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(54) Titre : ANTICORPS ANTI-STEAP1 ET LEURS UTILISATIONS

(54) Title: ANTI-STEAP1 ANTIBODIES AND USES THEREOF

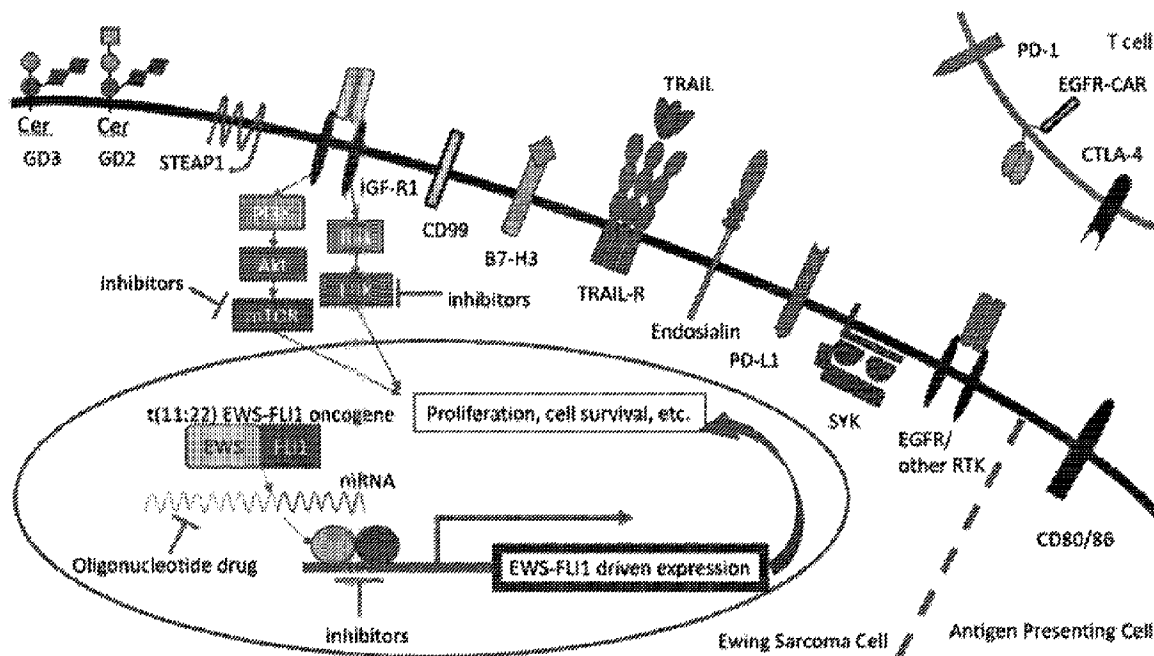


FIG. 1A

(57) **Abrégé/Abstract:**

The present technology relates generally to the preparation of immunoglobulin-related compositions (e.g., antibodies or antigen binding fragments thereof) that specifically bind STEAP1 protein and uses of the same. In particular, the present technology relates to the preparation of STEAP1 binding antibodies and their use in detecting and treating STEAP1 -associated cancers.

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Abstract:

The present technology relates generally to the preparation of immunoglobulin-related compositions (e.g., antibodies or antigen binding fragments thereof) that specifically bind STEAP1 protein and uses of the same. In particular, the present technology relates to the preparation of STEAP1 binding antibodies and their use in detecting and treating STEAP1 -associated cancers.

ANTI-STEAP1 ANTIBODIES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 62/896,415, filed September 5, 2019, the entire contents of which are incorporated herein by reference.

TECHNICAL FIELD

[0002] The present technology relates generally to the preparation of immunoglobulin-related compositions (*e.g.*, antibodies or antigen binding fragments thereof) that specifically bind STEAP1 protein and uses of the same. In particular, the present technology relates to the preparation of STEAP1 binding antibodies and their use in detecting and treating STEAP1-associated cancers.

BACKGROUND

[0003] The following description of the background of the present technology is provided simply as an aid in understanding the present technology and is not admitted to describe or constitute prior art to the present technology.

