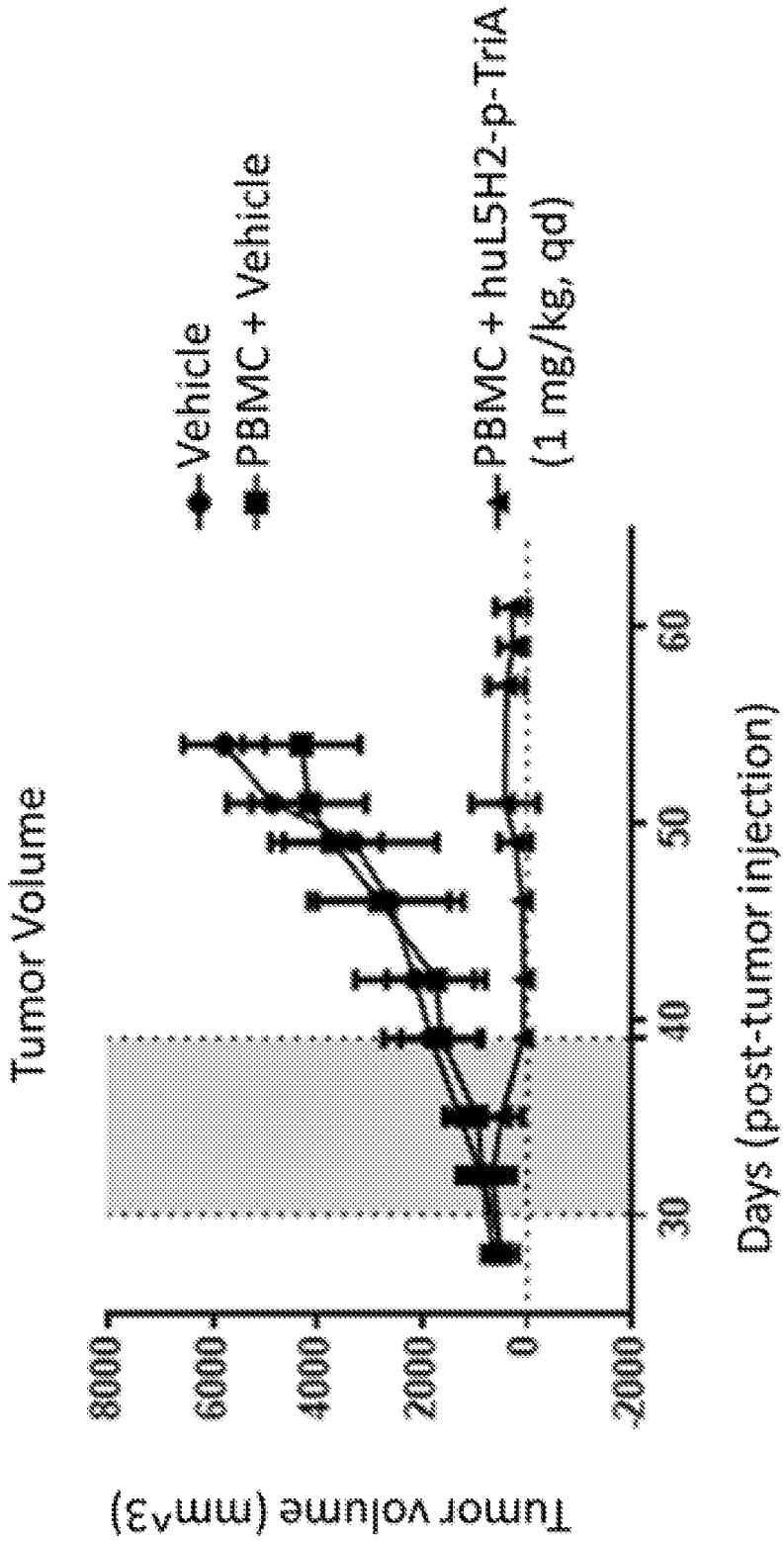

Tumor Volume
Vehicle
PBMC + Vehicle
PBMC + huL5H2-p-TriA
(1 mg/kg, qd)
Tumor volume (mm^3)
8000
6000
4000
2000
0
-2000
30
40
50
60
Days (post-tumor injection)

FIG. 14B

FIG. 15

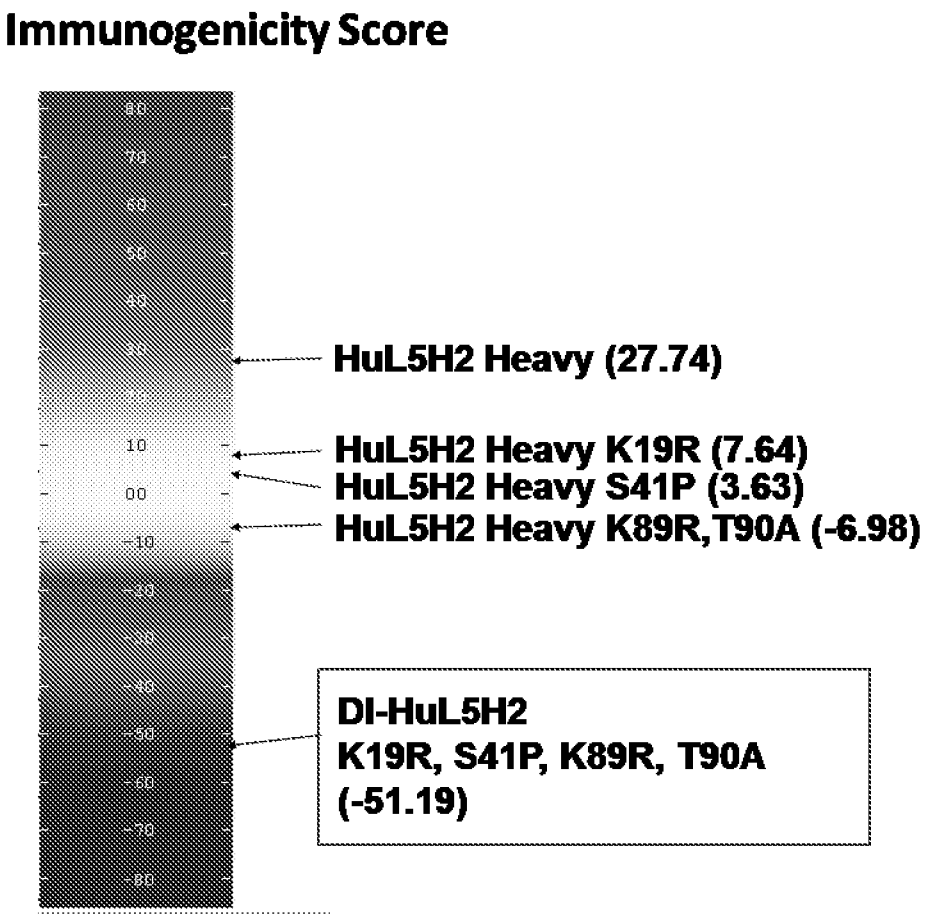

Immunogenicity Score
HuL5H2 Heavy (27.74)
HuL5H2 Heavy K19R (7.64)
HuL5H2 Heavy S41P (3.63)
HuL5H2 Heavy K89R,T90A (-6.98)
DI-HuL5H2
K19R, S41P, K89R, T90A
(-51.19)

FIG. 16

QTOF analysis

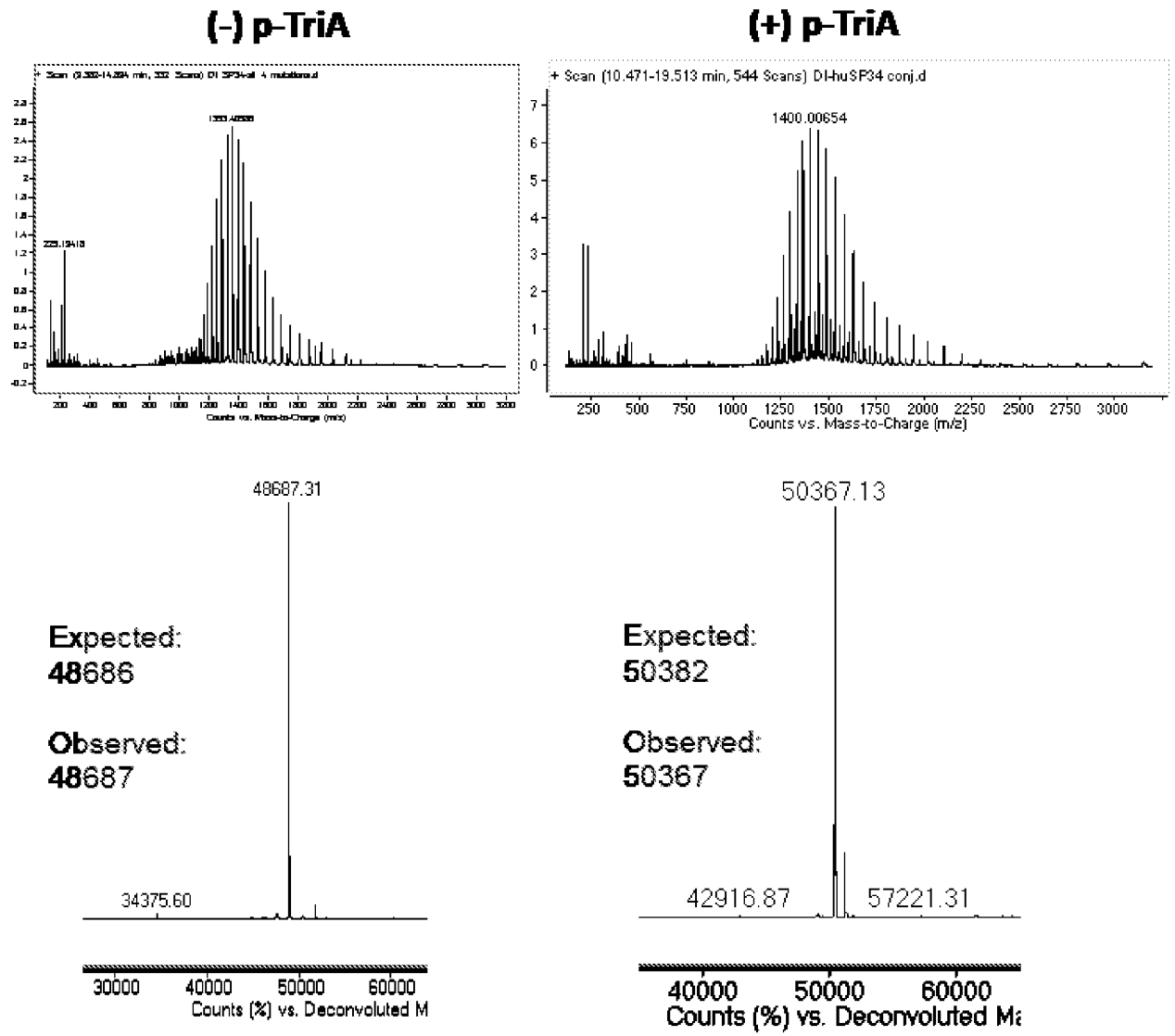


FIG. 17A

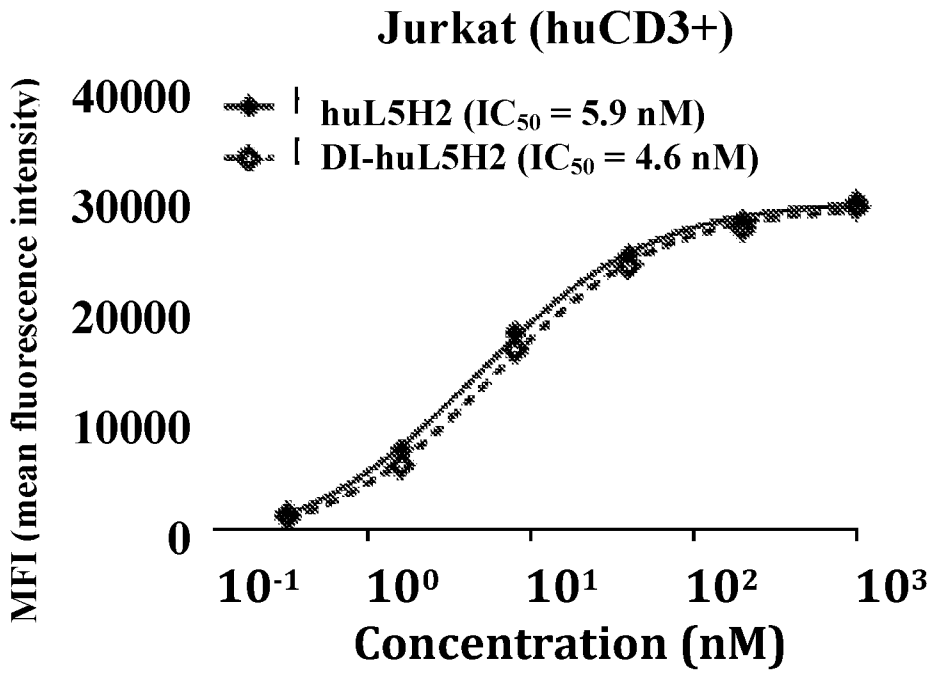
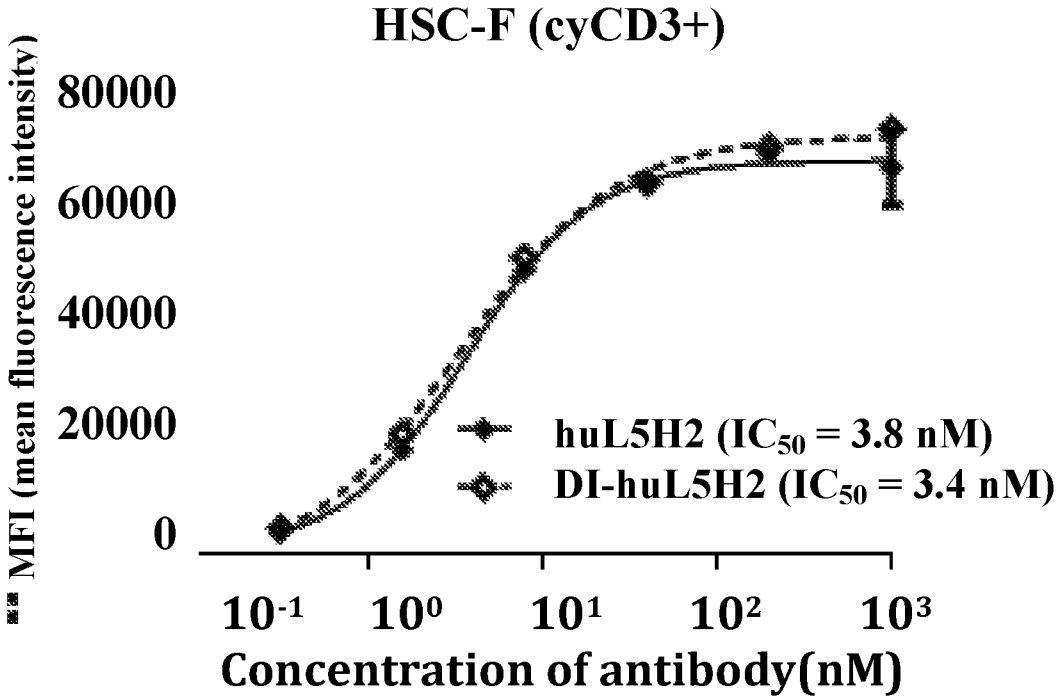


FIG. 17B



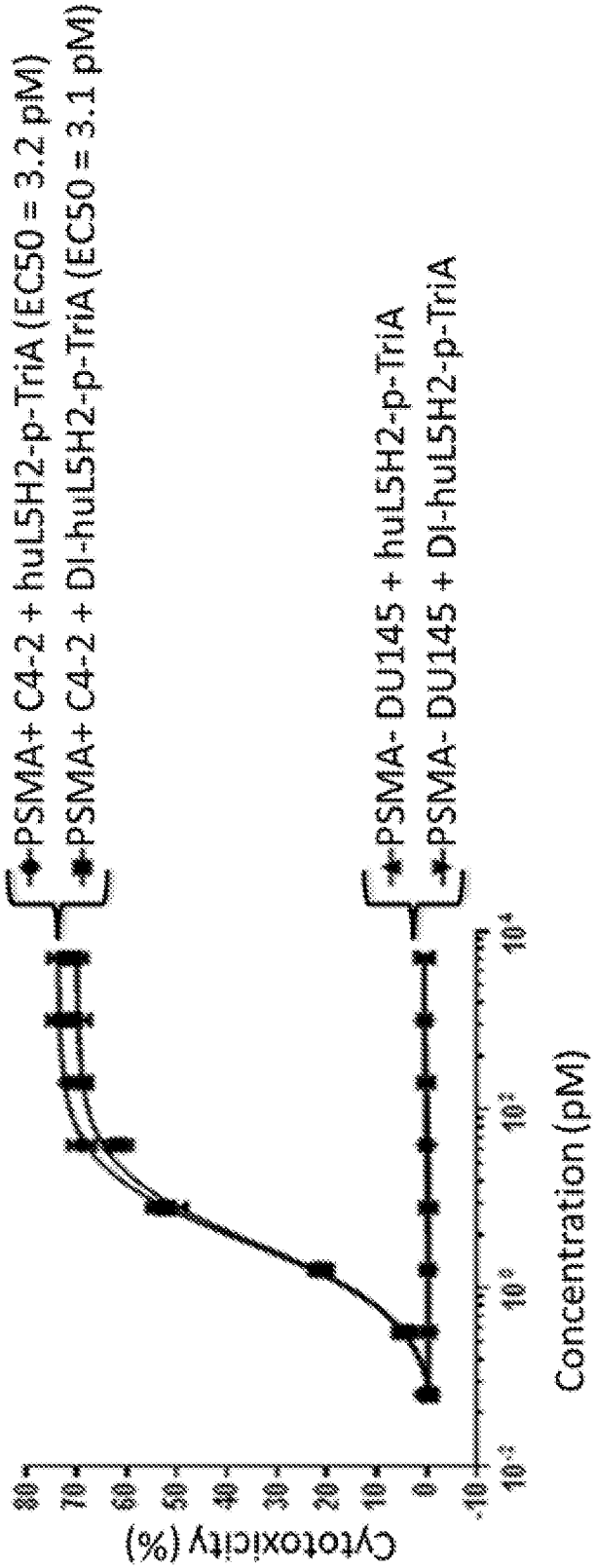

PSMA+ C4-2 + huL5H2-p-TriA (EC50 = 3.2 pM)
PSMA+ C4-2 + DI-huL5H2-p-TriA (EC50 = 3.1 pM)
PSMA- DU145 + huL5H2-p-TriA
PSMA- DU145 + DI-huL5H2-p-TriA
Cytotoxicity (%)
Concentration (pM)

FIG. 18

FIG. 19A

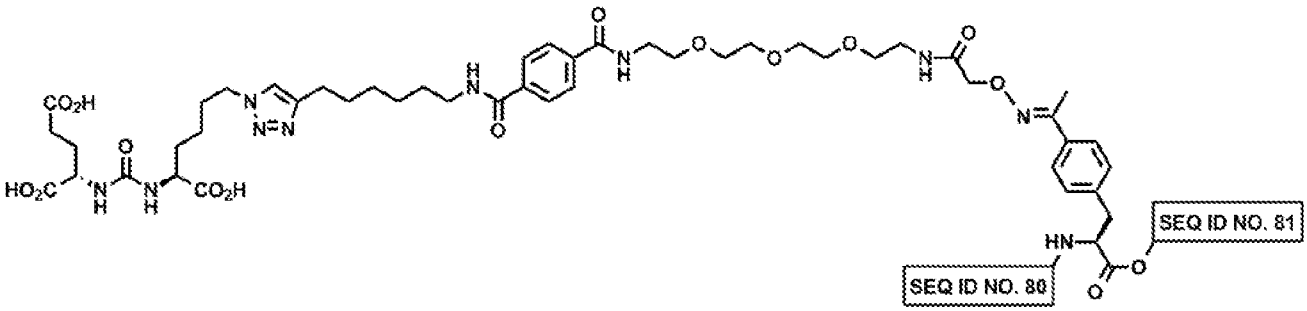


FIG. 19B

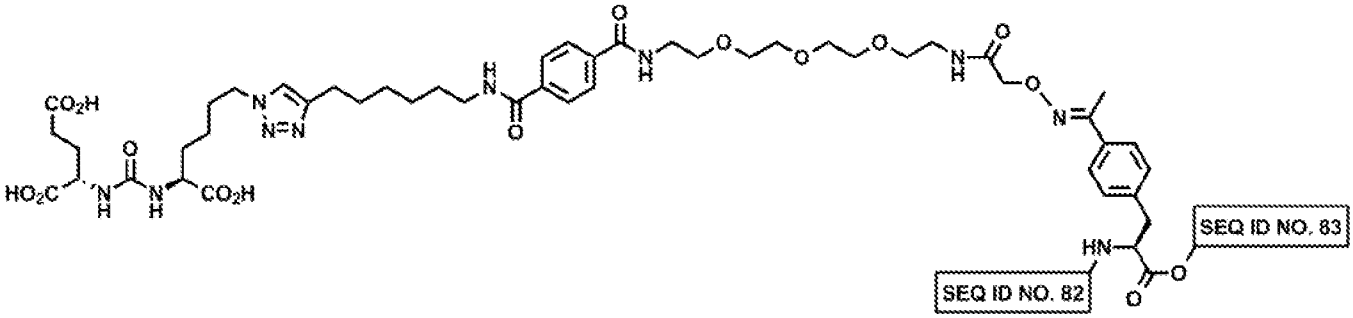


FIG. 19C

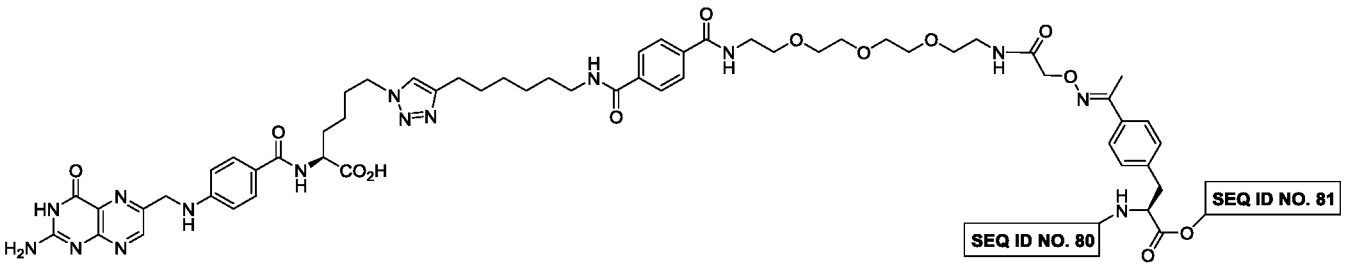


FIG. 19D

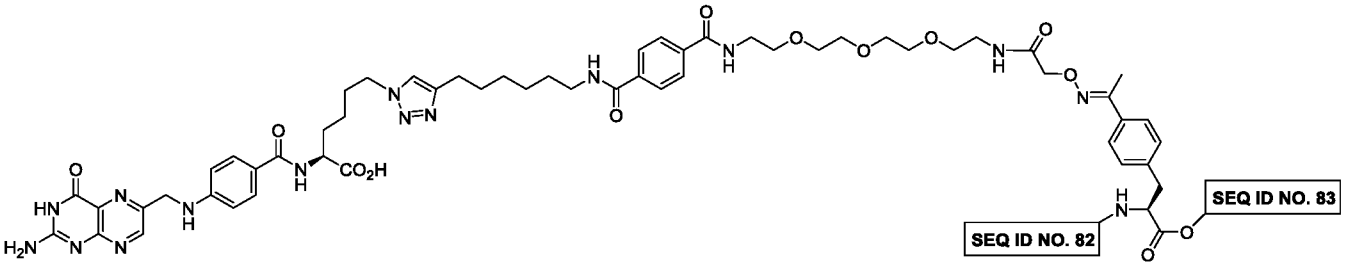
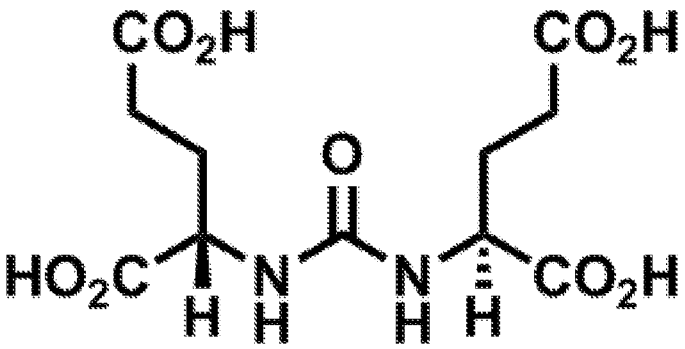
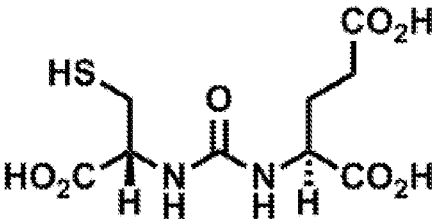


FIG. 20A

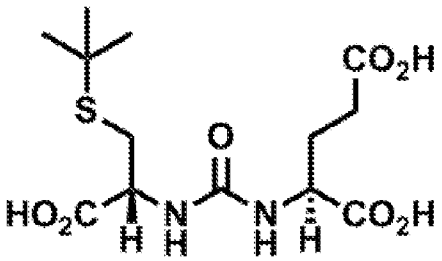


DUPA

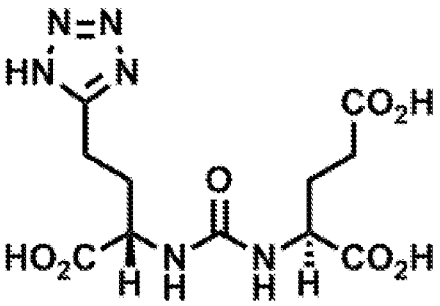
FIG. 20B



CMCG

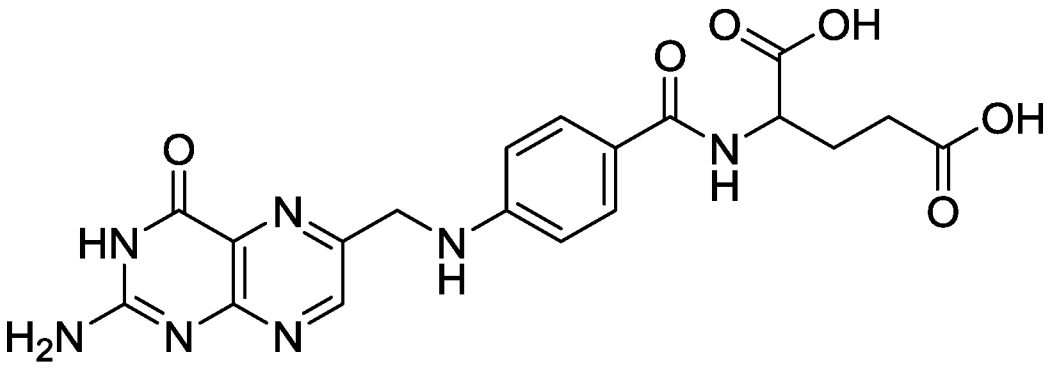


tBuCMCG



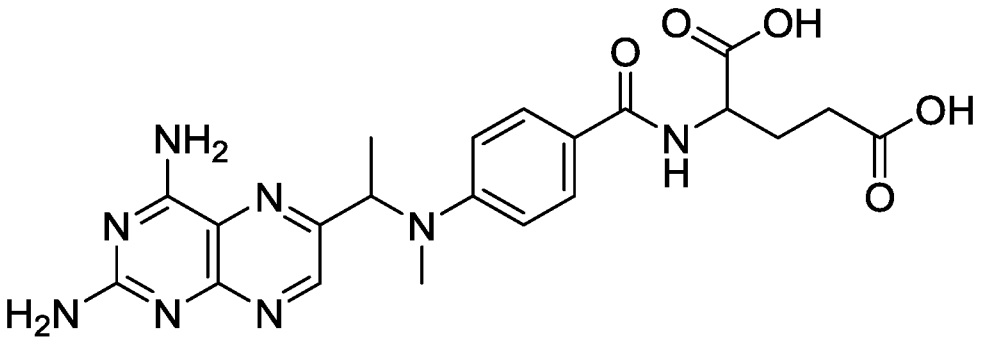
CTCG

FIG. 20C

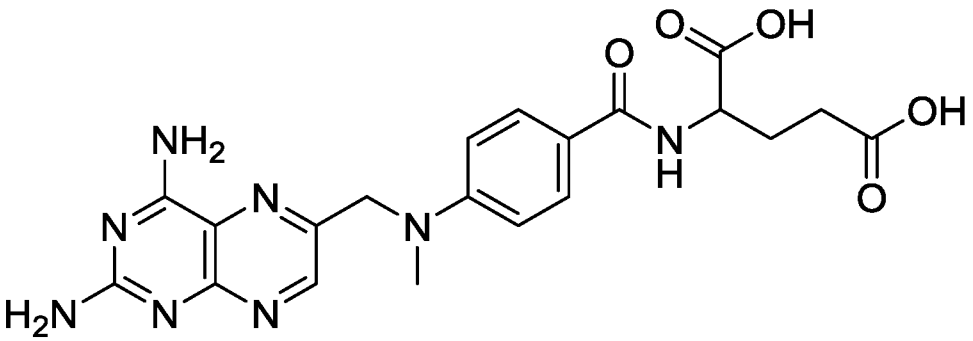


Folic acid

FIG. 20D



Denopterin



Methotrexate

FIG. 21A

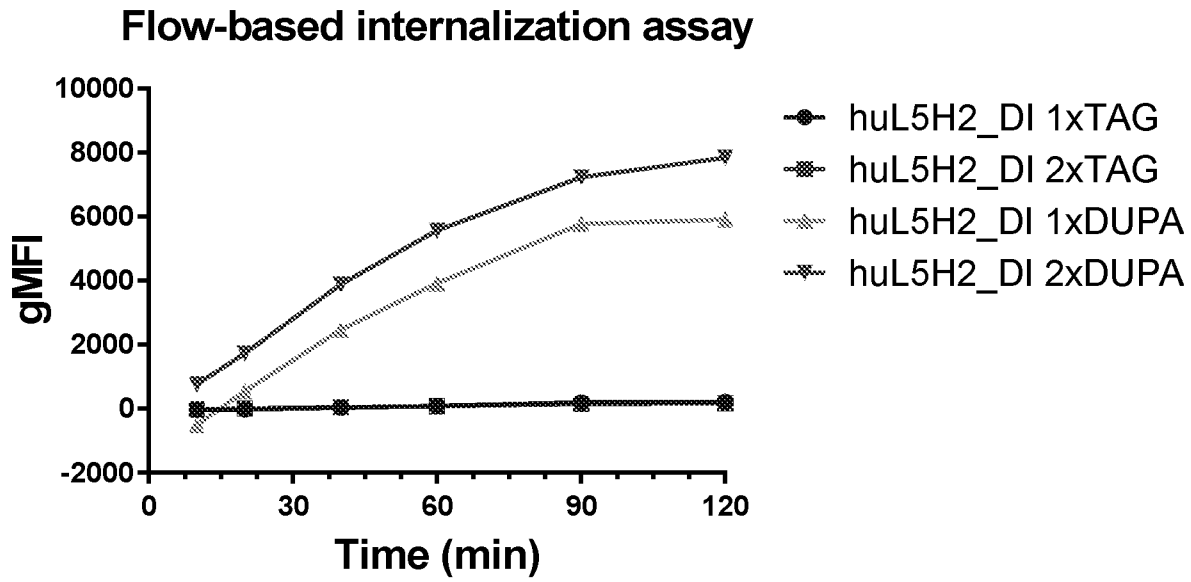


FIG. 21B

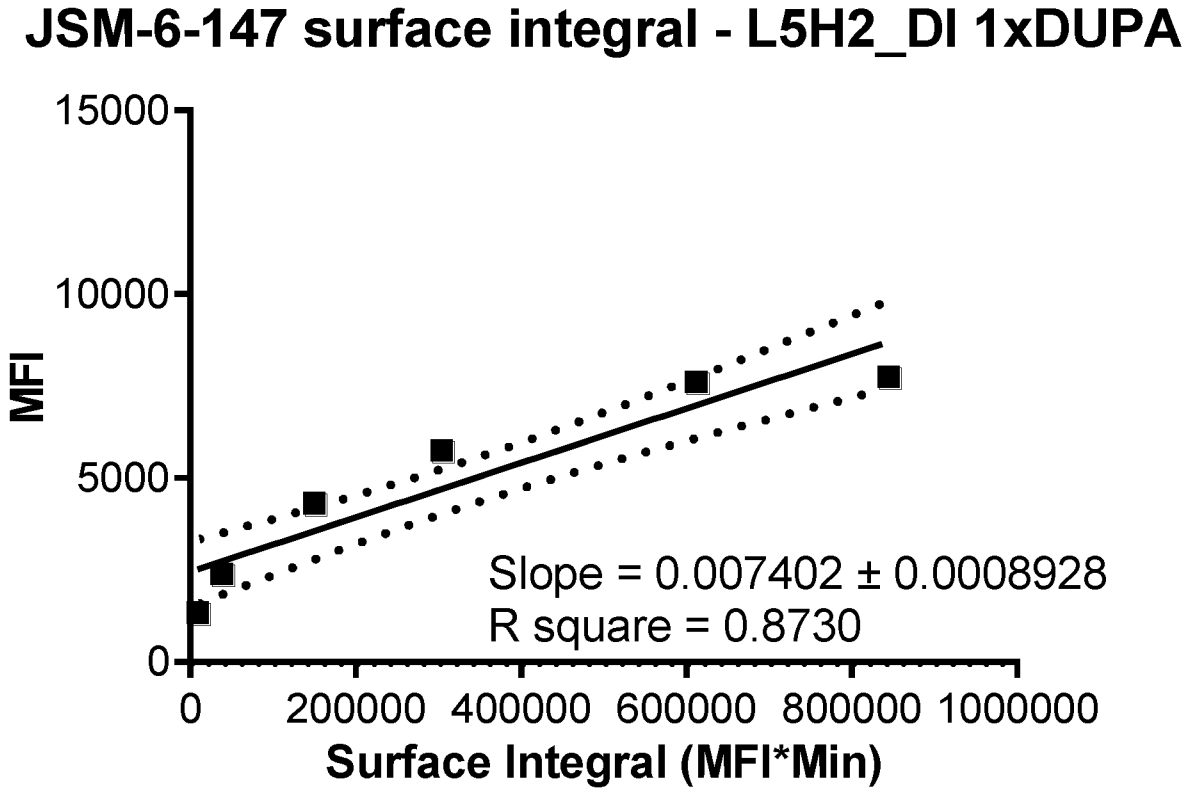


FIG. 21C

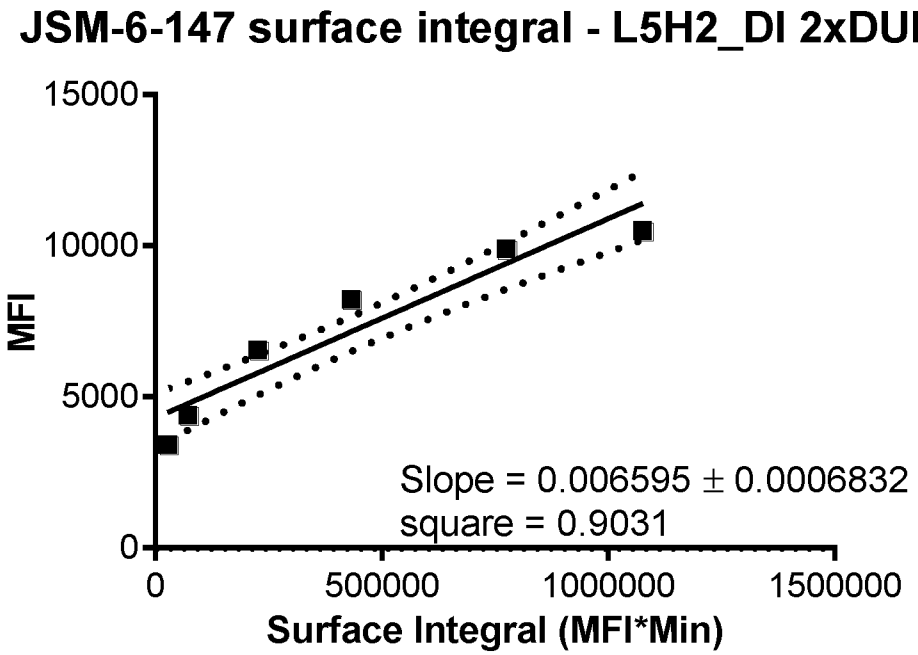
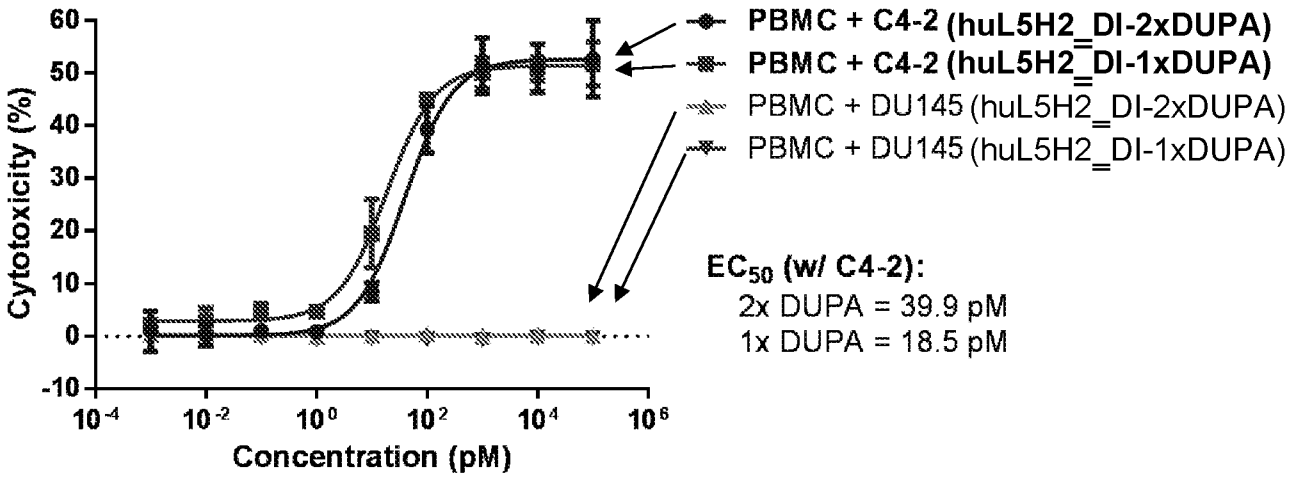


FIG. 22



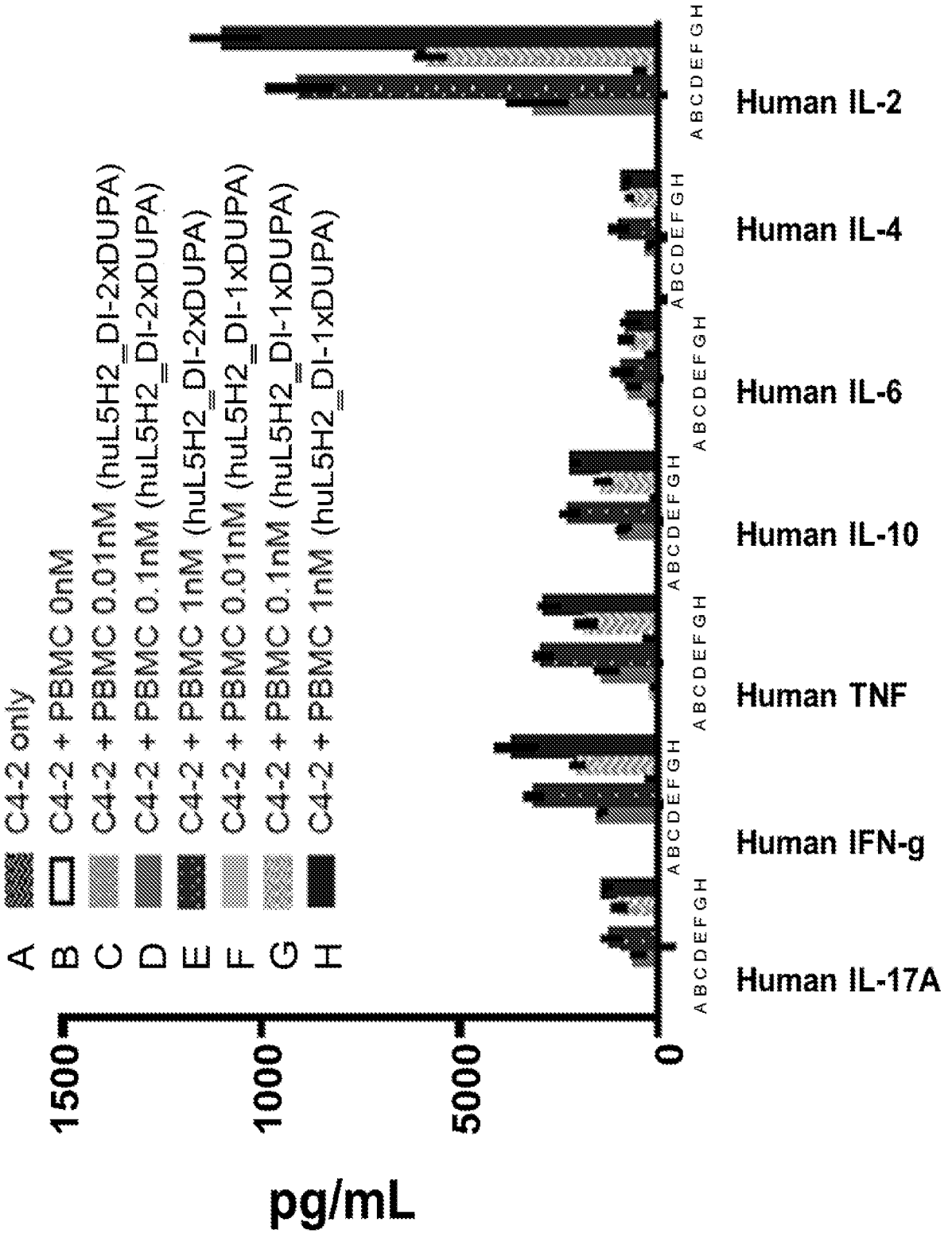

A C4-2 only
B C4-2 + PBMC 0nM
C C4-2 + PBMC 0.01nM (huL5H2_DI-2xDUPA)
D C4-2 + PBMC 0.1nM (huL5H2_DI-2xDUPA)
E C4-2 + PBMC 1nM (huL5H2_DI-2xDUPA)
F C4-2 + PBMC 0.01nM (huL5H2_DI-1xDUPA)
G C4-2 + PBMC 0.1nM (huL5H2_DI-1xDUPA)
H C4-2 + PBMC 1nM (huL5H2_DI-1xDUPA)
1500
1000
5000
0
pg/mL
A B C D E F G H
Human IL-2
Human IL-4
Human IL-6
Human IL-10
Human TNF
Human IFN-g
Human IL-17A

FIG. 23

FIG. 24

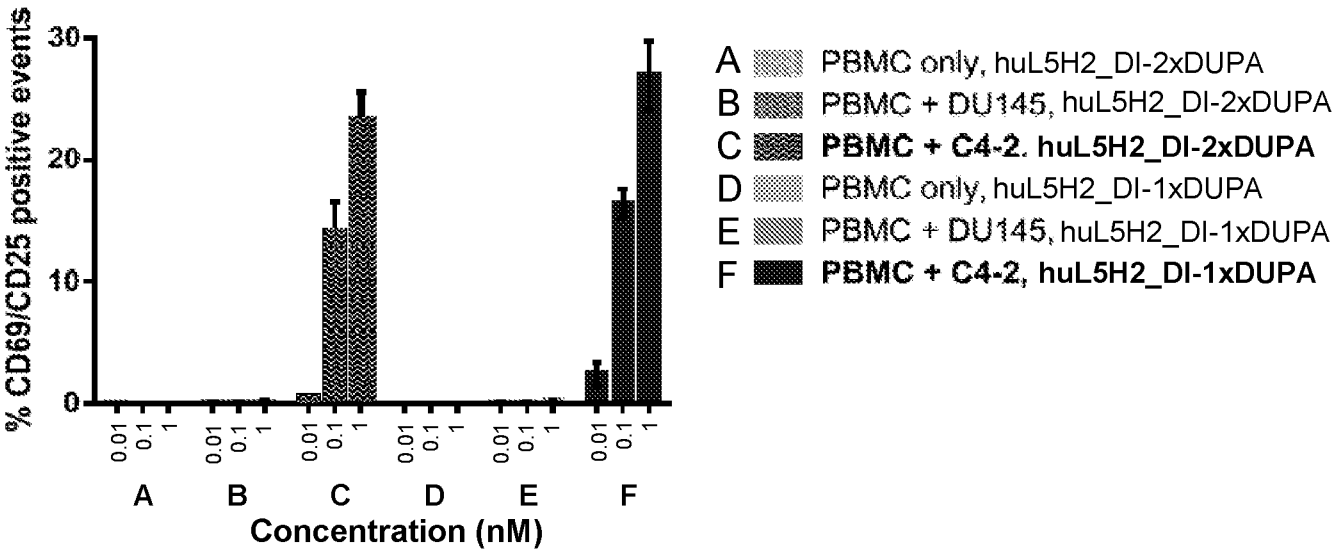

% CD69/CD25 positive events
30
20
10
0
0.01
0.1
1
A
B
C
D
E
F
Concentration (nM)
A PBMC only, huL5H2_DI-2xDUPA
B PBMC + DU145, huL5H2_DI-2xDUPA
C PBMC + C4-2. huL5H2_DI-2xDUPA
D PBMC only, huL5H2_DI-1xDUPA
E PBMC + DU145, huL5H2_DI-1xDUPA
F PBMC + C4-2, huL5H2_DI-1xDUPA

FIG. 25

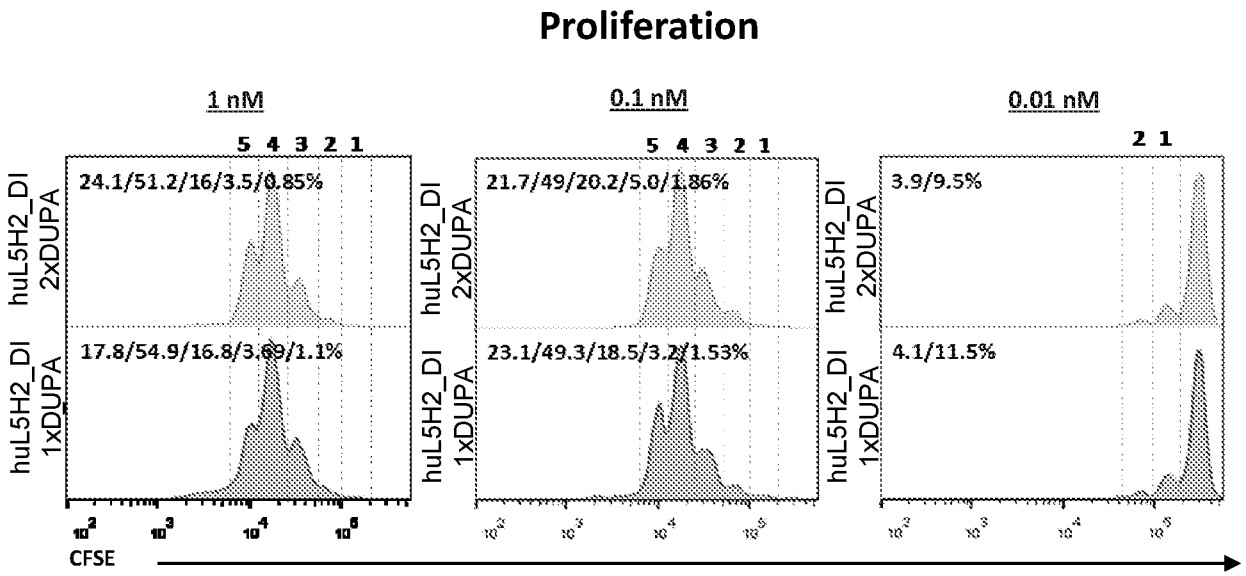

Proliferation
1 nM
0.1 nM
0.01 nM
5 4 3 2 1
2 1
huL5H2_DI 2xDUPA
huL5H2_DI 1xDUPA
24.1/51.2/16/3.5/0.85%
17.8/54.9/16.8/3.69/1.1%
21.7/49/20.2/5.0/1.86%
23.1/49.3/18.5/3.2/1.53%
3.9/9.5%
4.1/11.5%
CFSE