[0004] Ewing family of tumors (EFT) is a family of small round blue cell tumors that arise from bone or soft tissue. It represents the second most common malignant bone tumor in children and young adults, with an incidence of approximately 200 cases per year in the United States. Esiashvili *et al.*, *J Pediatr Hematol Oncol.* 30(6): 425-30 (2008). EFT is characterized by a specific translocation involving the EWS (Ewing's sarcoma gene) on chromosome 22 with one of the E26 transformation-specific transcription factor family genes. The EWS-FLI1 (Friend Leukemia Integration 1 transcription factor) fusion gene, t(11;22)(q24;q12) is found in approximately 85% of EFT tumors and plays a key role in the pathogenesis of EFT. Arvand and Denny, *Oncogene* 20(40): 5747-54 (2001); and May *et al.*, *Proc Natl Acad Sci U S A* 90(12): 5752-6 (1993).

SUMMARY OF THE PRESENT TECHNOLOGY

[0005] In one aspect, the present disclosure provides an antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light

chain immunoglobulin variable domain (V_L), wherein: (a) the V_H comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11; and/or (b) the V_L comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

[0006] In any of the above embodiments, the antibody may further comprise an Fc domain of an isotype selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, IgD, and IgE. In some embodiments, the antibody comprises an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A and K322A. Additionally or alternatively, in some embodiments, the antibody comprises an IgG4 constant region comprising a S228P mutation. In certain embodiments, the antigen binding fragment is selected from the group consisting of Fab, F(ab')₂, Fab', scFv, and Fv. In some embodiments, the antibody is a monoclonal antibody, chimeric antibody, humanized antibody, or a bispecific antibody. In certain embodiments, the antibody or antigen binding fragment binds to a STEAP1 polypeptide comprising amino acids 185 to 216 of any of SEQ ID NOs: 41, 42, or 60 (*e.g.*, second extra cellular domain of a STEAP1 polypeptide).

[0007] In another aspect, the present disclosure provides an antibody comprising a heavy chain (HC) amino acid sequence comprising SEQ ID NO: 22, SEQ ID NO: 26, or a variant thereof having one or more conservative amino acid substitutions, and/or a light chain (LC) amino acid sequence comprising SEQ ID NO: 21, SEQ ID NO: 24, SEQ ID NO: 27, SEQ ID NO: 28, or a variant thereof having one or more conservative amino acid substitutions.

[0008] In certain embodiments, the antibody comprises a HC amino acid sequence and a LC amino acid sequence selected from the group consisting of: SEQ ID NO: 22 and SEQ ID NO: 21; SEQ ID NO: 22 and SEQ ID NO: 24; SEQ ID NO: 22 and SEQ ID NO: 27; SEQ ID NO: 22 and SEQ ID NO: 28; SEQ ID NO: 26 and SEQ ID NO: 21; SEQ ID NO: 26 and SEQ ID NO: 24; SEQ ID NO: 26 and SEQ ID NO: 27; and SEQ ID NO: 26 and SEQ ID NO: 28, respectively.

[0009] In one aspect, the present disclosure provides an antibody comprising (a) a light chain immunoglobulin variable domain sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the light chain immunoglobulin variable

domain sequence of any one of SEQ ID NOs: 17, 18, 19, or 20; and/or (b) a heavy chain immunoglobulin variable domain sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the heavy chain immunoglobulin variable domain sequence of any one of SEQ ID NOs: 6, 7, 8, 9, 10, or 11.

[0010] In another aspect, the present disclosure provides an antibody comprising (a) a LC sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the LC sequence present in any one of SEQ ID NO: 21, SEQ ID NO: 24, SEQ ID NO: 27, or SEQ ID NO: 28; and/or (b) a HC sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the HC sequence present in SEQ ID NO: 22 or SEQ ID NO: 26.

[0011] In any of the above embodiments, the antibody is a chimeric antibody, a humanized antibody, or a bispecific antibody. Additionally or alternatively, in some embodiments, the antibody comprises an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A and K322A. In certain embodiments, the antibody of the present technology comprises an IgG4 constant region comprising a S228P mutation. In any of the above embodiments, the antibody binds to a STEAP1 polypeptide comprising amino acids 185 to 216 of any of SEQ ID NOs: 41, 42, or 60 (*e.g.*, second extra cellular domain of a STEAP1 polypeptide). Additionally or alternatively, in some embodiments, the antibody of the present technology lacks α -1,6-fucose modifications.

[0012] Additionally or alternatively, in certain embodiments, the bispecific antibody (or antigen binding fragment thereof) comprises an additional V_H and/or V_L comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, and SEQ ID NO: 79. In some embodiments, the bispecific antibody (or antigen binding fragment thereof) comprises an additional V_H sequence and an additional V_L sequence comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 76 and SEQ ID NO: 77, and SEQ ID NO: 78, and SEQ ID NO: 79.