FIG. 26

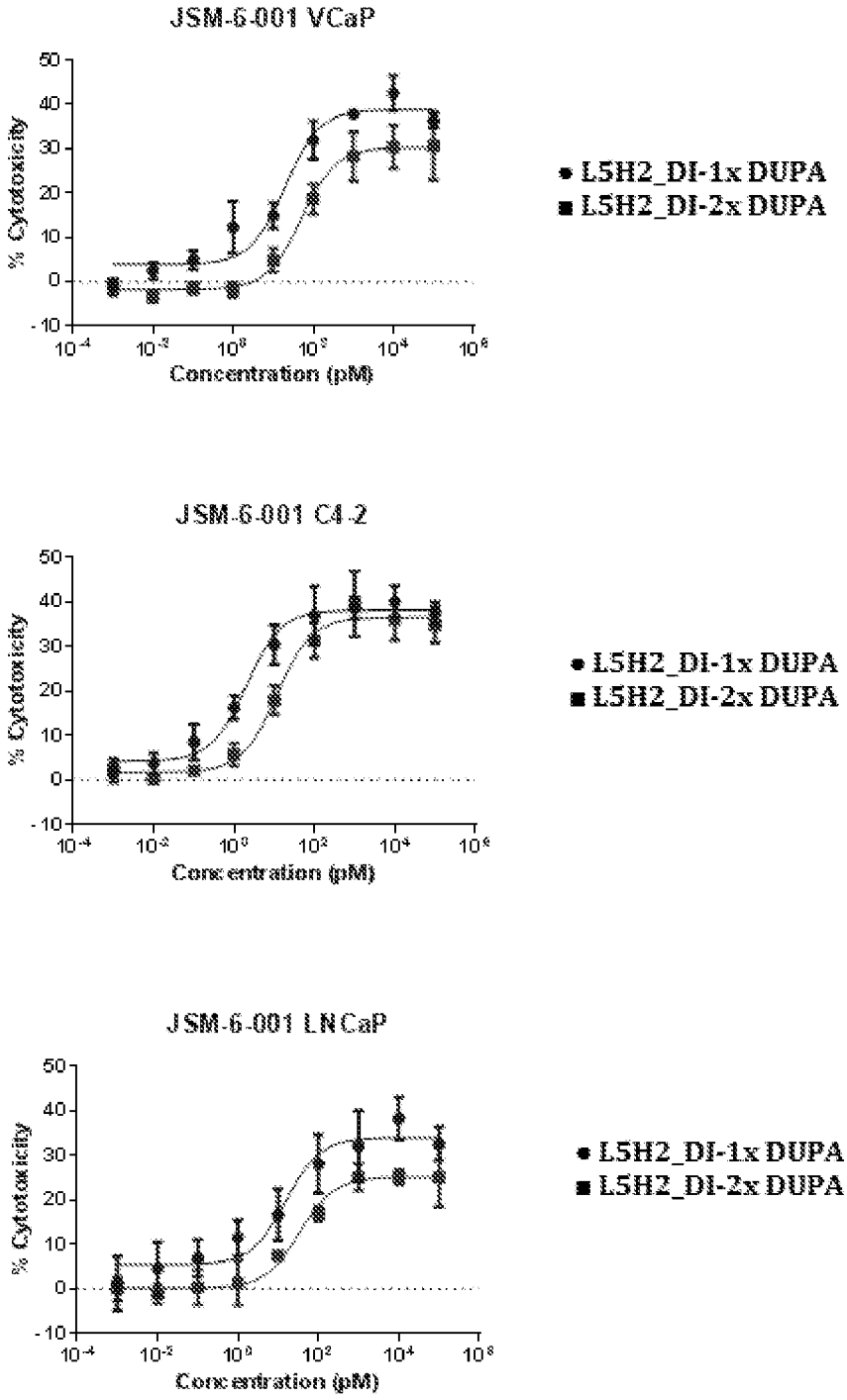

JSM-6-001 VCaP
% Cytotoxicity
Concentration (pM)
L5H2_DI-1x DUPA
L5H2_DI-2x DUPA
JSM-6-001 C4-2
% Cytotoxicity
Concentration (pM)
L5H2_DI-1x DUPA
L5H2_DI-2x DUPA
JSM-6-001 LNCaP
% Cytotoxicity
Concentration (pM)
L5H2_DI-1x DUPA
L5H2_DI-2x DUPA

FIG. 27

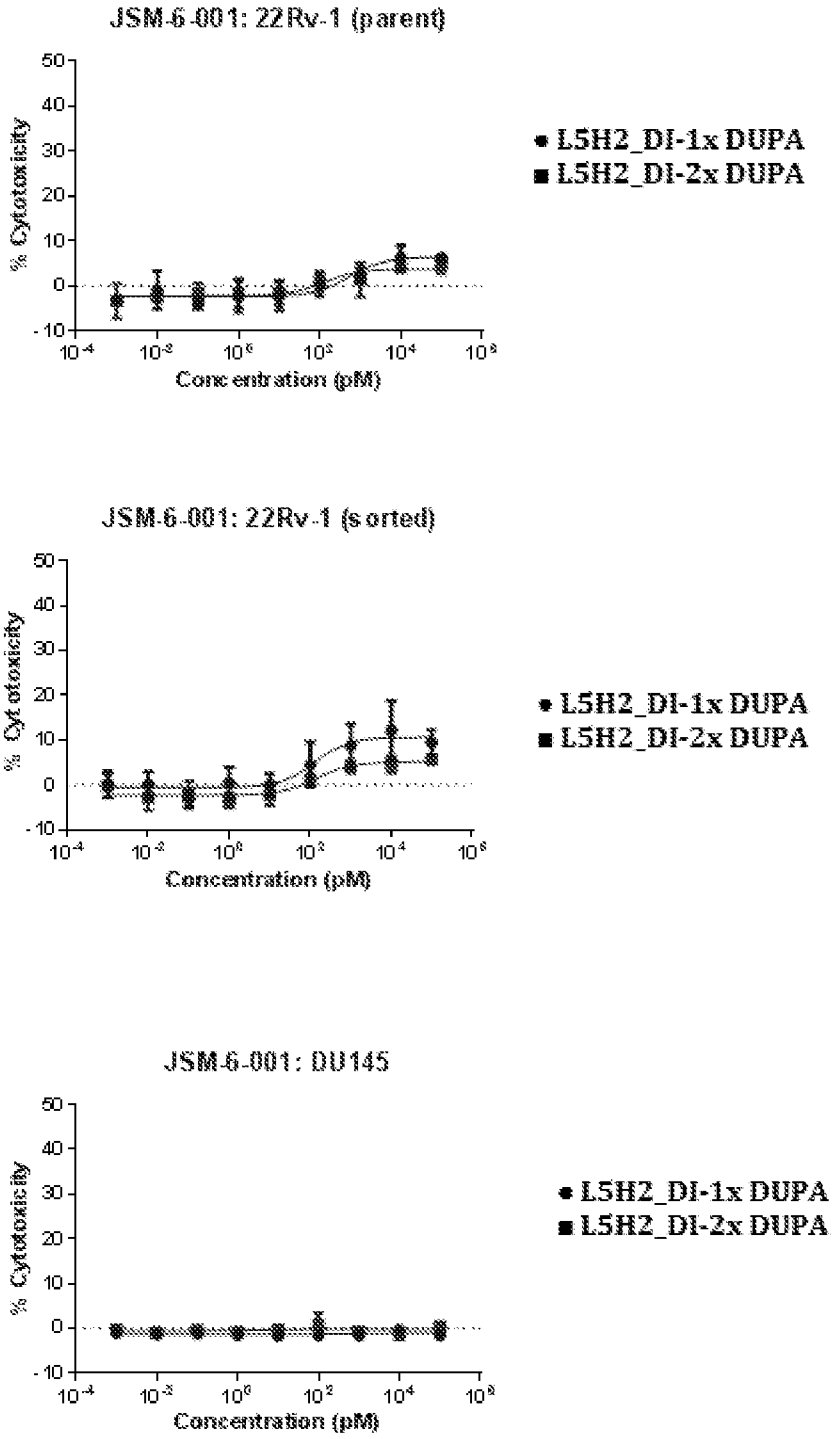

JSM-6-001: 22Rv-1 (parent)
% Cytotoxicity
Concentration (pM)
L5H2_DI-1x DUPA
L5H2_DI-2x DUPA
JSM-6-001: 22Rv-1 (sorted)
% Cytotoxicity
Concentration (pM)
L5H2_DI-1x DUPA
L5H2_DI-2x DUPA
JSM-6-001: DU145
% Cytotoxicity
Concentration (pM)
L5H2_DI-1x DUPA
L5H2_DI-2x DUPA

FIG. 28

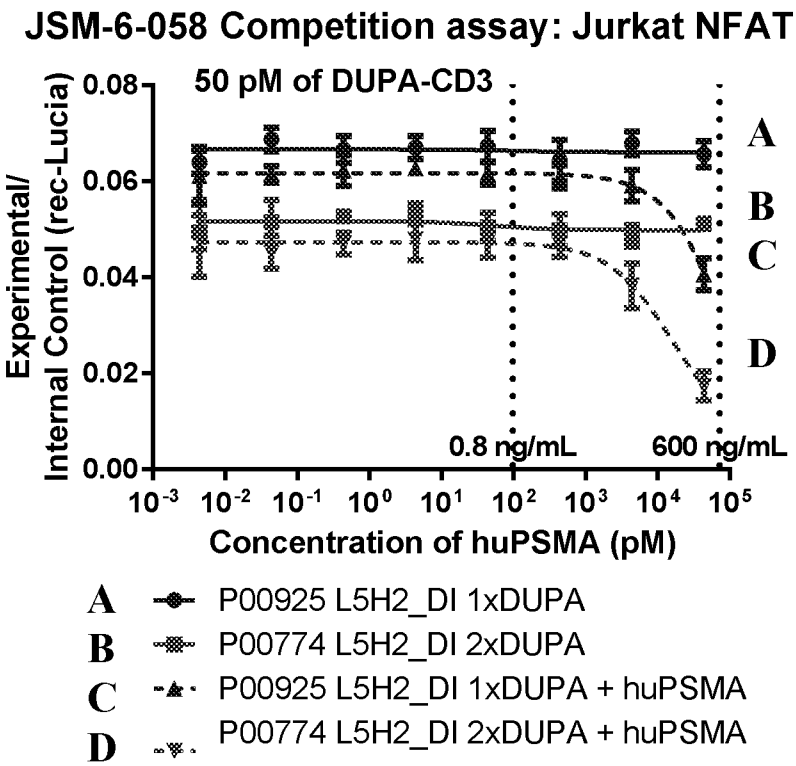
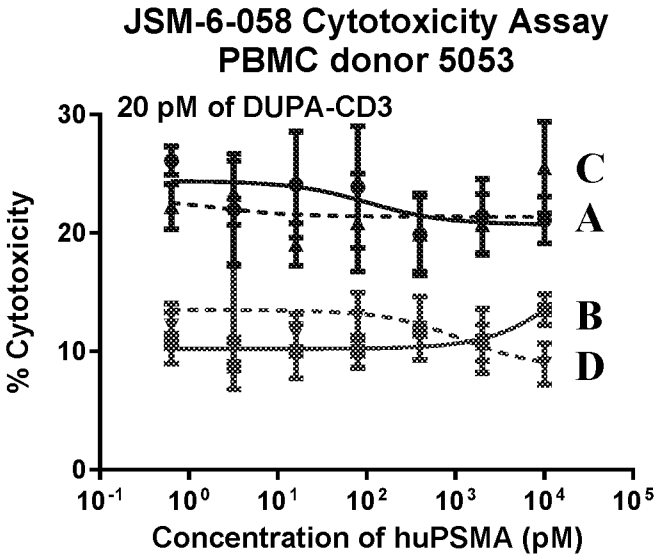


FIG. 29



24hr LDH cytotoxicity assay
Effector cells: PBL (Fresh)
E:T: 10:1
Background killing: donor 5053, 1.3-3.4%

A P00925 L5H2_DI 1xDUPA
B P00774 L5H2_DI 2xDUPA
C P00925 L5H2_DI 1xDUPA + huPSMA
D P00774 L5H2_DI 2xDUPA + huPSMA

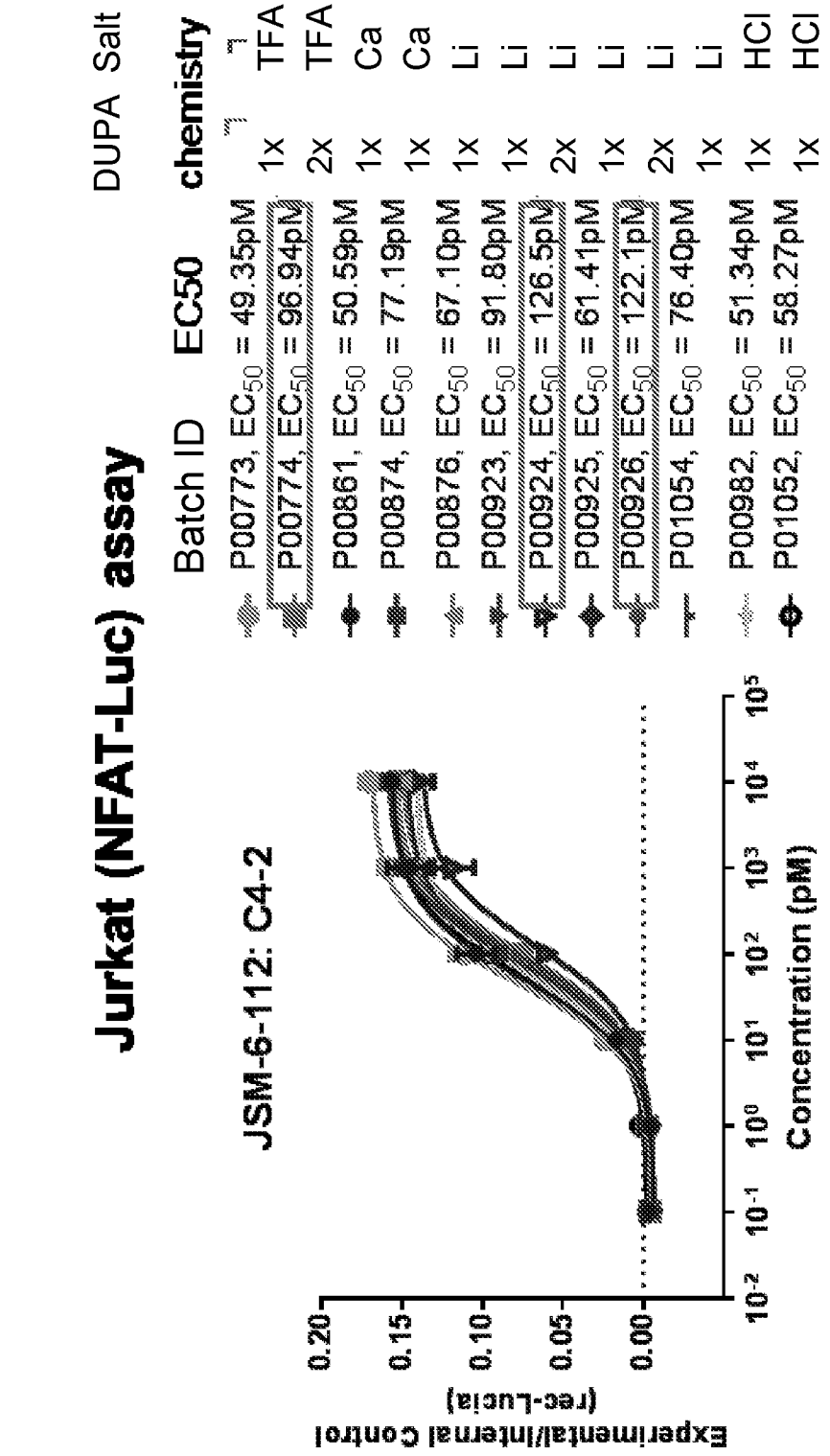

Jurkat (NFAT-Luc) assay
JSM-6-112: C4-2
DUPA Salt
Batch ID EC50 chemistry
P00773, EC50 = 49.35pM 1x TFA
P00774, EC50 = 96.94pM 2x TFA
P00861, EC50 = 50.59pM 1x Ca
P00874, EC50 = 77.19pM 1x Ca
P00876, EC50 = 67.10pM 1x Li
P00923, EC50 = 91.80pM 1x Li
P00924, EC50 = 126.5pM 2x Li
P00925, EC50 = 61.41pM 1x Li
P00926, EC50 = 122.1pM 2x Li
P01054, EC50 = 76.40pM 1x Li
P00982, EC50 = 51.34pM 1x HCl
P01052, EC50 = 58.27pM 1x HCl
Experimental/Internal Control (rec-Lucia)
0.20
0.15
0.10
0.05
0.00
10-2 10-1 100 101 102 103 104 105
Concentration (pM)

FIG. 30

FIG. 31A

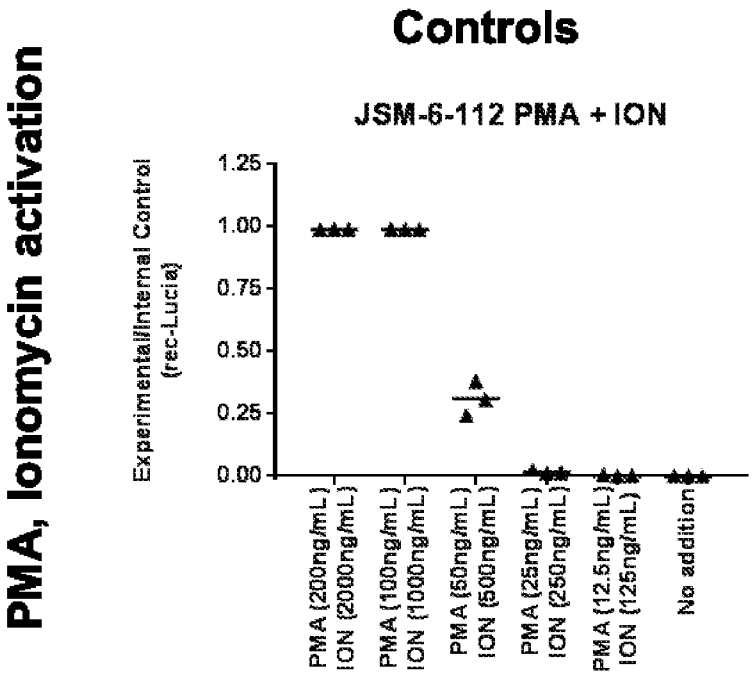


FIG. 31B

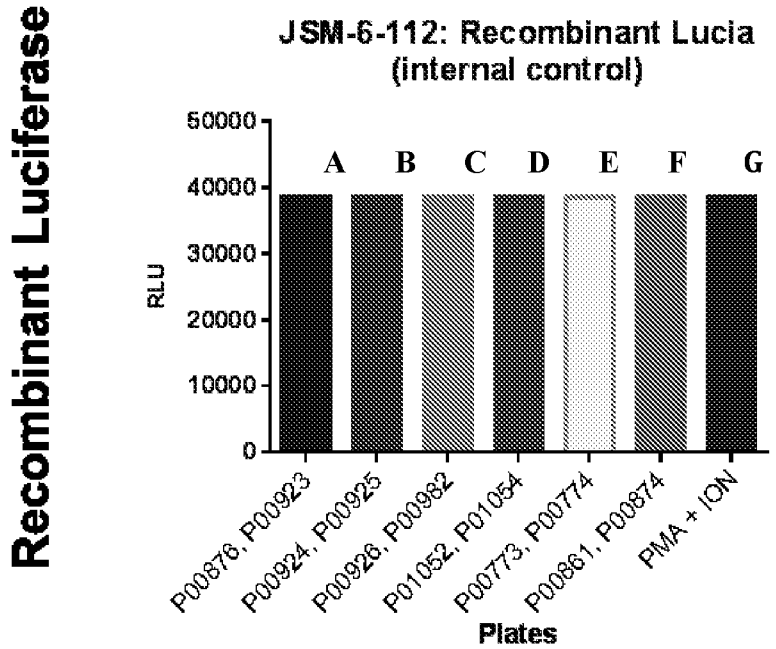


FIG. 32

High resolution mass spec

48 hr incubation in each serum

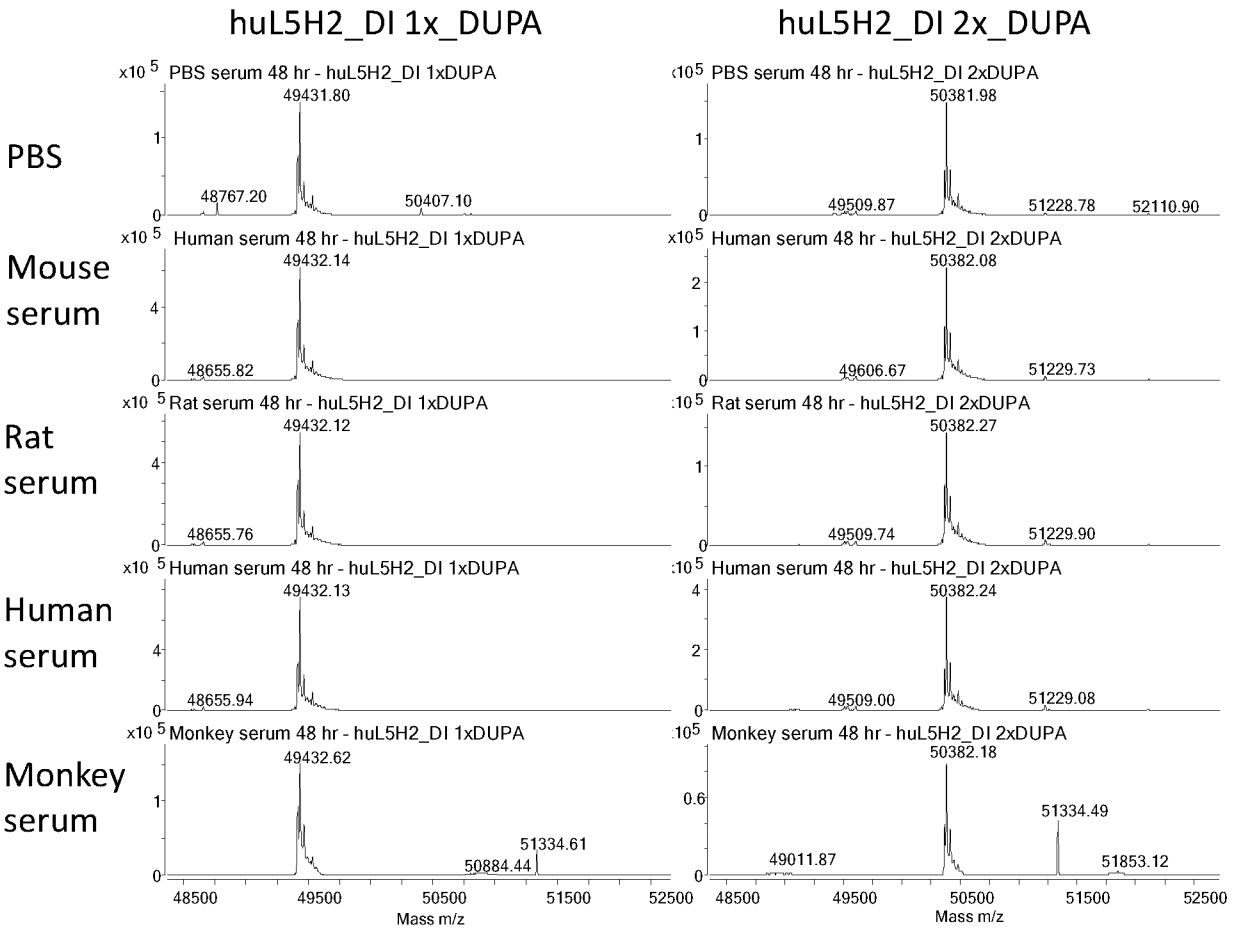


FIG. 33A

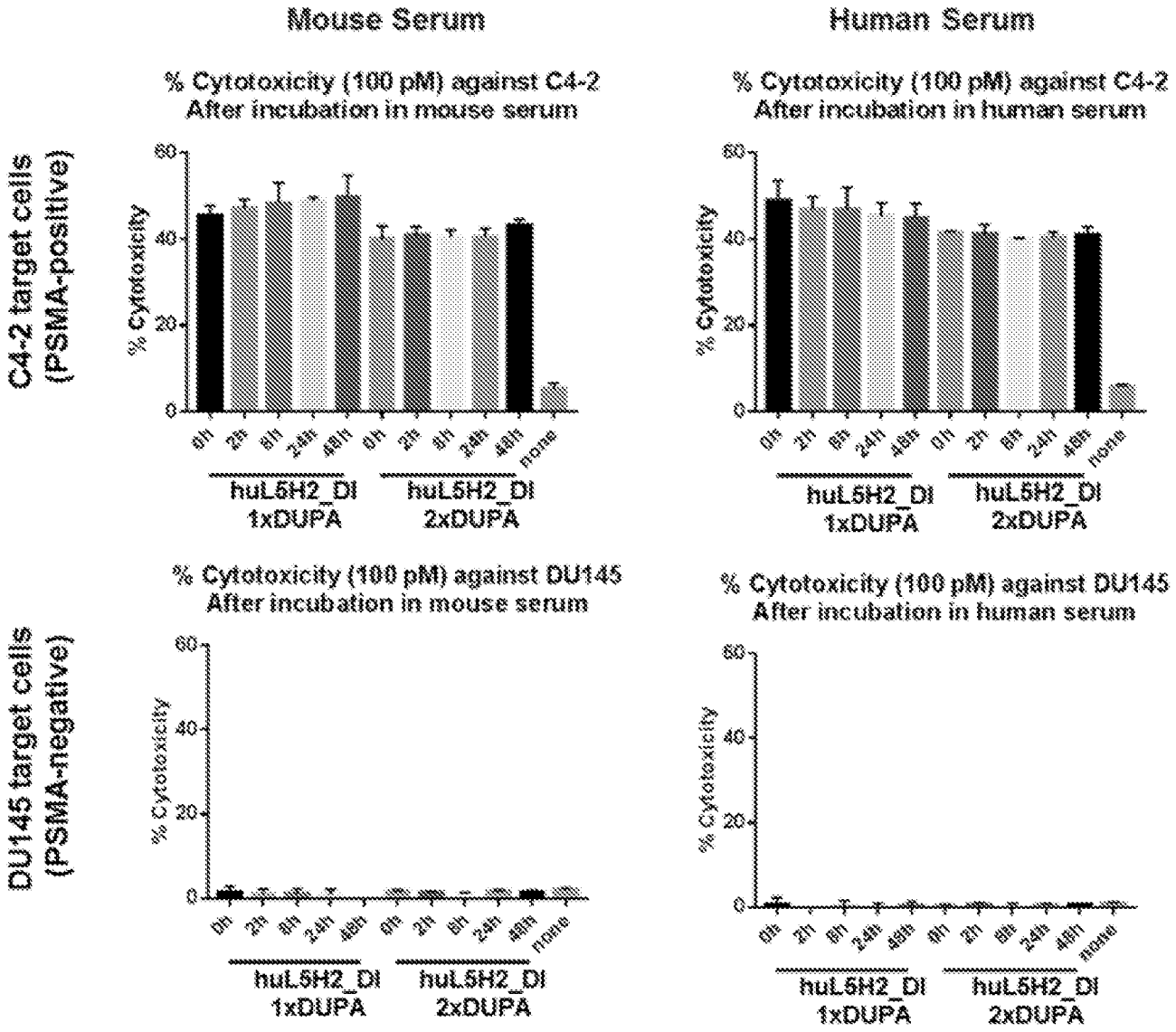

Mouse Serum
Human Serum
C4-2 target cells (PSMA-positive)
DU145 target cells (PSMA-negative)
% Cytotoxicity (100 pM) against C4-2
After incubation in mouse serum
% Cytotoxicity (100 pM) against C4-2
After incubation in human serum
% Cytotoxicity (100 pM) against DU145
After incubation in mouse serum
% Cytotoxicity (100 pM) against DU145
After incubation in human serum
% Cytotoxicity
0
20
40
60
0h
2h
8h
24h
48h
none
huL5H2_DI 1xDUPA
huL5H2_DI 2xDUPA

FIG. 33B

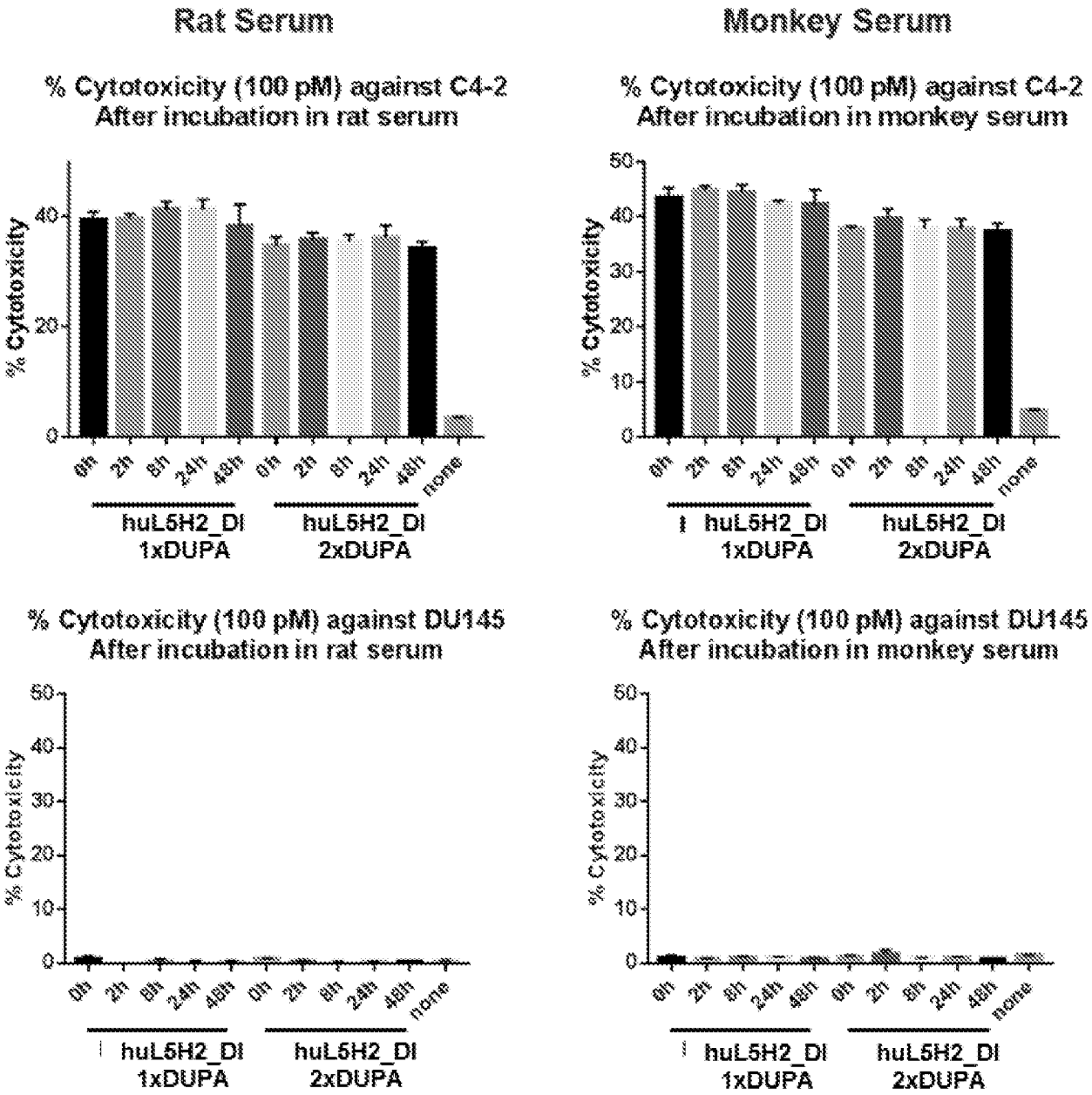

Rat Serum
% Cytotoxicity (100 pM) against C4-2
After incubation in rat serum
% Cytotoxicity
0
20
40
0h
2h
8h
24h
48h
0h
2h
8h
24h
48h
none
huL5H2_DI
1xDUPA
huL5H2_DI
2xDUPA
Monkey Serum
% Cytotoxicity (100 pM) against C4-2
After incubation in monkey serum
% Cytotoxicity
0
10
20
30
40
50
0h
2h
8h
24h
48h
0h
2h
8h
24h
48h
none
huL5H2_DI
1xDUPA
huL5H2_DI
2xDUPA
% Cytotoxicity (100 pM) against DU145
After incubation in rat serum
% Cytotoxicity
0
10
20
30
40
50
0h
2h
8h
24h
48h
0h
2h
8h
24h
48h
none
huL5H2_DI
1xDUPA
huL5H2_DI
2xDUPA
% Cytotoxicity (100 pM) against DU145
After incubation in monkey serum
% Cytotoxicity
0
10
20
30
40
50
0h
2h
8h
24h
48h
0h
2h
8h
24h
48h
none
huL5H2_DI
1xDUPA
huL5H2_DI
2xDUPA

FIG. 34A

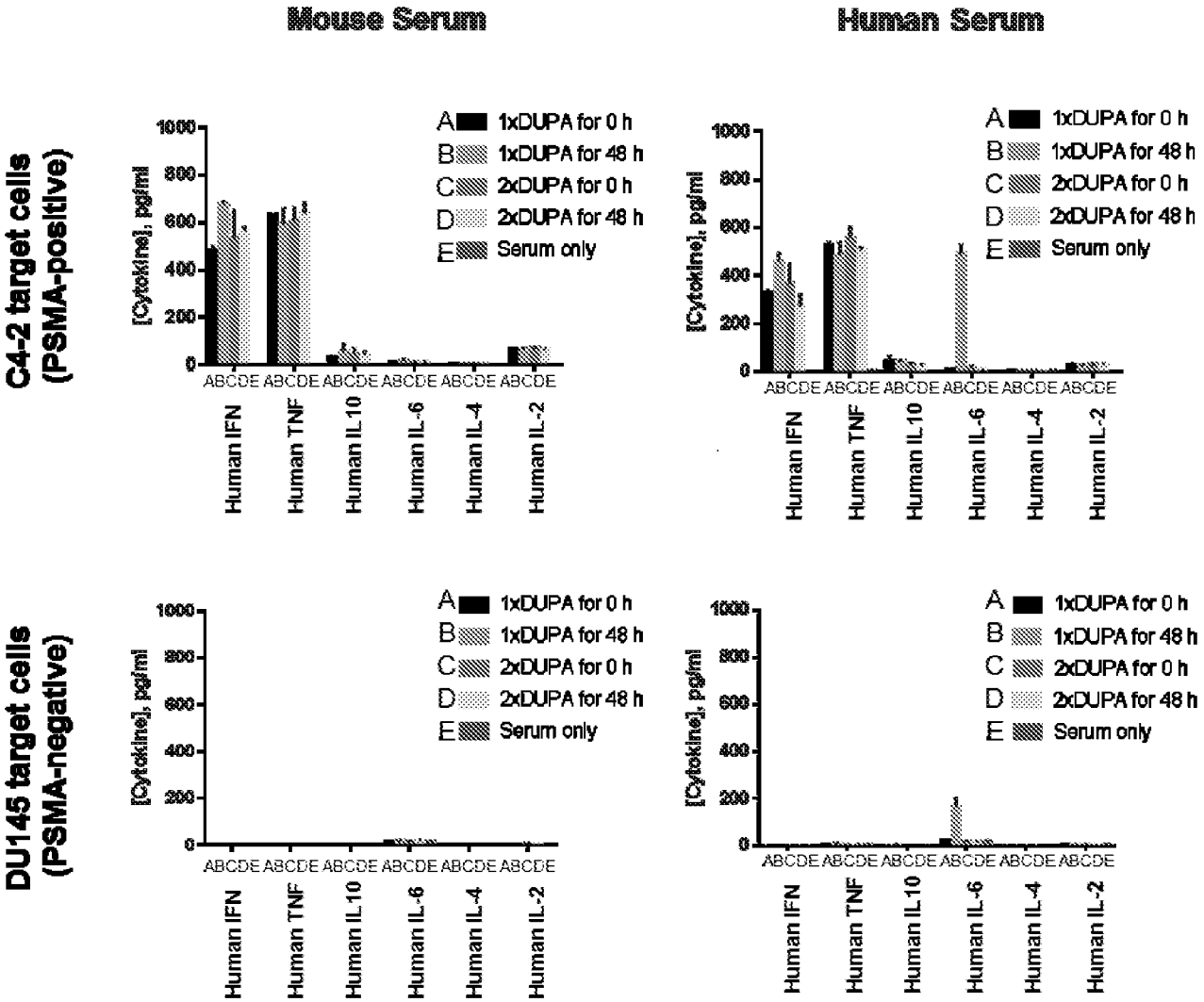
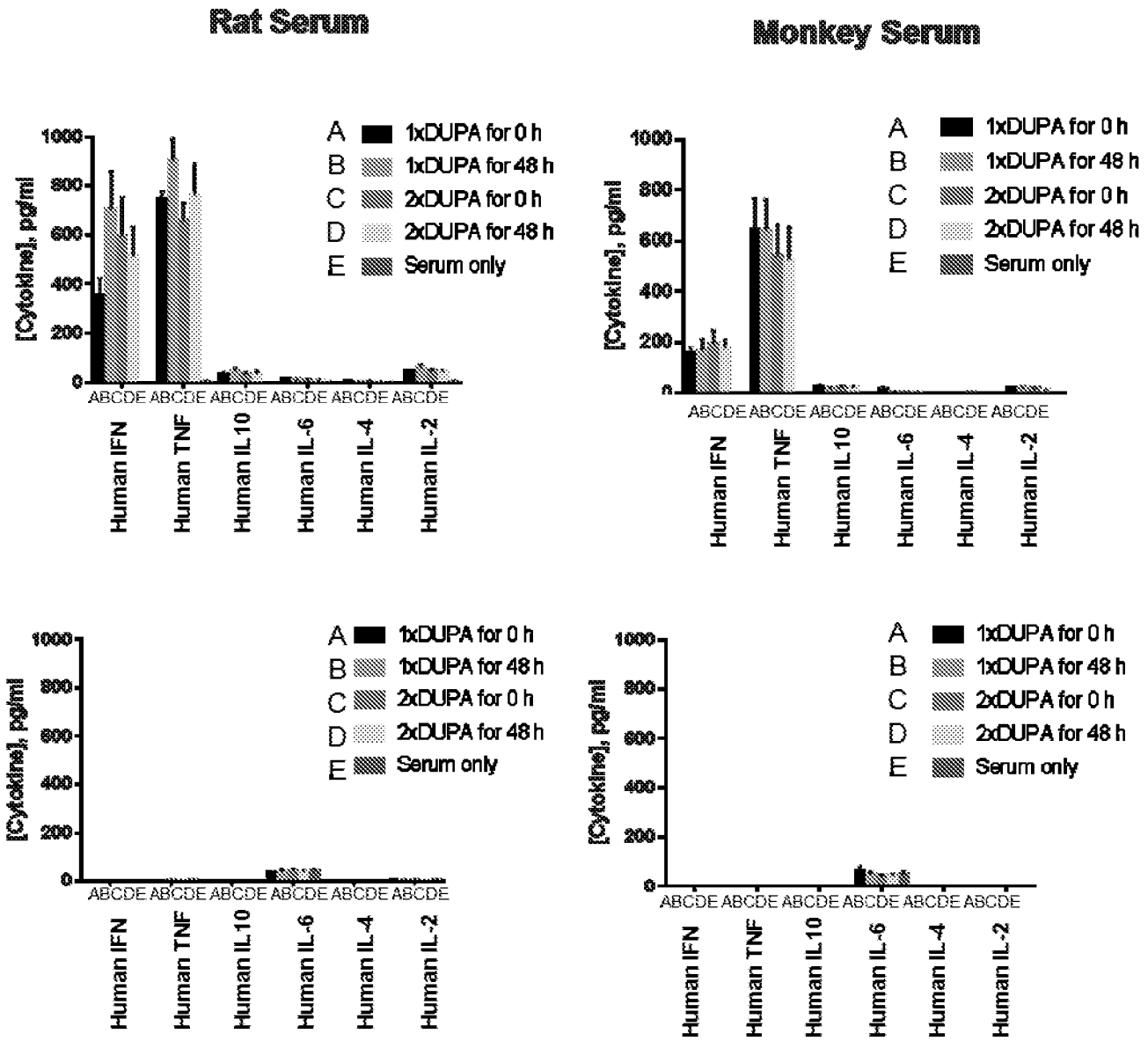

Mouse Serum
Human Serum
C4-2 target cells (PSMA-positive)
DU145 target cells (PSMA-negative)
[Cytokine], pg/ml
A 1xDUPA for 0 h
B 1xDUPA for 48 h
C 2xDUPA for 0 h
D 2xDUPA for 48 h
E Serum only
Human IFN
Human TNF
Human IL10
Human IL-6
Human IL-4
Human IL-2

FIG. 34B


Rat Serum
Monkey Serum
[Cytokine], pg/ml
A 1xDUPA for 0 h
B 1xDUPA for 48 h
C 2xDUPA for 0 h
D 2xDUPA for 48 h
E Serum only
ABCDE ABCDE ABCDE ABCDE ABCDE ABCDE
Human IFN
Human TNF
Human IL10
Human IL-6
Human IL-4
Human IL-2

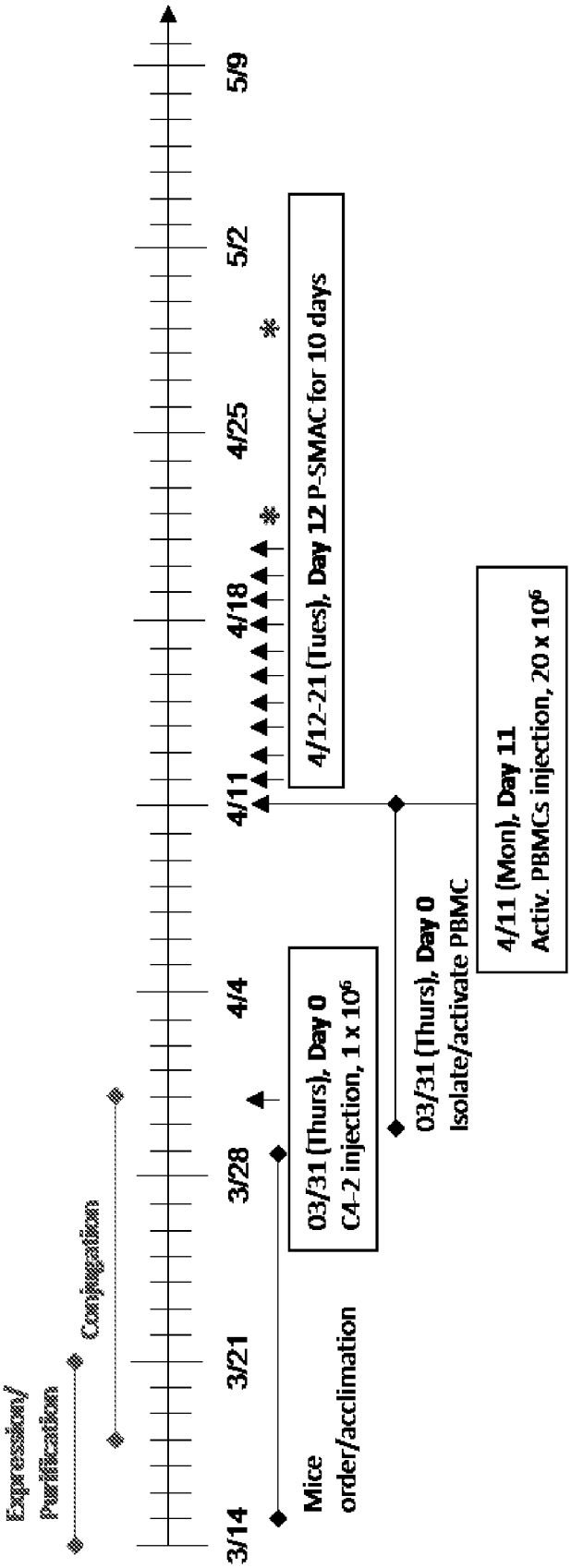

3/14
3/21
3/28
4/4
4/11
4/18
4/25
5/2
5/9
Expression/ Purification
Conjugation
Mice order/acclimation
03/31 (Thurs), Day 0 C4-2 injection, 1 x 10^6
03/31 (Thurs), Day 0 Isolate/activate PBMC
4/11 (Mon), Day 11 Activ. PBMCs injection, 20 x 10^6
4/12-21 (Tues), Day 12 P-SMAC for 10 days

FIG. 35A

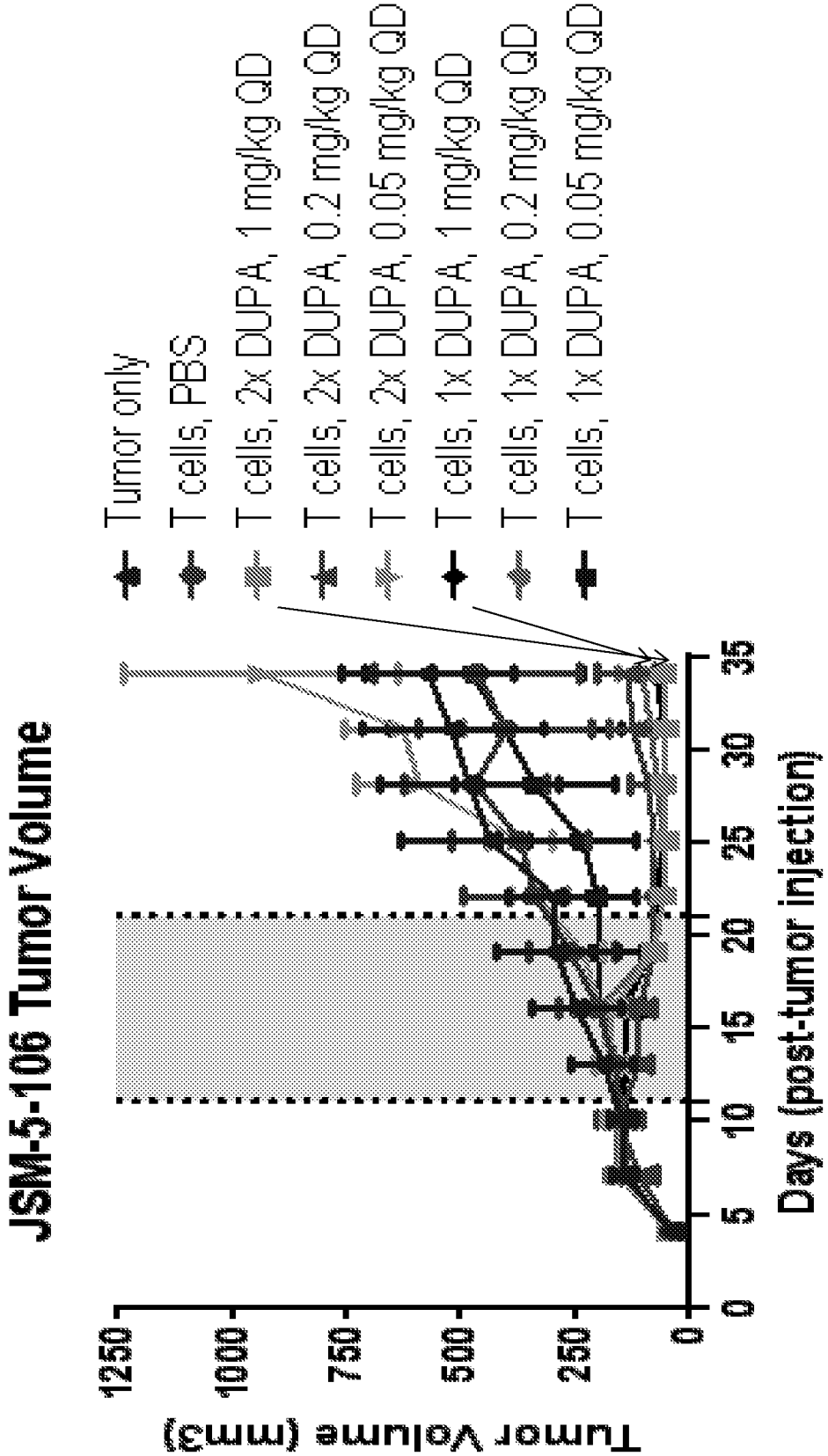

JSM-5-106 Tumor Volume
Tumor Volume (mm3)
1250
1000
750
500
250
0
0
5
10
15
20
25
30
35
Days (post-tumor injection)
Tumor only
T cells, PBS
T cells, 2x DUPA, 1 mg/kg QD
T cells, 2x DUPA, 0.2 mg/kg QD
T cells, 2x DUPA, 0.05 mg/kg QD
T cells, 1x DUPA, 1 mg/kg QD
T cells, 1x DUPA, 0.2 mg/kg QD
T cells, 1x DUPA, 0.05 mg/kg QD