[0013] In one aspect, the present disclosure provides a bispecific antibody or antigen binding fragment comprising an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the an amino acid sequence selected from any one of SEQ ID NOs: 29-40 or 61-64. In certain embodiments, the bispecific antibody or

antigen binding fragment comprises an amino acid sequence selected from any one of SEQ ID NOs: 29-40 or 61-64.

[0014] In one aspect, the present disclosure provides a bispecific antigen binding fragment comprising a first polypeptide chain, wherein: the first polypeptide chain comprises in the N-terminal to C-terminal direction: (i) a heavy chain variable domain of a first immunoglobulin that is capable of specifically binding to a first epitope; (ii) a flexible peptide linker comprising the amino acid sequence (GGGGS)₆; (iii) a light chain variable domain of the first immunoglobulin; (iv) a flexible peptide linker comprising the amino acid sequence (GGGGS)₄; (v) a heavy chain variable domain of a second immunoglobulin that is capable of specifically binding to a second epitope; (vi) a flexible peptide linker comprising the amino acid sequence (GGGGS)₆; (vii) a light chain variable domain of the second immunoglobulin; (viii) a flexible peptide linker sequence comprising the amino acid sequence TPLGDTTHT; and (ix) a self-assembly disassembly (SADA) polypeptide, wherein the heavy chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and/or the light chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20.

[0015] In another aspect, the present disclosure provides a bispecific antigen binding fragment comprising a first polypeptide chain, wherein: the first polypeptide chain comprises in the N-terminal to C-terminal direction: (i) a light chain variable domain of a first immunoglobulin that is capable of specifically binding to a first epitope; (ii) a flexible peptide linker comprising the amino acid sequence (GGGGS)₆; (iii) a heavy chain variable domain of the first immunoglobulin; (iv) a flexible peptide linker comprising the amino acid sequence (GGGGS)₄; (v) a heavy chain variable domain of a second immunoglobulin that is capable of specifically binding to a second epitope; (vi) a flexible peptide linker comprising the amino acid sequence (GGGGS)₆; (vii) a light chain variable domain of the second immunoglobulin; (viii) a flexible peptide linker sequence comprising the amino acid sequence TPLGDTTHT; and (ix) a self-assembly disassembly (SADA) polypeptide, wherein the heavy chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and/or

the light chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20.

[0016] In certain embodiments of the bispecific antigen binding fragments disclosed herein, the SADA polypeptide comprises a tetramerization, pentamerization, or hexamerization domain. In some embodiments, the SADA polypeptide comprises a tetramerization domain of any one of p53, p63, p73, hnRNPC, SNA-23, Stefin B, KCNQ4, and CBFA2T1. Additionally or alternatively, in some embodiments, the bispecific antigen binding fragment comprises an amino acid sequence selected from SEQ ID NOs: 29-40 or 61-64.

[0017] In one aspect, the present disclosure provides a bispecific antibody comprising a first polypeptide chain, a second polypeptide chain, a third polypeptide chain and a fourth polypeptide chain, wherein the first and second polypeptide chains are covalently bonded to one another, the second and third polypeptide chains are covalently bonded to one another, and the third and fourth polypeptide chain are covalently bonded to one another, and wherein: (a) each of the first polypeptide chain and the fourth polypeptide chain comprises in the N-terminal to C-terminal direction: (i) a light chain variable domain of a first immunoglobulin that is capable of specifically binding to a first epitope; (ii) a light chain constant domain of the first immunoglobulin; (iii) a flexible peptide linker comprising the amino acid sequence (GGGGS)₃; and (iv) a light chain variable domain of a second immunoglobulin that is linked to a complementary heavy chain variable domain of the second immunoglobulin, or a heavy chain variable domain of a second immunoglobulin that is linked to a complementary light chain variable domain of the second immunoglobulin, wherein the light chain and heavy chain variable domains of the second immunoglobulin are capable of specifically binding to a second epitope, and are linked together via a flexible peptide linker comprising the amino acid sequence (GGGGS)₆ to form a single-chain variable fragment; and (b) each of the second polypeptide chain and the third polypeptide chain comprises in the N-terminal to C-terminal direction: (i) a heavy chain variable domain of the first immunoglobulin that is capable of specifically binding to the first epitope; and (ii) a heavy chain constant domain of the first immunoglobulin; and wherein the heavy chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and/or the light chain

variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In certain embodiments, the second immunoglobulin binds to CD3, CD4, CD8, CD20, CD19, CD21, CD23, CD46, CD80, HLA-DR, CD74, CD22, CD14, CD15, CD16, CD123, TCR gamma/delta, NKp46, KIR, or a small molecule DOTA hapten.