FIG. 35B

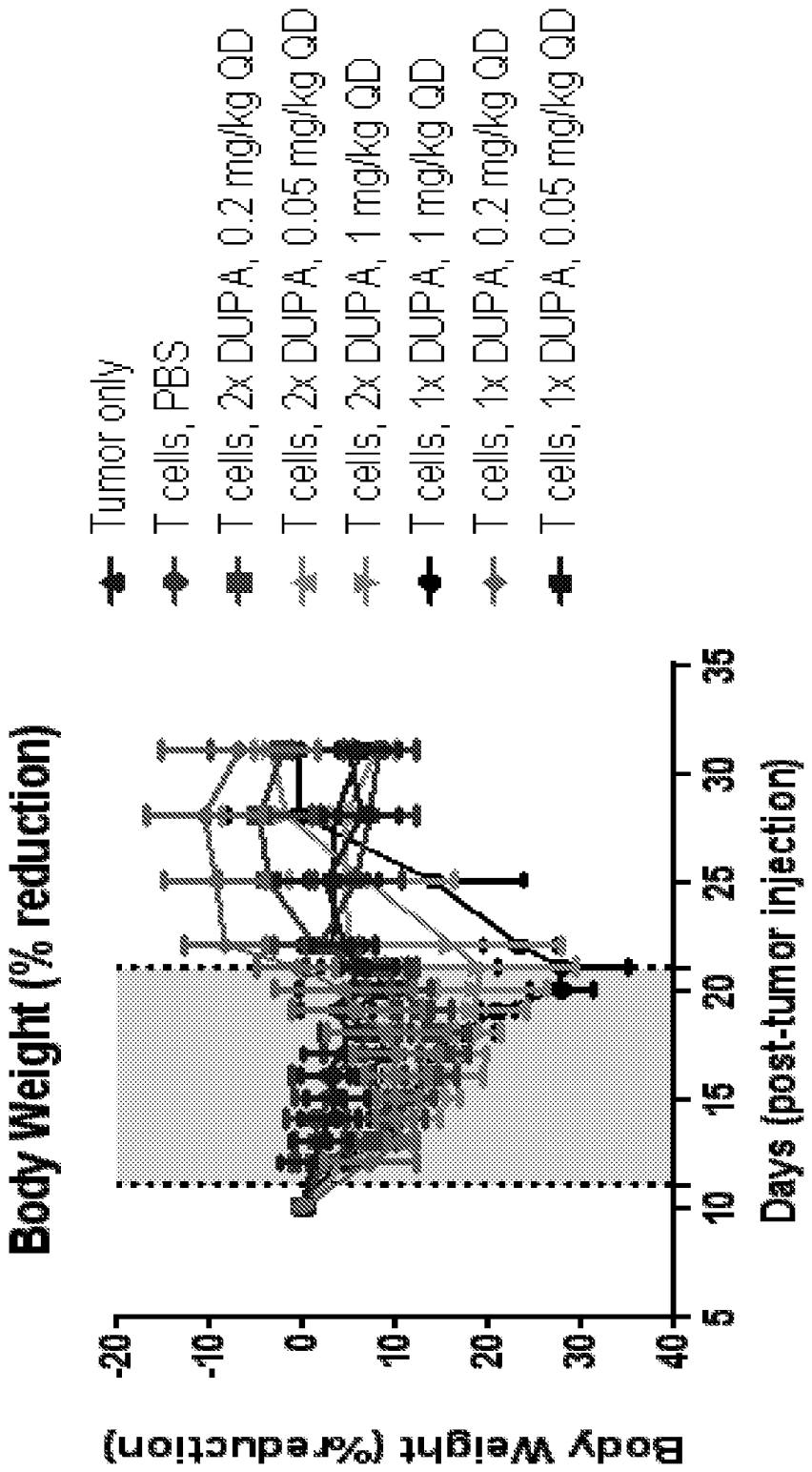

Body Weight (% reduction)
Days (post-tumor injection)
Tumor only
T cells, PBS
T cells, 2x DUPA, 0.2 mg/kg QD
T cells, 2x DUPA, 0.05 mg/kg QD
T cells, 2x DUPA, 1 mg/kg QD
T cells, 1x DUPA, 1 mg/kg QD
T cells, 1x DUPA, 0.2 mg/kg QD
T cells, 1x DUPA, 0.05 mg/kg QD
-20
-10
0
10
20
30
40
5
10
15
20
25
30
35

FIG. 35C

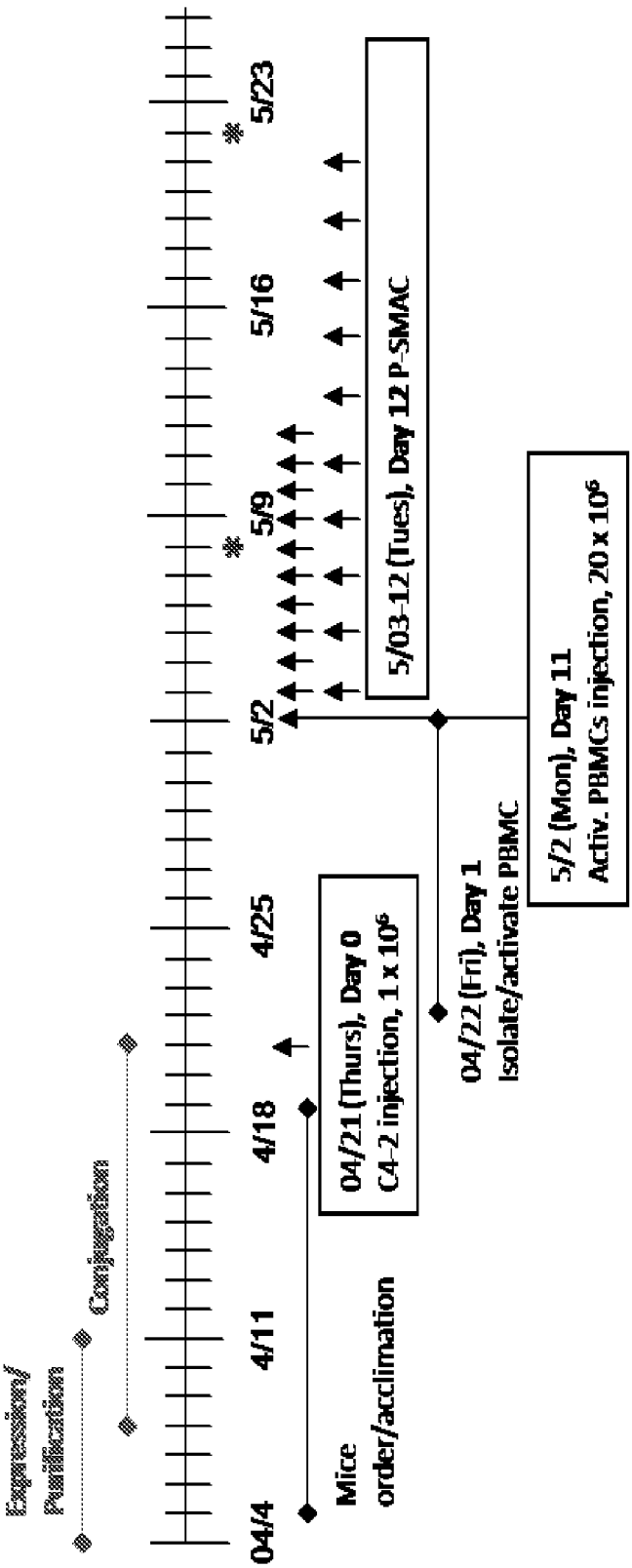

04/4
4/11
4/18
4/25
5/2
5/9
5/16
5/23
Expression/
Purification
Conjugation
Mice
order/acclimation
04/21 (Thurs), Day 0
C4-2 injection, 1 x 10^6
04/22 (Fri), Day 1
Isolate/activate PBMC
5/2 (Mon), Day 11
Activ. PBMCs injection, 20 x 10^6
5/03-12 (Tues), Day 12 P-SMAC

FIG. 36A

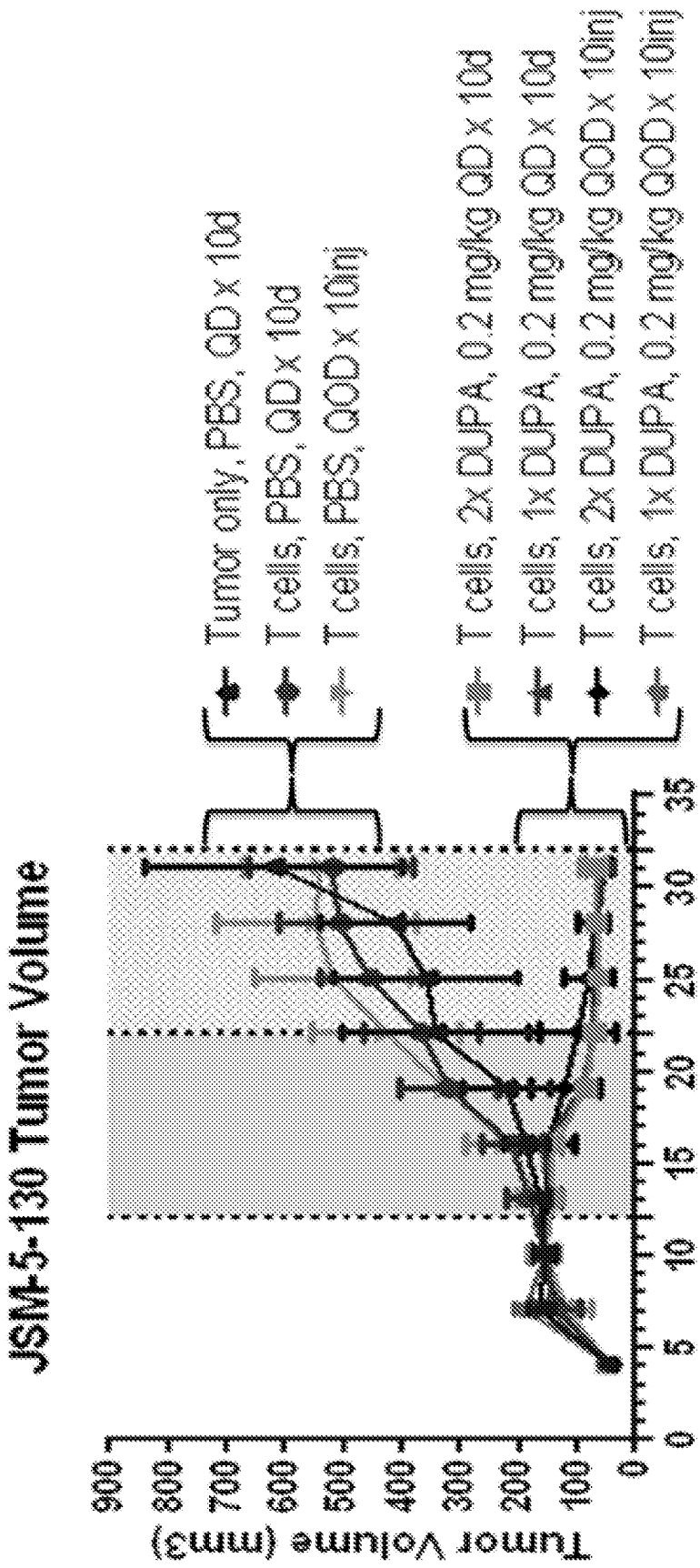

JSM-5-130 Tumor Volume
Tumor Volume (mm3)
900
800
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
Days (post-tumor injection)
Tumor only, PBS, QD x 10d
T cells, PBS, QD x 10d
T cells, PBS, QOD x 10inj
T cells, 2x DUPA, 0.2 mg/kg QD x 10d
T cells, 1x DUPA, 0.2 mg/kg QD x 10d
T cells, 2x DUPA, 0.2 mg/kg QOD x 10inj
T cells, 1x DUPA, 0.2 mg/kg QOD x 10inj

FIG. 36B

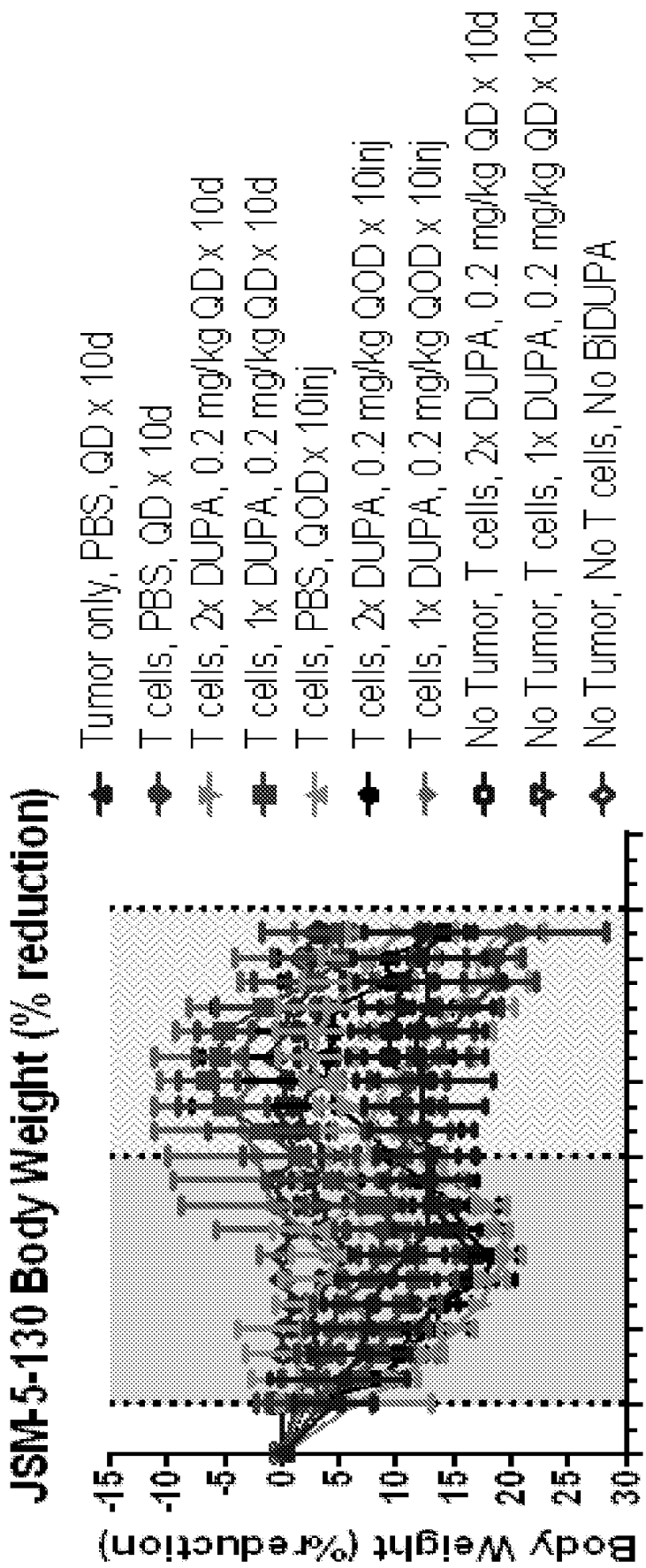

JSM-5-130 Body Weight (% reduction)
Body Weight (%reduction)
-15
-10
-5
0
5
10
15
20
25
30
10
15
20
25
30
35
Days (post-tumor injection)
Tumor only, PBS, QD x 10d
T cells, PBS, QD x 10d
T cells, 2x DUPA, 0.2 mg/kg QD x 10d
T cells, 1x DUPA, 0.2 mg/kg QD x 10d
T cells, PBS, QOD x 10inj
T cells, 2x DUPA, 0.2 mg/kg QOD x 10inj
T cells, 1x DUPA, 0.2 mg/kg QOD x 10inj
No Tumor, T cells, 2x DUPA, 0.2 mg/kg QD x 10d
No Tumor, T cells, 1x DUPA, 0.2 mg/kg QD x 10d
No Tumor, No T cells, No BiDUPA

FIG. 36C

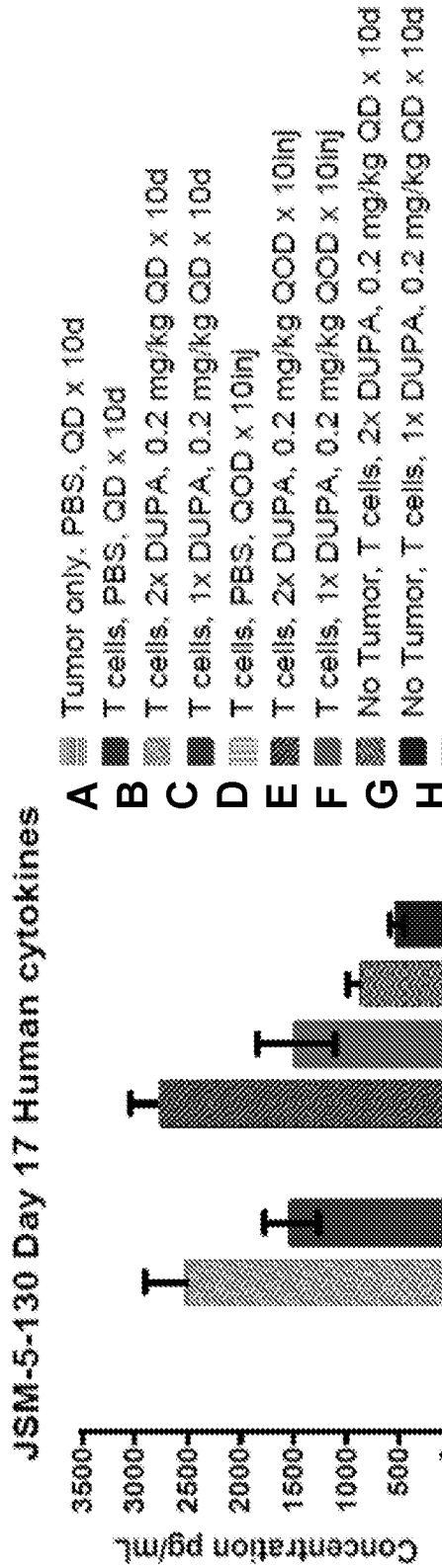


FIG. 37A

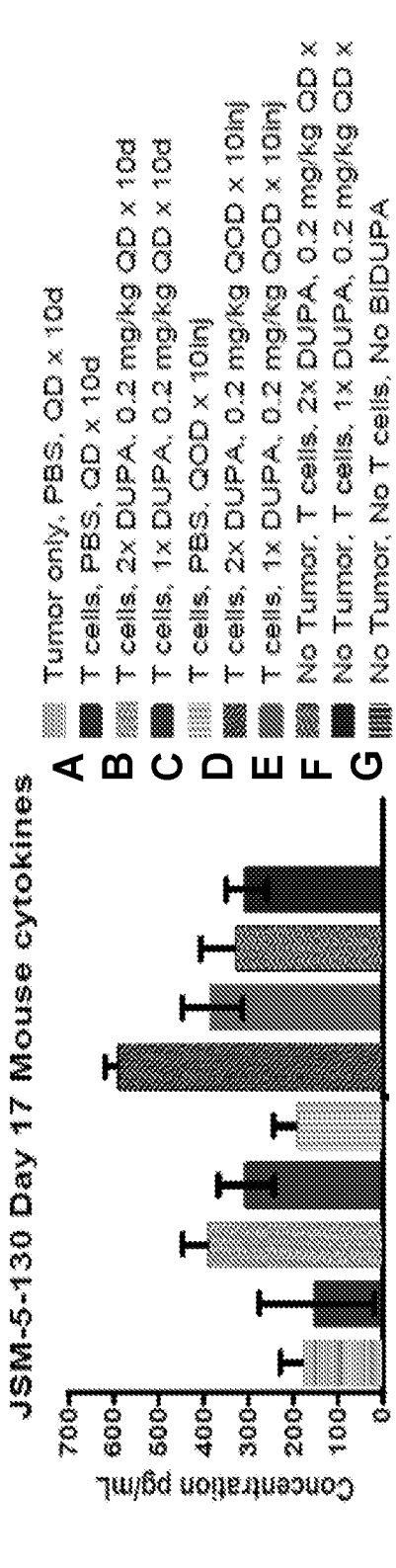


FIG. 37B

FIG. 38A

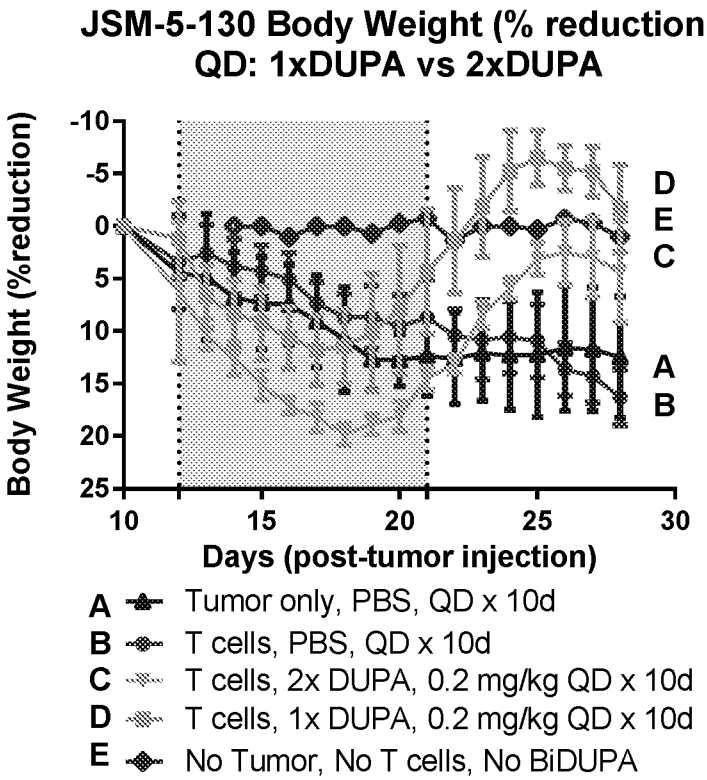


FIG. 38B

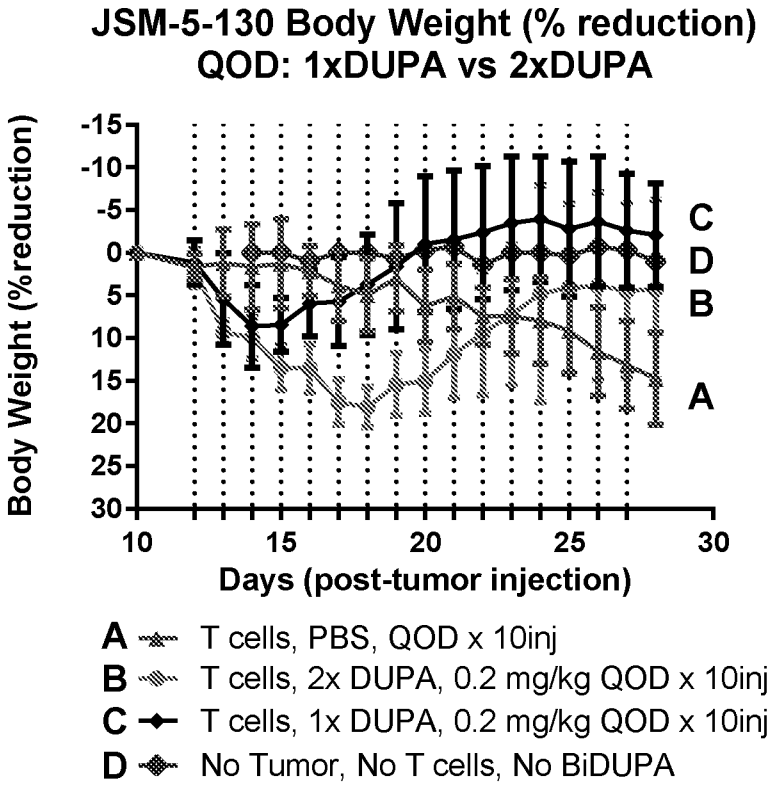


FIG. 38C

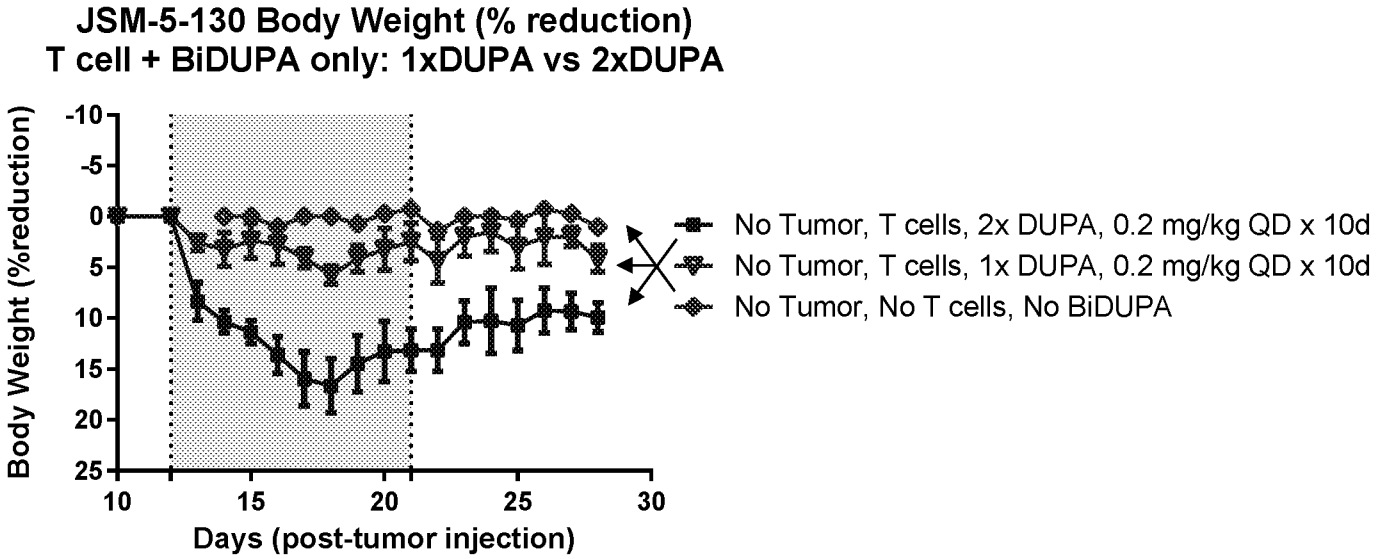


FIG. 39A

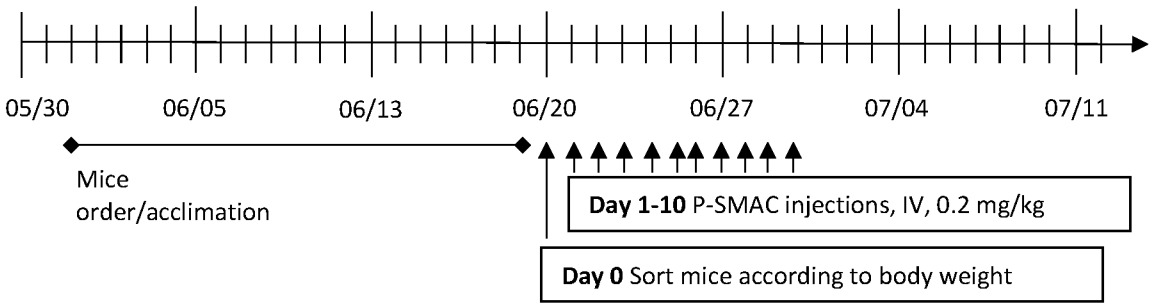


FIG. 39B

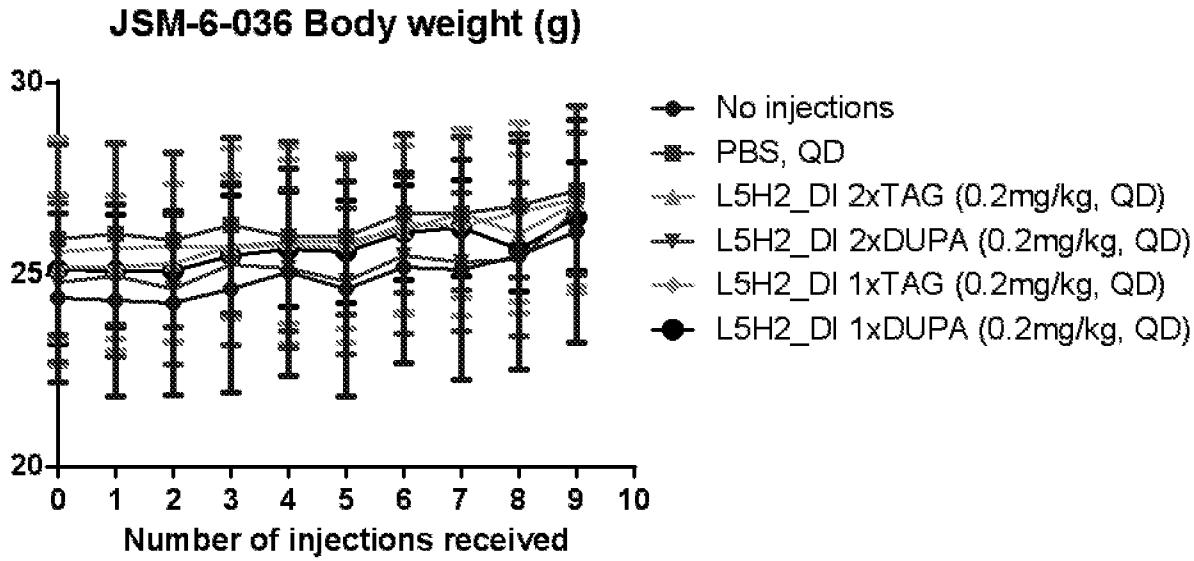


FIG. 39C

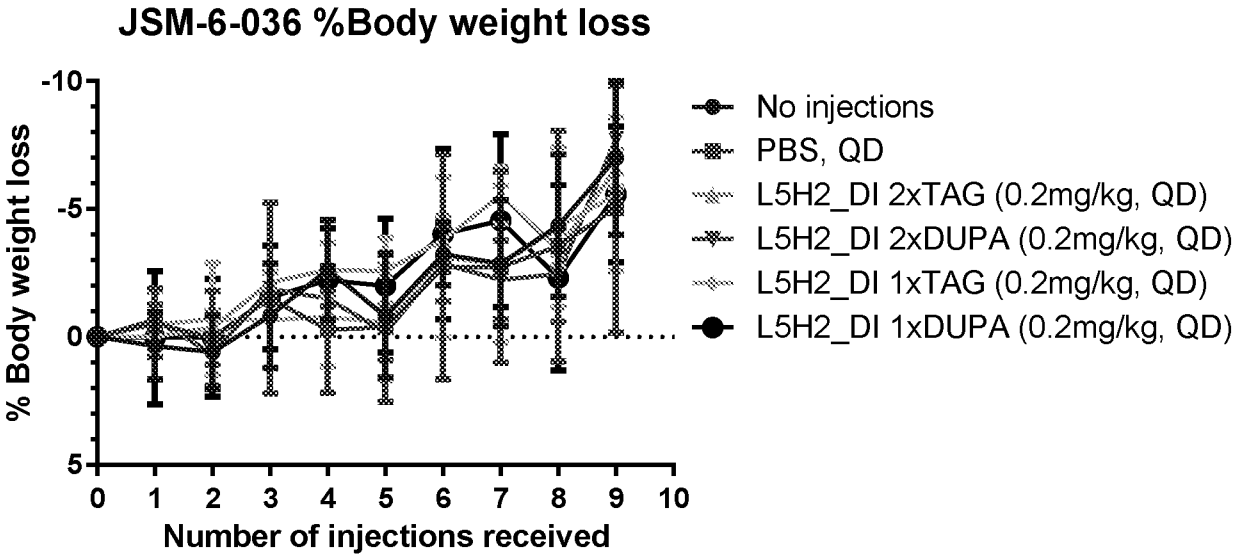
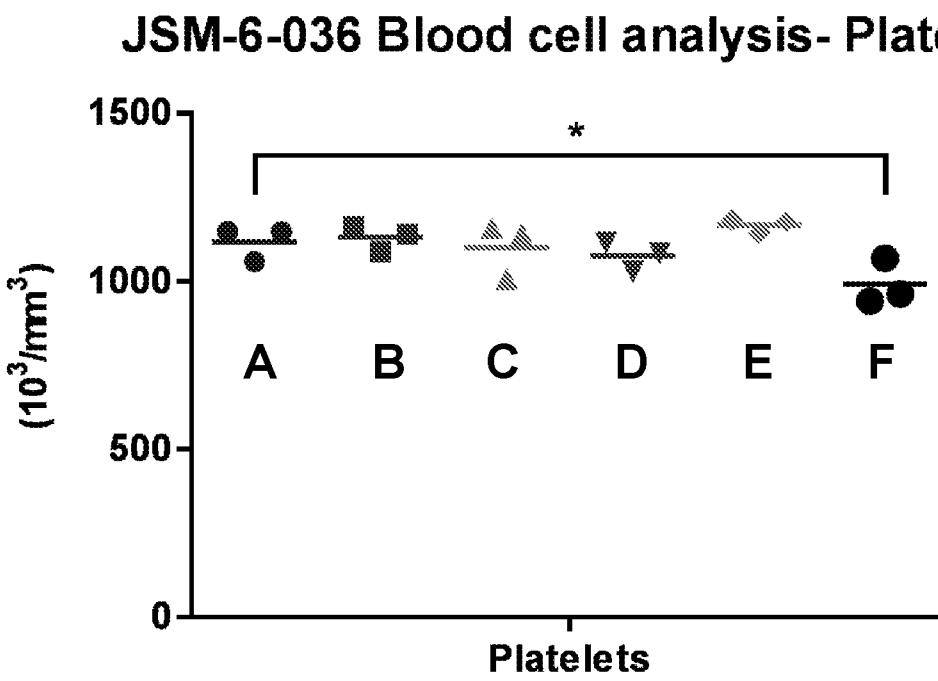


FIG. 40A



* = n.s., p>0.05 in two-sample t-test and Wilcoxon test.

A	No Injections
B	PBS, QD
C	L5H2_DI-2xTAG (0.2mg/kg, QD)
D	L5H2_DI-2xDUPA (0.2mg/kg, QD)
E	L5H2_DI-1xTAG (0.2mg/kg, QD)
F	L5H2_DI-2xDUPA (0.2mg/kg, QD)

FIG. 40B

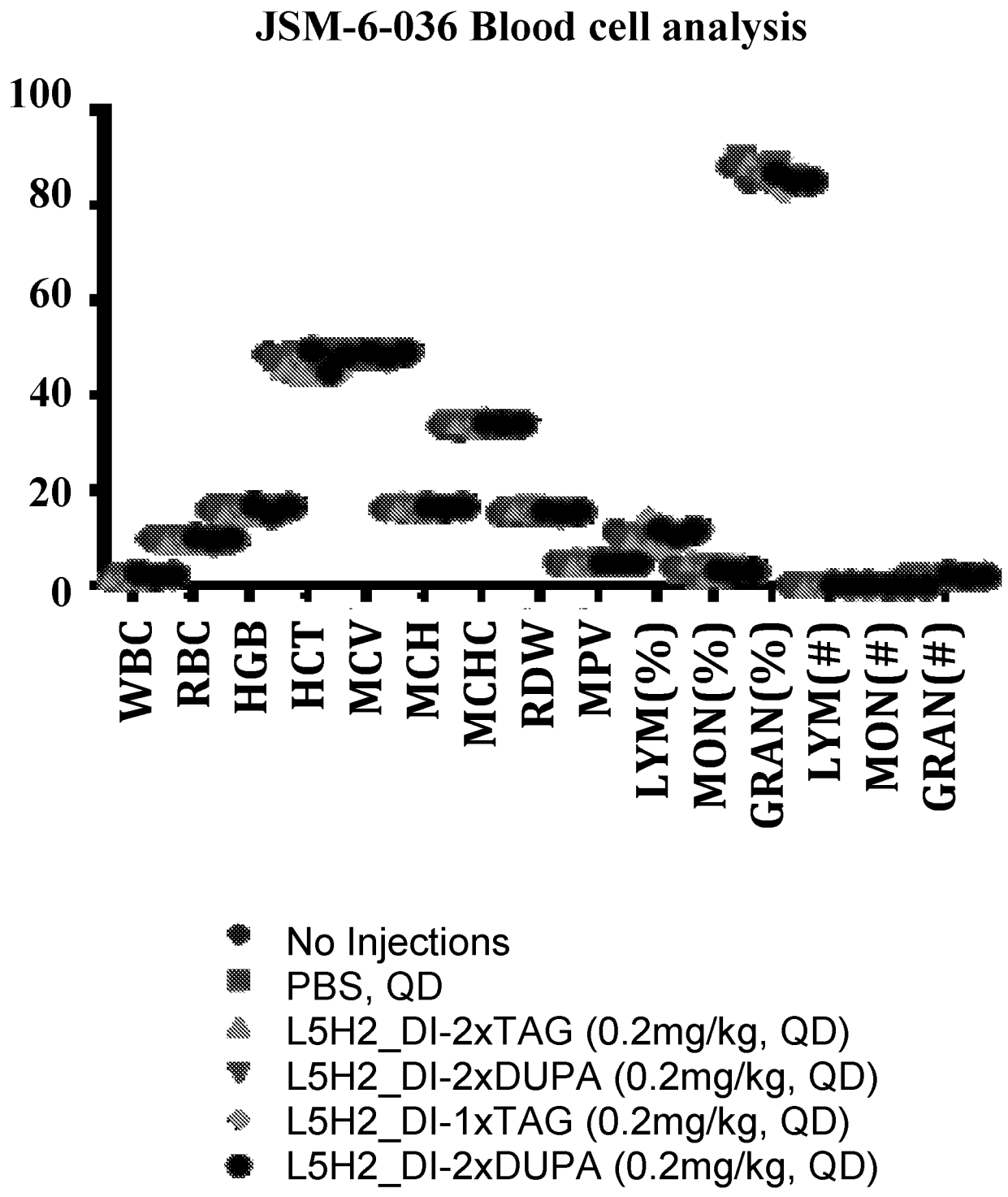

JSM-6-036 Blood cell analysis
100
80
60
40
20
0
WBC
RBC
HGB
HCT
MCV
MCH
MCHC
RDW
MPV
LYM(%)
MON(%)
GRAN(%)
LYM(#)
MON(#)
GRAN(#)
No Injections
PBS, QD
L5H2_DI-2xTAG (0.2mg/kg, QD)
L5H2_DI-2xDUPA (0.2mg/kg, QD)
L5H2_DI-1xTAG (0.2mg/kg, QD)
L5H2_DI-2xDUPA (0.2mg/kg, QD)

FIG. 40C

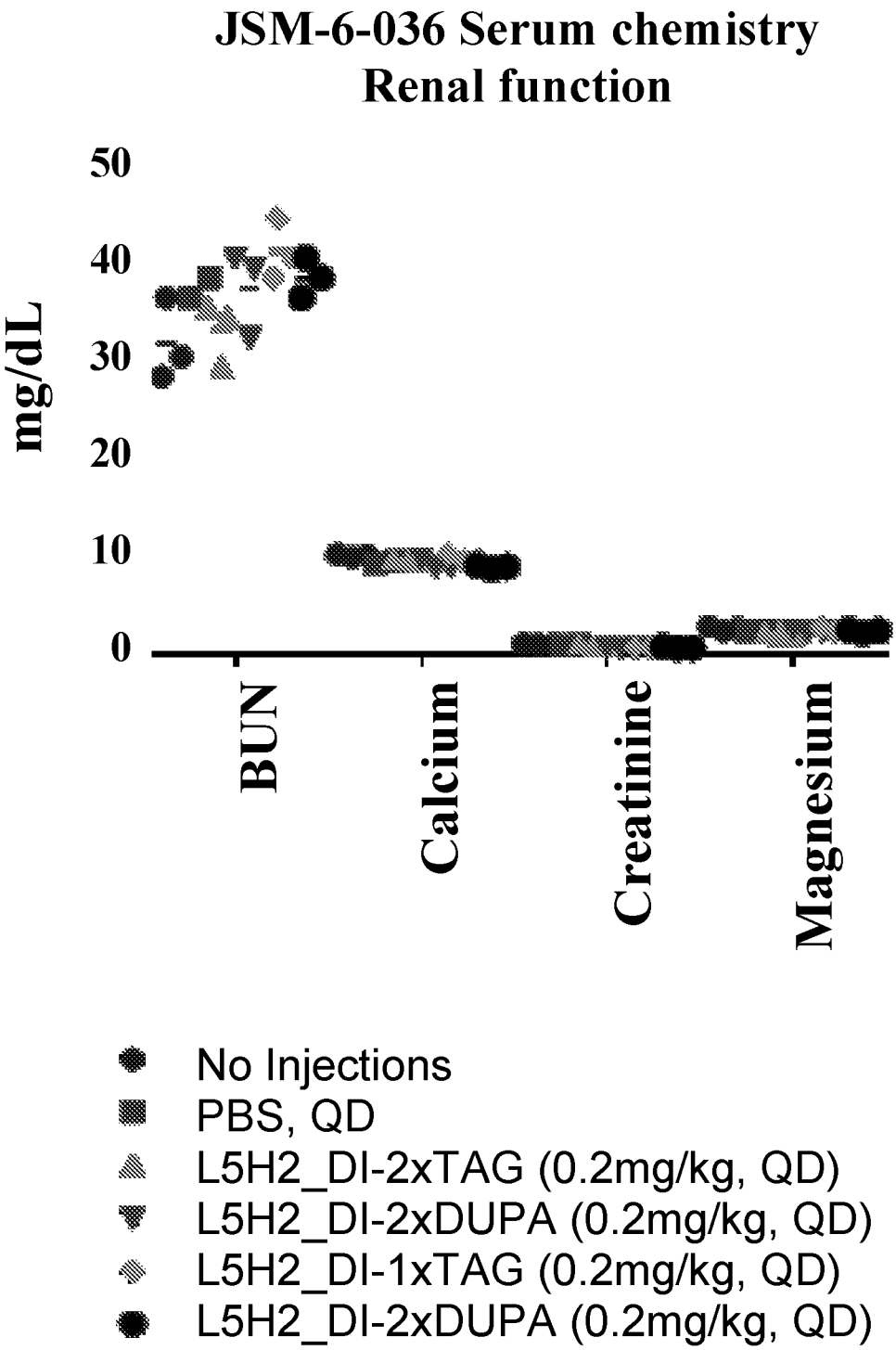

JSM-6-036 Serum chemistry
Renal function
mg/dL
50
40
30
20
10
0
BUN
Calcium
Creatinine
Magnesium
No Injections
PBS, QD
L5H2_DI-2xTAG (0.2mg/kg, QD)
L5H2_DI-2xDUPA (0.2mg/kg, QD)
L5H2_DI-1xTAG (0.2mg/kg, QD)
L5H2_DI-2xDUPA (0.2mg/kg, QD)

FIG. 40D

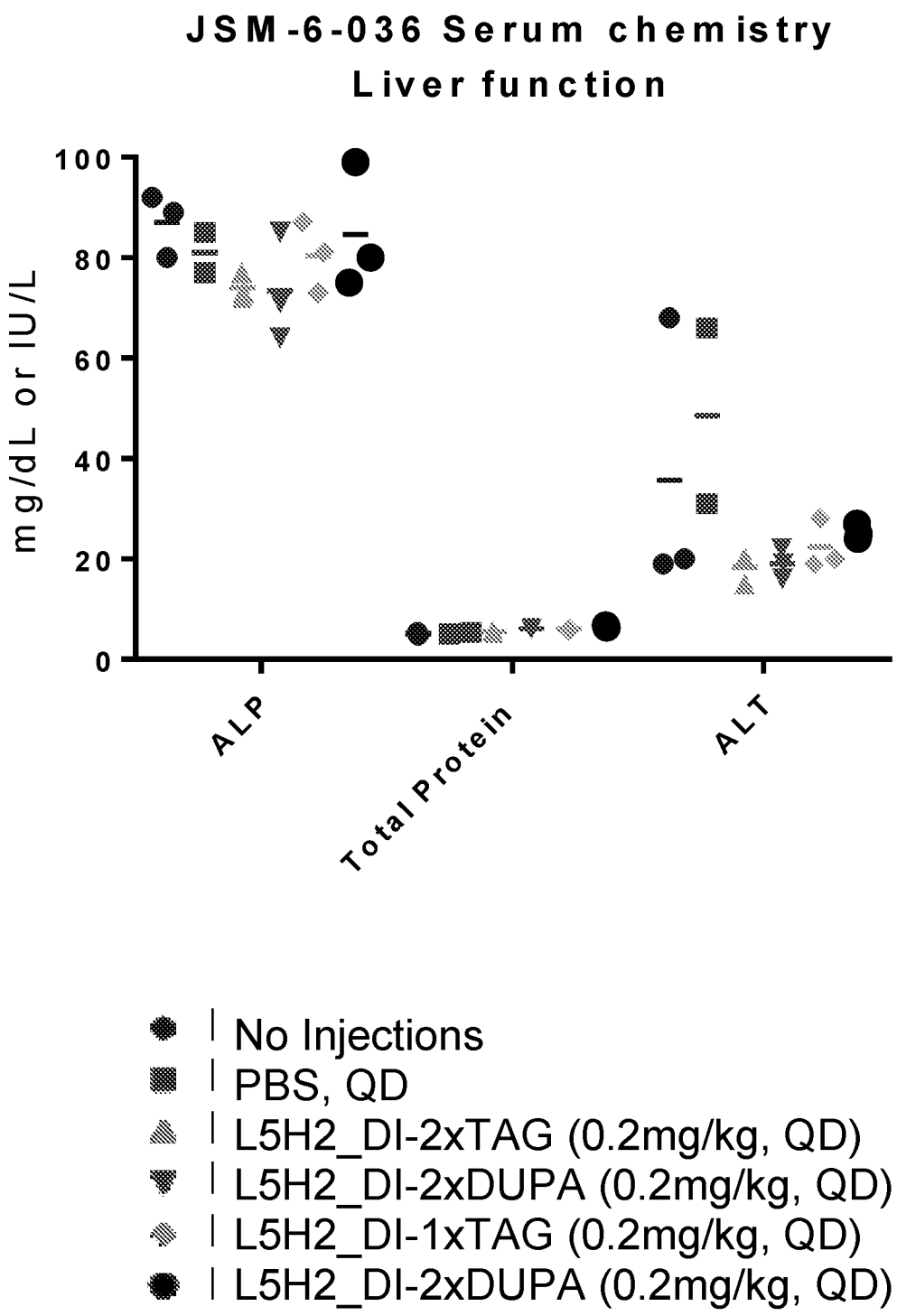
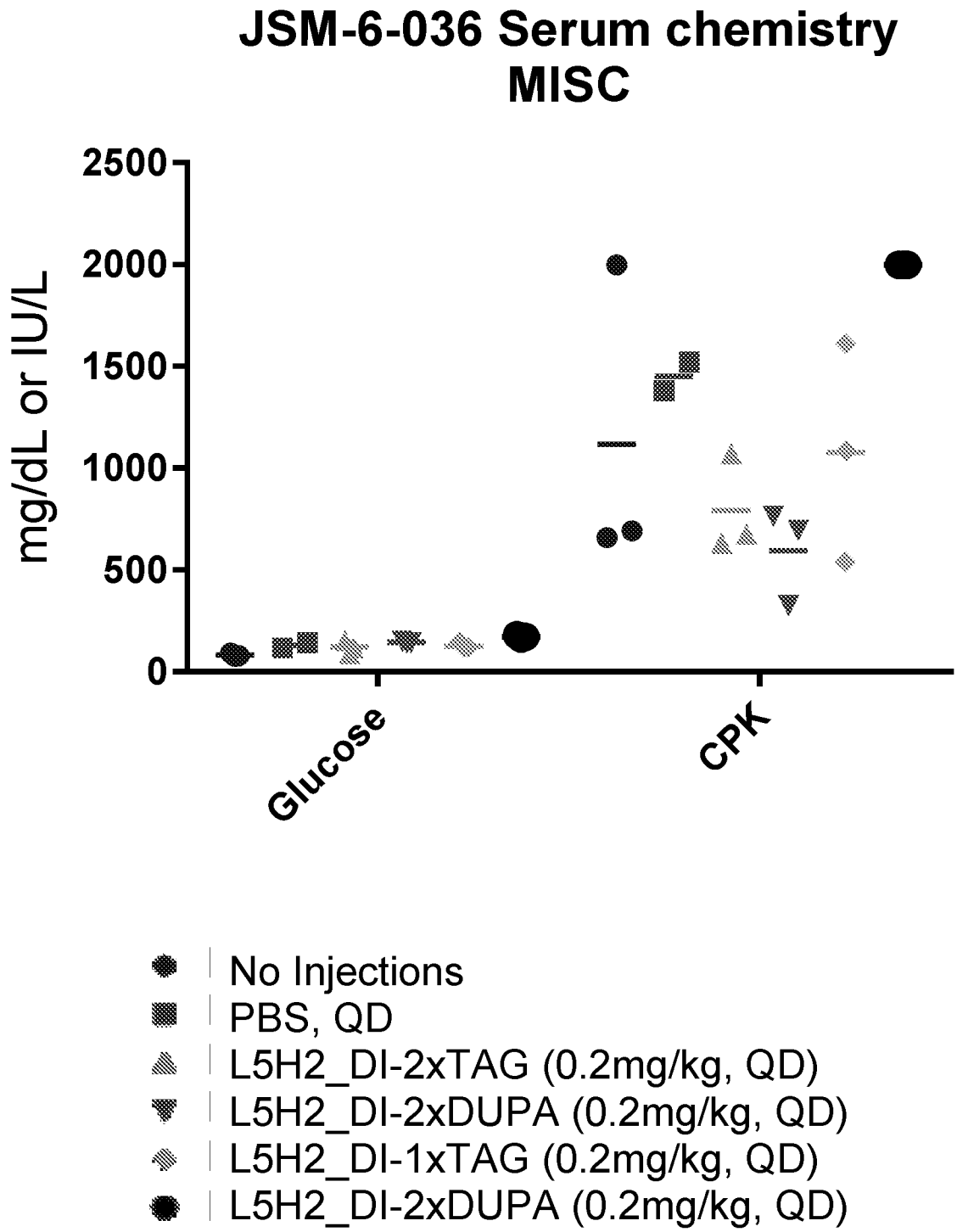