[0018] In one aspect, the present disclosure provides a recombinant nucleic acid sequence encoding any of the antibodies or antigen binding fragments described herein. In some embodiments, the recombinant nucleic acid sequence is selected from the group consisting of: SEQ ID NOs: 23 and 25.

[0019] In another aspect, the present disclosure provides a host cell or vector comprising any of the recombinant nucleic acid sequences disclosed herein.

[0020] In one aspect, the present disclosure provides a composition comprising an antibody or antigen binding fragment of the present technology and a pharmaceutically-acceptable carrier, wherein the antibody or antigen binding fragment is optionally conjugated to an agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof.

[0021] In some embodiments of the bispecific antibody or antigen binding fragment of the present technology, the bispecific antibody binds to T cells, B-cells, myeloid cells, plasma cells, or mast-cells. Additionally or alternatively, in some embodiments, the bispecific antibody or antigen binding fragment binds to CD3, CD4, CD8, CD20, CD19, CD21, CD23, CD46, CD80, HLA-DR, CD74, CD22, CD14, CD15, CD16, CD123, TCR gamma/delta, NKp46, KIR, or a small molecule DOTA hapten. The small molecule DOTA hapten may be selected from the group consisting of DOTA, DOTA-Bn, DOTA-desferrioxamine, DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH₂, Ac-Lys(HSG)-D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH₂, DOTA-D-Asp-D-Lys(HSG)-D-Asp-D-Lys(HSG)-NH₂, DOTA-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂, DOTA-D-Tyr-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂, DOTA-D-Ala-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂, DOTA-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-NH₂, Ac-D-Phe-D-Lys(DOTA)-D-Tyr-D-Lys(DOTA)-NH₂, Ac-D-Phe-D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-NH₂, Ac-D-Phe-D-Lys(Bz-DTPA)-D-Tyr-D-Lys(Bz-

DTPA)-NH₂, Ac-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(Tscg-Cys)-NH₂, DOTA-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(Tscg-Cys)-NH₂, (Tscg-Cys)-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(DOTA)-NH₂, Tscg-D-Cys-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂, (Tscg-Cys)-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂, Ac-D-Cys-D-Lys(DOTA)-D-Tyr-D-Ala-D-Lys(DOTA)-D-Cys-NH₂, Ac-D-Cys-D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-NH₂, Ac-D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-D-Lys(Tscg-Cys)-NH₂, and Ac-D-Lys(DOTA)-D-Tyr-D-Lys(DOTA)-D-Lys(Tscg-Cys)-NH₂.

[0022] In another aspect, the present disclosure provides a method for treating a STEAP1-associated cancer in a subject in need thereof, comprising administering to the subject an effective amount of any one of the antibodies or antigen binding fragments disclosed herein. In certain embodiments, the antibody comprises a HC amino acid sequence and a LC amino acid sequence selected from the group consisting of: SEQ ID NO: 22 and SEQ ID NO: 21; SEQ ID NO: 22 and SEQ ID NO: 24; SEQ ID NO: 22 and SEQ ID NO: 27; SEQ ID NO: 22 and SEQ ID NO: 28; SEQ ID NO: 26 and SEQ ID NO: 21; SEQ ID NO: 26 and SEQ ID NO: 24; SEQ ID NO: 26 and SEQ ID NO: 27; and SEQ ID NO: 26 and SEQ ID NO: 28, respectively, wherein the antibody specifically binds to STEAP1. In some embodiments, the antibody or antigen binding fragment comprises an amino acid sequence selected from any one of SEQ ID NOs. 29-40 or 61-64.

[0023] In some embodiments, the STEAP1-associated cancer is Ewing's sarcoma (ES), prostate cancer, osteosarcoma, bladder cancer, breast cancer, ovary cancer, colon cancer, lung cancer, or kidney cancer.

[0024] Additionally or alternatively, in some embodiments of the method, the antibody or antigen binding fragment is administered to the subject separately, sequentially or simultaneously with an additional therapeutic agent. Examples of additional therapeutic agents include one or more of alkylating agents, platinum agents, taxanes, vinca agents, anti-estrogen drugs, aromatase inhibitors, ovarian suppression agents, VEGF/VEGFR inhibitors, EGF/EGFR inhibitors, PARP inhibitors, cytostatic alkaloids, cytotoxic antibiotics, antimetabolites, endocrine/hormonal agents, bisphosphonate therapy agents.