JSM-6-036 Serum chemistry
Liver function
mg/dL or IU/L
100
80
60
40
20
0
ALP
Total Protein
ALT
No Injections
PBS, QD
L5H2_DI-2xTAG (0.2mg/kg, QD)
L5H2_DI-2xDUPA (0.2mg/kg, QD)
L5H2_DI-1xTAG (0.2mg/kg, QD)
L5H2_DI-2xDUPA (0.2mg/kg, QD)

FIG. 40E


JSM-6-036 Serum chemistry
MISC
mg/dL or IU/L
2500
2000
1500
1000
500
0
Glucose
CPK
No Injections
PBS, QD
L5H2_DI-2xTAG (0.2mg/kg, QD)
L5H2_DI-2xDUPA (0.2mg/kg, QD)
L5H2_DI-1xTAG (0.2mg/kg, QD)
L5H2_DI-2xDUPA (0.2mg/kg, QD)

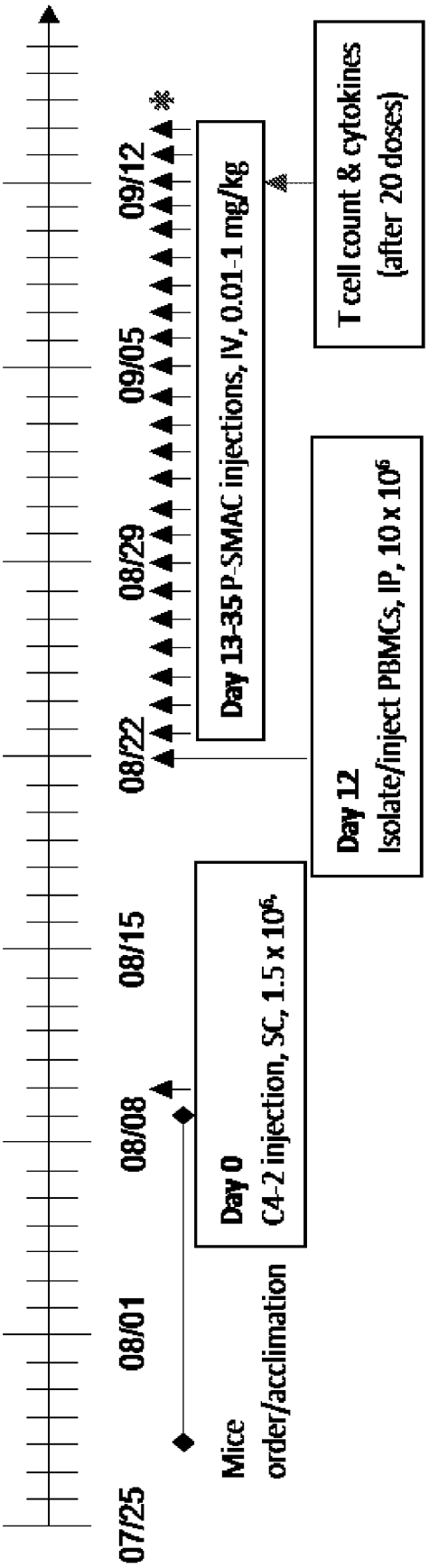
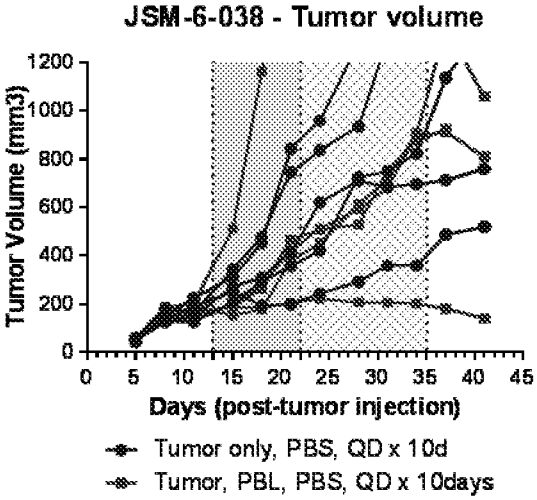

07/25
08/01
08/08
08/15
08/22
08/29
09/05
09/12
*
Mice order/acclimation
Day 0
C4-2 injection, SC, 1.5 x 10^6
Day 12
Isolate/inject PBMCs, IP, 10 x 10^6
Day 13-35 P-SMAC injections, IV, 0.01-1 mg/kg
T cell count & cytokines (after 20 doses)

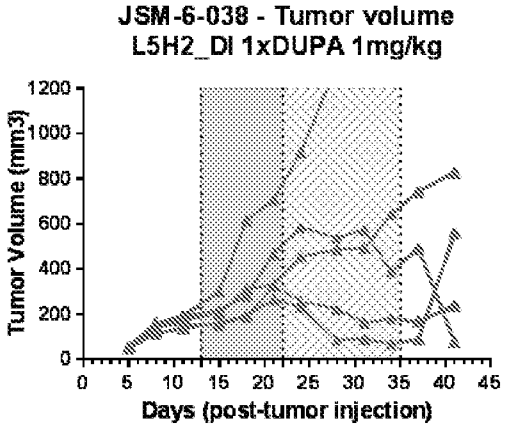
FIG. 41

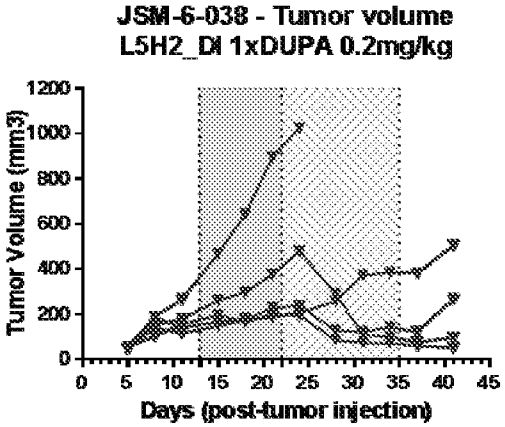
FIG. 42

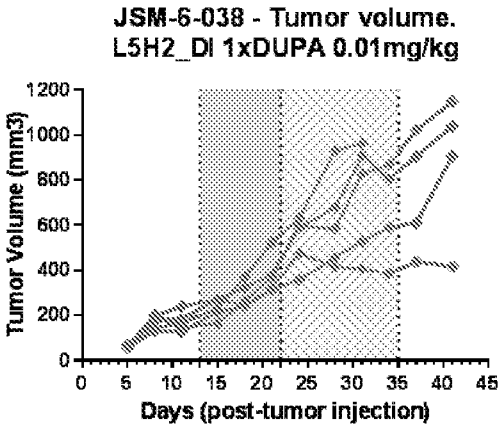
Controls



Treatment groups







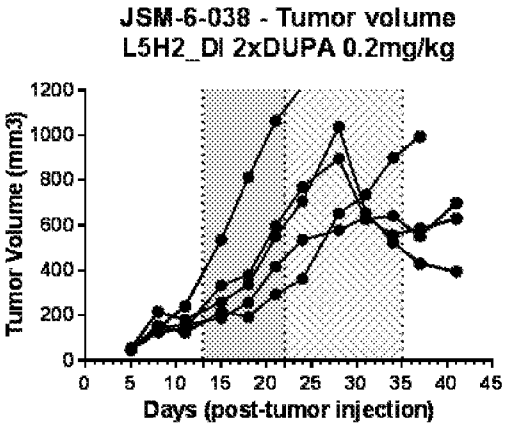
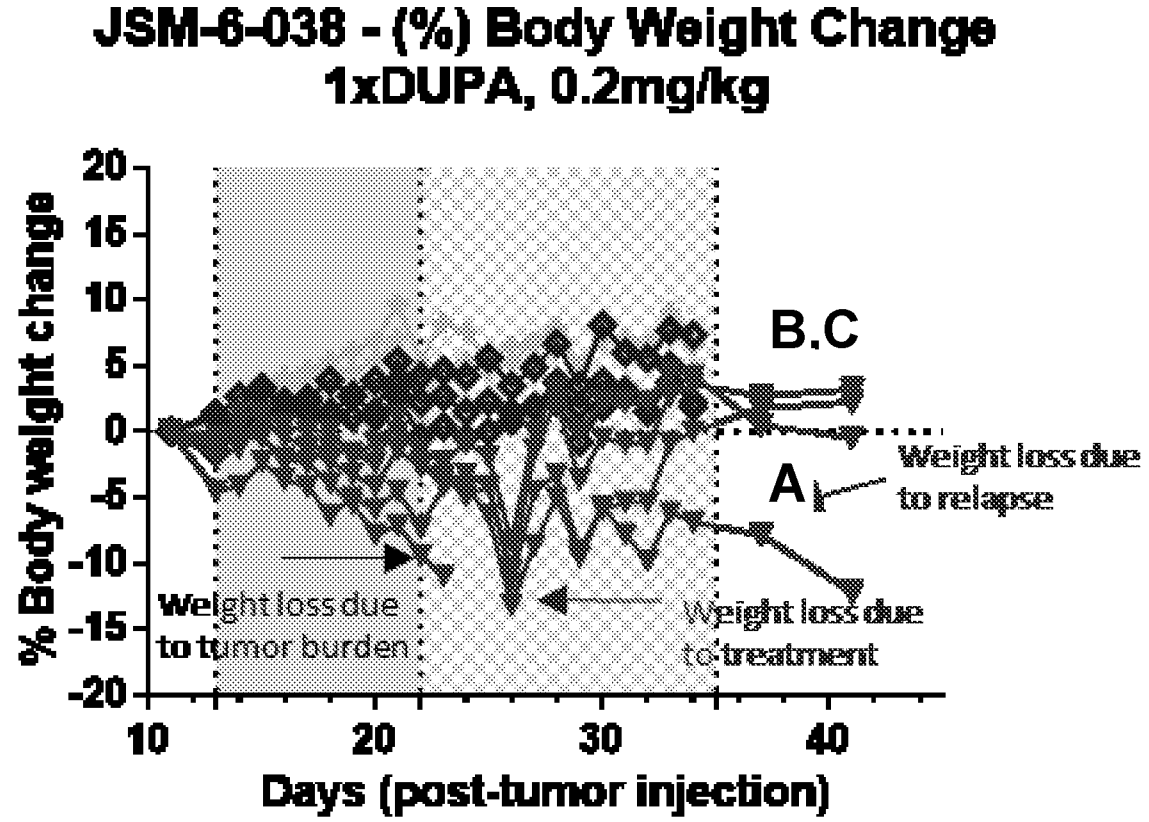


FIG. 43


JSM-6-038 - (%) Body Weight Change
1xDUPA, 0.2mg/kg
% Body weight change
20
15
10
5
0
-5
-10
-15
-20
10
20
30
40
Days (post-tumor injection)
B.C
A
Weight loss due to relapse
Weight loss due to tumor burden
Weight loss due to treatment
A Tumor, PBL, L5H2_DI 1xDUPA 0.2mg/kg QD x 10days
B PBL, L5H2_DI 1xDUPA 0.2mg/kg QD x 10days
C L5H2_DI 1xDUPA 0.2mg/kg QD x 10days

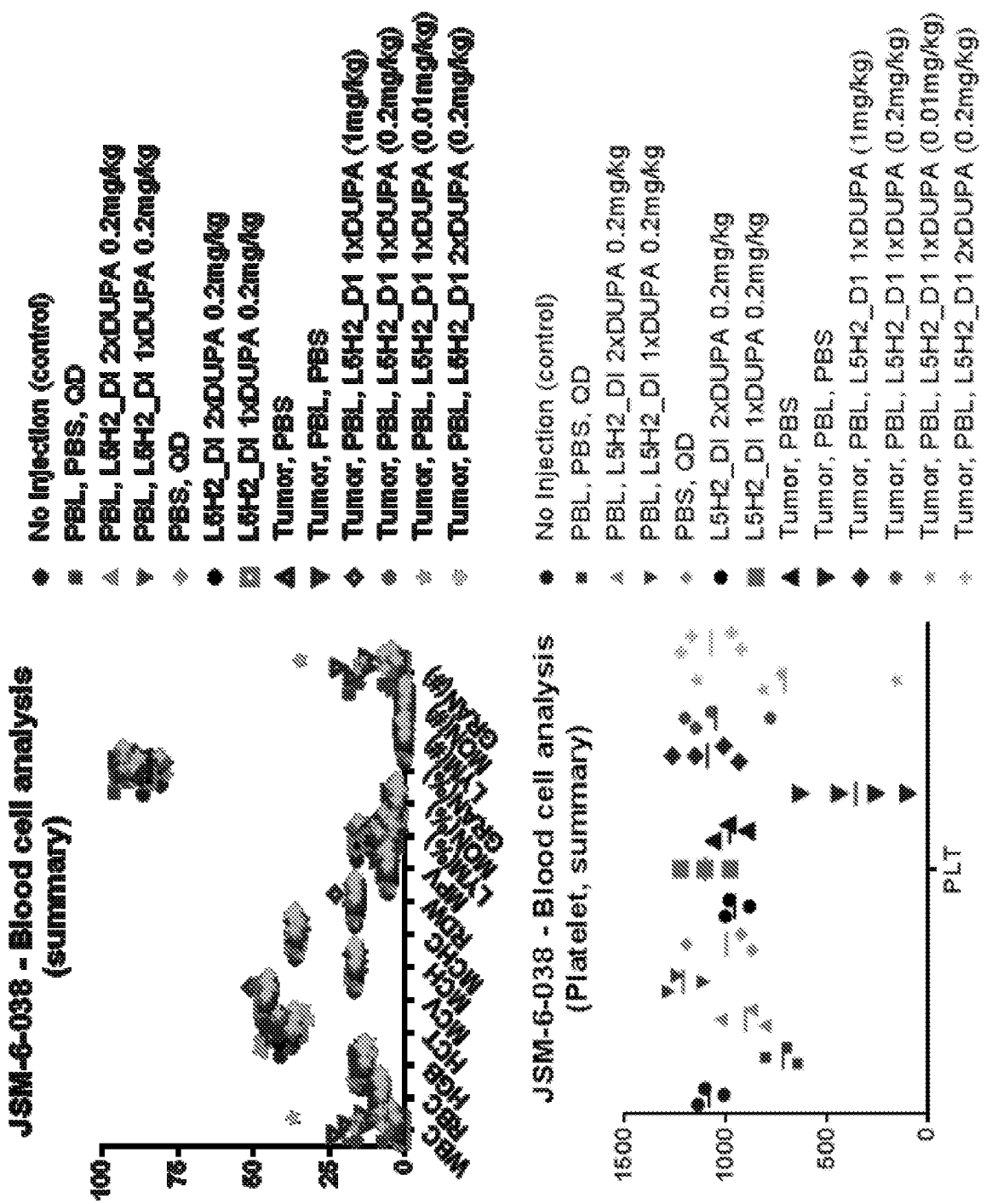

JSM-6-038 - Blood cell analysis (summary)
No Injection (control)
PBL, PBS, QD
PBL, L5H2_D1 2xDUPA 0.2mg/kg
PBL, L5H2_D1 1xDUPA 0.2mg/kg
PBS, QD
L5H2_D1 2xDUPA 0.2mg/kg
L5H2_D1 1xDUPA 0.2mg/kg
Tumor, PBS
Tumor, PBL, PBS
Tumor, PBL, L5H2_D1 1xDUPA (1mg/kg)
Tumor, PBL, L5H2_D1 1xDUPA (0.2mg/kg)
Tumor, PBL, L5H2_D1 1xDUPA (0.01mg/kg)
Tumor, PBL, L5H2_D1 2xDUPA (0.2mg/kg)
100
75
50
25
0
JSM-6-038 - Blood cell analysis (Platelet, summary)
No Injection (control)
PBL, PBS, QD
PBL, L5H2_D1 2xDUPA 0.2mg/kg
PBL, L5H2_D1 1xDUPA 0.2mg/kg
PBS, QD
L5H2_D1 2xDUPA 0.2mg/kg
L5H2_D1 1xDUPA 0.2mg/kg
Tumor, PBS
Tumor, PBL, PBS
Tumor, PBL, L5H2_D1 1xDUPA (1mg/kg)
Tumor, PBL, L5H2_D1 1xDUPA (0.2mg/kg)
Tumor, PBL, L5H2_D1 1xDUPA (0.01mg/kg)
Tumor, PBL, L5H2_D1 2xDUPA (0.2mg/kg)
1500
1000
500
0
PLT

FIG. 44A

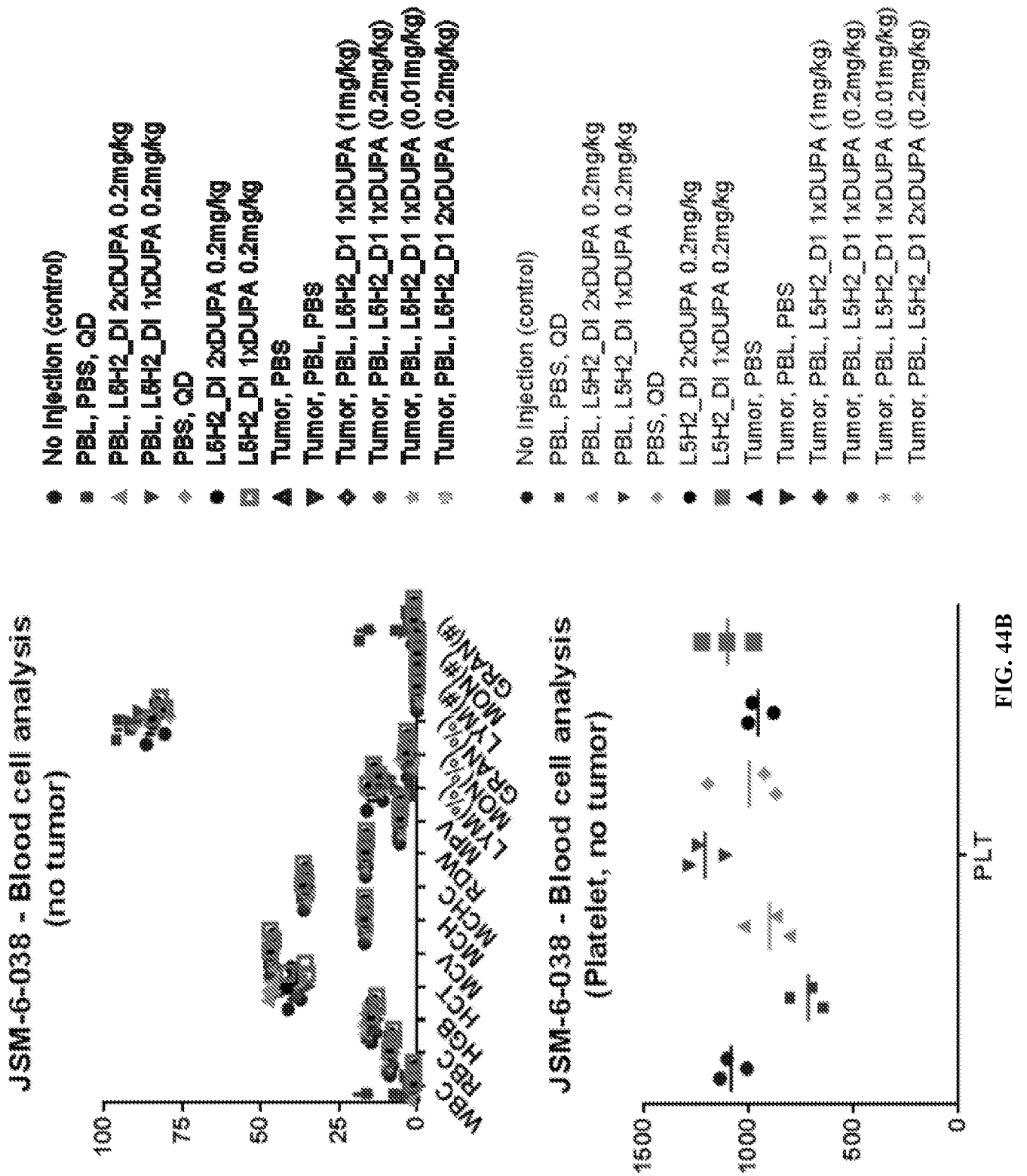

JSM-6-038 - Blood cell analysis
(no tumor)
100
75
50
25
0
WBC
RBC
HGB
HCT
MCV
MCH
MCHC
RDW
MPV
LYM(%)
MON(%)
GRAN(%)
LYM(#)
MON(#)
GRAN(#)
No Injection (control)
PBL, PBS, QD
PBL, L6H2_DI 2xDUPA 0.2mg/kg
PBL, L6H2_DI 1xDUPA 0.2mg/kg
PBS, QD
L6H2_DI 2xDUPA 0.2mg/kg
L6H2_DI 1xDUPA 0.2mg/kg
Tumor, PBS
Tumor, PBL, PBS
Tumor, PBL, L6H2_D1 1xDUPA (1mg/kg)
Tumor, PBL, L6H2_D1 1xDUPA (0.2mg/kg)
Tumor, PBL, L6H2_D1 1xDUPA (0.01mg/kg)
Tumor, PBL, L6H2_D1 2xDUPA (0.2mg/kg)
JSM-6-038 - Blood cell analysis
(Platelet, no tumor)
1500
1000
500
0
PLT
No Injection (control)
PBL, PBS, QD
PBL, L5H2_DI 2xDUPA 0.2mg/kg
PBL, L5H2_DI 1xDUPA 0.2mg/kg
PBS, QD
L5H2_DI 2xDUPA 0.2mg/kg
L5H2_DI 1xDUPA 0.2mg/kg
Tumor, PBS
Tumor, PBL, PBS
Tumor, PBL, L5H2_D1 1xDUPA (1mg/kg)
Tumor, PBL, L5H2_D1 1xDUPA (0.2mg/kg)
Tumor, PBL, L5H2_D1 1xDUPA (0.01mg/kg)
Tumor, PBL, L5H2_D1 2xDUPA (0.2mg/kg)

FIG. 44B

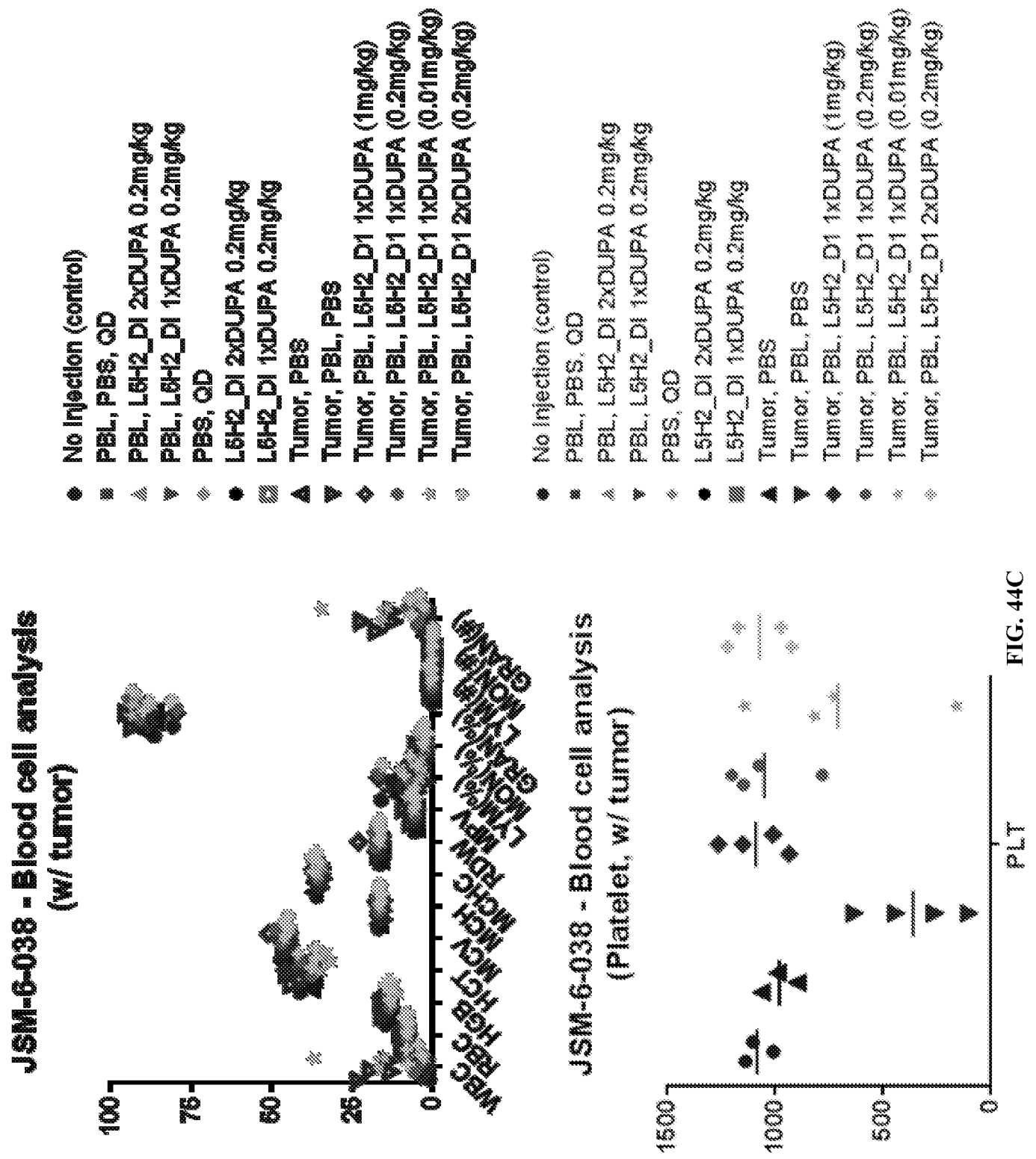

JSM-6-038 - Blood cell analysis
(w/ tumor)
100
75
50
25
0
WBC
RBC
HGB
HCT
MCV
MCH
MCHC
RDW
PLT
MPV
LYM#
MON#
NEU#
EOS#
BAS#
GRA#
No Injection (control)
PBL, PBS, QD
PBL, L6H2_DI 2xDUPA 0.2mg/kg
PBL, L6H2_DI 1xDUPA 0.2mg/kg
PBS, QD
L6H2_DI 2xDUPA 0.2mg/kg
L6H2_DI 1xDUPA 0.2mg/kg
Tumor, PBS
Tumor, PBL, PBS
Tumor, PBL, L6H2_D1 1xDUPA (1mg/kg)
Tumor, PBL, L6H2_D1 1xDUPA (0.2mg/kg)
Tumor, PBL, L6H2_D1 1xDUPA (0.01mg/kg)
Tumor, PBL, L6H2_D1 2xDUPA (0.2mg/kg)
JSM-6-038 - Blood cell analysis
(Platelet, w/ tumor)
1500
1000
500
0
PLT
No Injection (control)
PBL, PBS, QD
PBL, L5H2_DI 2xDUPA 0.2mg/kg
PBL, L5H2_DI 1xDUPA 0.2mg/kg
PBS, QD
L5H2_DI 2xDUPA 0.2mg/kg
L5H2_DI 1xDUPA 0.2mg/kg
Tumor, PBS
Tumor, PBL, PBS
Tumor, PBL, L5H2_D1 1xDUPA (1mg/kg)
Tumor, PBL, L5H2_D1 1xDUPA (0.2mg/kg)
Tumor, PBL, L5H2_D1 1xDUPA (0.01mg/kg)
Tumor, PBL, L5H2_D1 2xDUPA (0.2mg/kg)

FIG. 44C

FIG. 45A

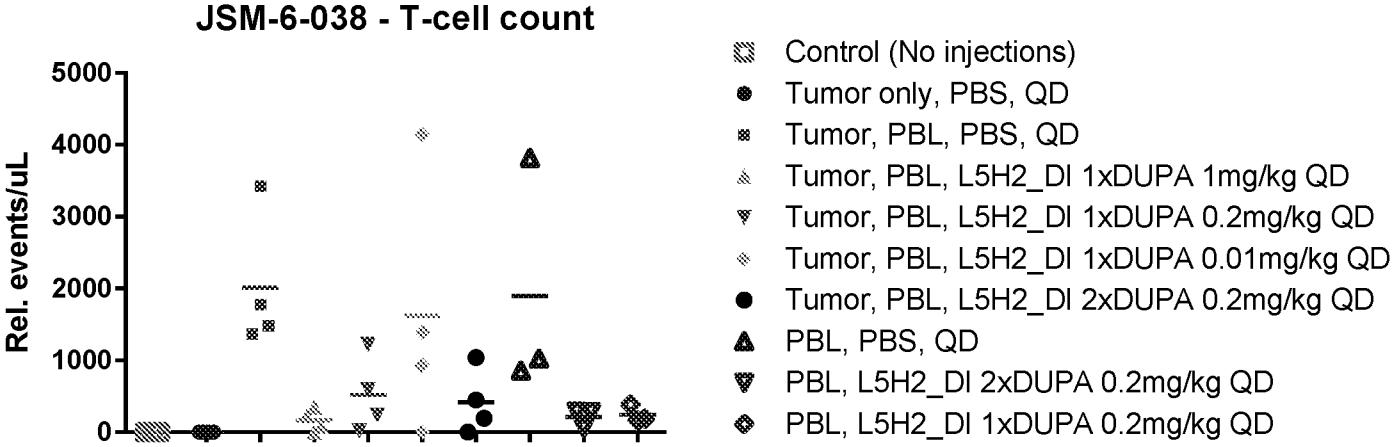


FIG. 45B

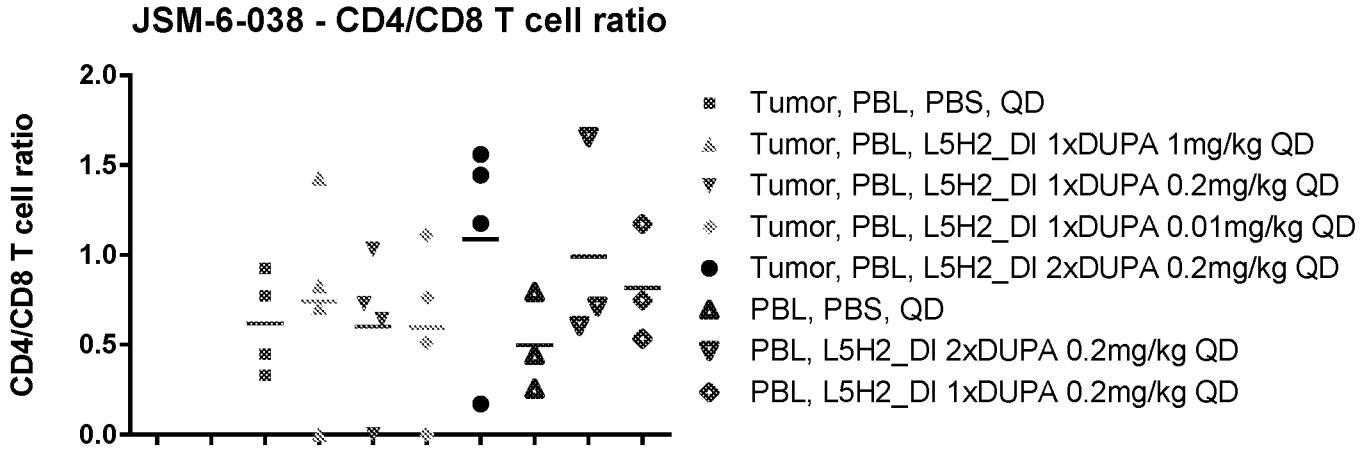


FIG. 45C

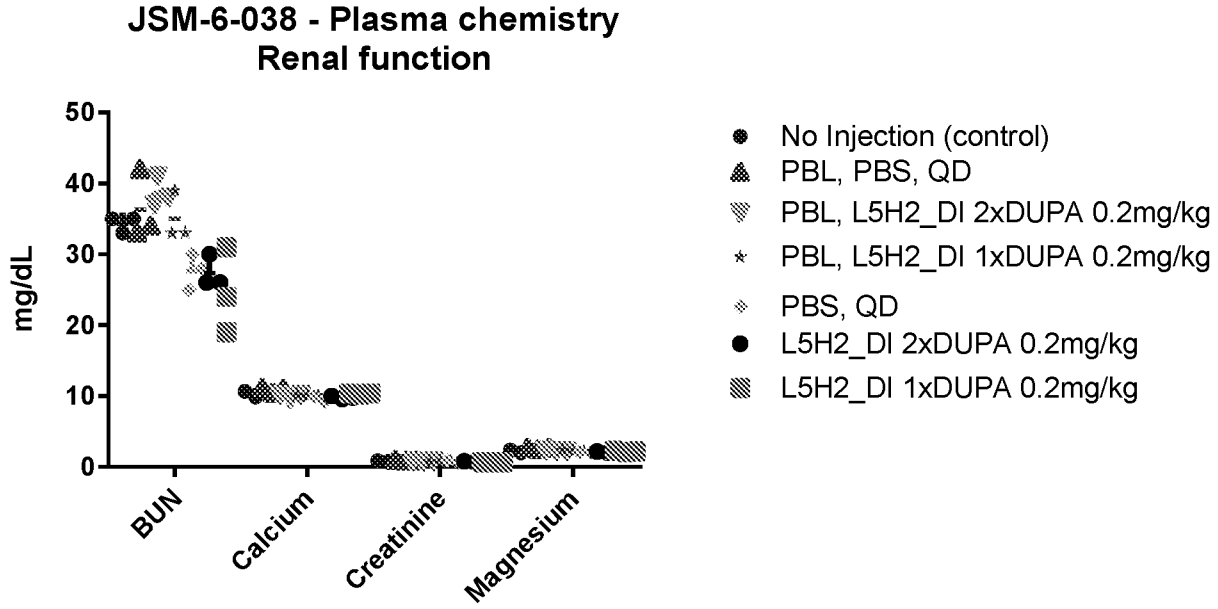


FIG. 45D

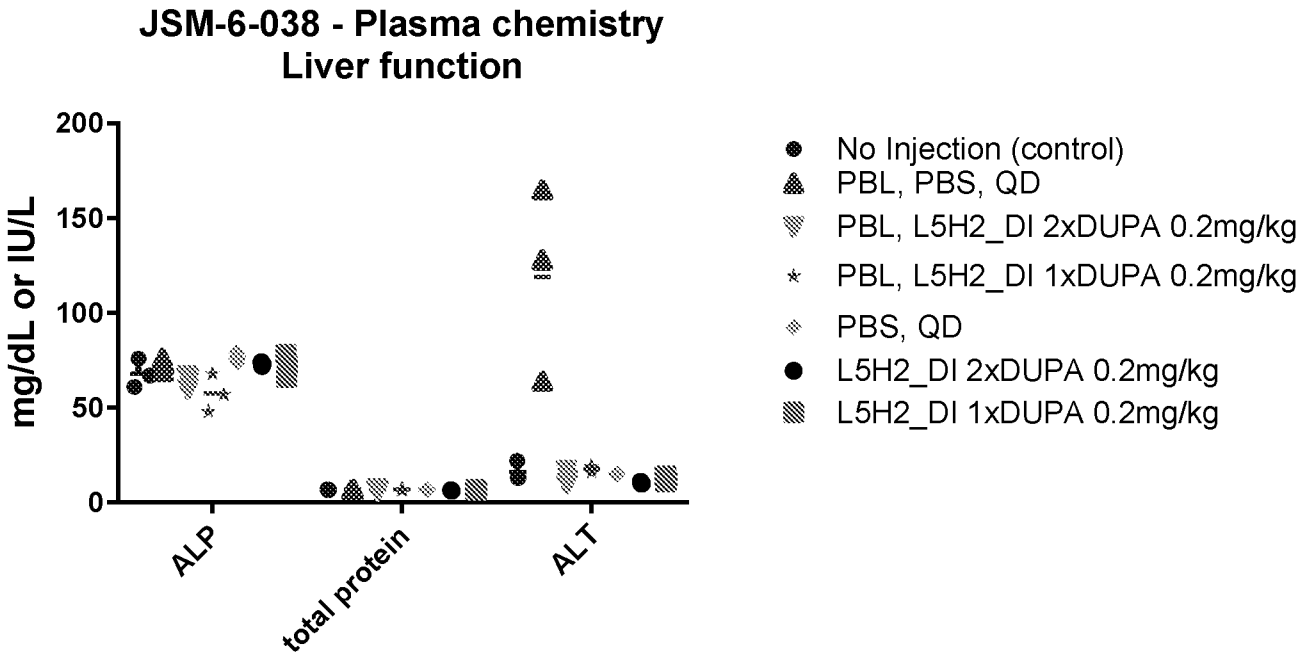


FIG. 45E

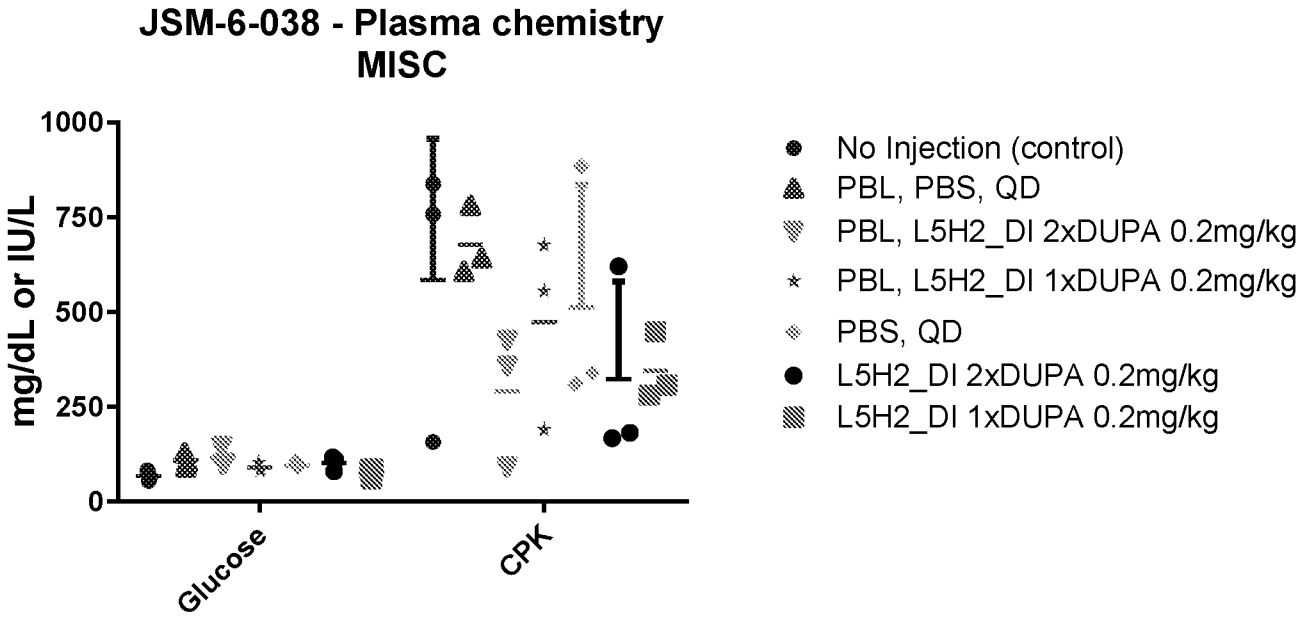
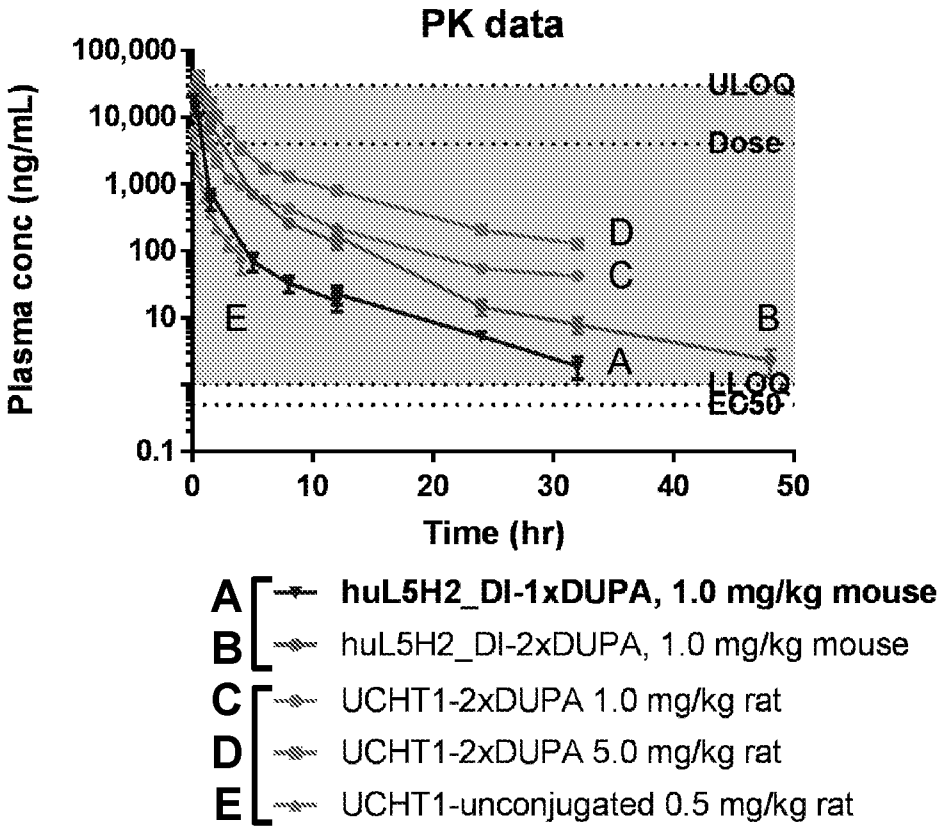
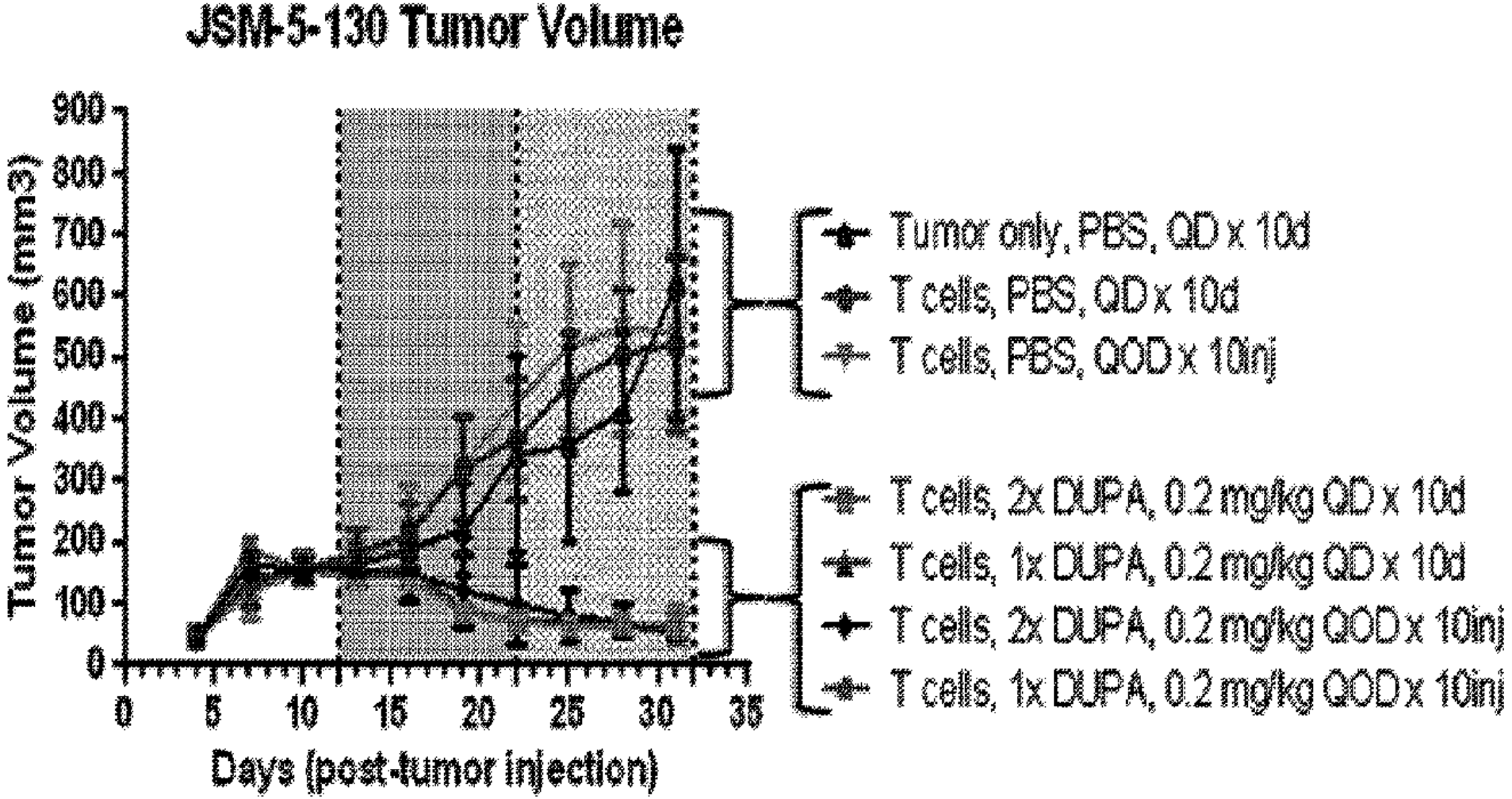


FIG. 46



Dose line: *In vivo* dosing in efficacy models at 0.2 mg/kg (approx. 20 ug/mL or 400 nM)
EC50 line: cytotoxicity (activated T cells) approx. 10 pM (0.5 ng/mL) (40 pM for PBMC)
ULOQ and LLOQ = upper & lower limits of quantification for current assay


JSM-5-130 Tumor Volume
Tumor Volume (mm3)
900
800
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
Days (post-tumor injection)
Tumor only, PBS, QD x 10d
T cells, PBS, QD x 10d
T cells, PBS, QOD x 10inj
T cells, 2x DUPA, 0.2 mg/kg QD x 10d
T cells, 1x DUPA, 0.2 mg/kg QD x 10d
T cells, 2x DUPA, 0.2 mg/kg QOD x 10inj
T cells, 1x DUPA, 0.2 mg/kg QOD x 10inj