[0025] In another aspect, the present disclosure provides a method for detecting a tumor in a subject *in vivo* comprising (a) administering to the subject an effective amount of an antibody or antigen binding fragment of the present technology, wherein the antibody or

antigen binding fragment is configured to localize to a tumor expressing STEAP1 and is labeled with a radioisotope; and (b) detecting the presence of a tumor in the subject by detecting radioactive levels emitted by the antibody or antigen binding fragment that are higher than a reference value. In some embodiments, the subject is diagnosed with or is suspected of having cancer. Radioactive levels emitted by the antibody or antigen binding fragment may be detected using positron emission tomography or single photon emission computed tomography.

[0026] Additionally or alternatively, in some embodiments, the method further comprises administering to the subject an effective amount of an immunoconjugate comprising an antibody or antigen binding fragment of the present technology conjugated to a radionuclide. In some embodiments, the radionuclide is an alpha particle-emitting isotope, a beta particle-emitting isotope, an Auger-emitter, or any combination thereof. Examples of beta particle-emitting isotopes include ^{86}Y , ^{90}Y , ^{89}Sr , ^{165}Dy , ^{186}Re , ^{188}Re , ^{177}Lu , and ^{67}Cu . In some embodiments of the method, nonspecific FcR-dependent binding in normal tissues is eliminated or reduced (*e.g.*, via N297A mutation in Fc region, which results in aglycosylation).

[0027] Also disclosed herein are kits for the detection and/or treatment of STEAP1-associated cancers, comprising at least one immunoglobulin-related composition of the present technology (*e.g.*, any antibody or antigen binding fragment described herein), or a functional variant (*e.g.*, substitutional variant) thereof and instructions for use. In certain embodiments, the immunoglobulin-related composition is coupled to one or more detectable labels. In one embodiment, the one or more detectable labels comprise a radioactive label, a fluorescent label, or a chromogenic label.

[0028] Additionally or alternatively, in some embodiments, the kit further comprises a secondary antibody that specifically binds to an anti-STEAP1 immunoglobulin-related composition described herein. In some embodiments, the secondary antibody is coupled to at least one detectable label selected from the group consisting of a radioactive label, a fluorescent label, or a chromogenic label.

[0029] In another aspect, the present disclosure provides a method for selecting a subject for pretargeted radioimmunotherapy comprising (a) administering to the subject an effective amount of a complex comprising a radiolabeled DOTA hapten and a bispecific antibody or

antigen binding fragment of the present technology that binds to the radiolabeled DOTA hapten and a STEAP1 antigen, wherein the complex is configured to localize to a tumor expressing the STEAP1 antigen recognized by the bispecific antibody or antigen binding fragment of the complex; (b) detecting radioactive levels emitted by the complex; and (c) selecting the subject for pretargeted radioimmunotherapy when the radioactive levels emitted by the complex are higher than a reference value.

[0030] In one aspect, the present disclosure provides a method for increasing tumor sensitivity to radiation therapy in a subject diagnosed with a STEAP1-associated cancer comprising administering to the subject an effective amount of a complex comprising a radiolabeled-DOTA hapten and a bispecific antibody or antigen binding fragment of the present technology that recognizes and binds to the radiolabeled-DOTA hapten and a STEAP1 target antigen, wherein the complex is configured to localize to a tumor expressing the STEAP1 target antigen recognized by the bispecific antibody or antigen binding fragment of the complex.

[0031] In another aspect, the present disclosure provides a method for treating cancer in a subject in need thereof comprising administering to the subject an effective amount of a complex comprising a radiolabeled-DOTA hapten and a bispecific antibody or antigen binding fragment of the present technology that recognizes and binds to the radiolabeled-DOTA hapten and a STEAP1 target antigen, wherein the complex is configured to localize to a tumor expressing the STEAP1 target antigen recognized by the bispecific antibody or antigen binding fragment of the complex.

[0032] In any of the above embodiments of the methods disclosed herein, the complex is administered intravenously, intramuscularly, intraarterially, intrathecally, intracapsularly, intraorbitally, intradermally, intraperitoneally, transtracheally, subcutaneously, intracerebroventricularly, orally, intratumorally, or intranasally. In some embodiments of the methods disclosed herein, the subject is human. Additionally or alternatively, in any of the above embodiments of the methods disclosed herein, the radiolabeled-DOTA hapten comprises ^{213}Bi , ^{211}At , ^{225}Ac , ^{152}Dy , ^{212}Bi , ^{223}Ra , ^{219}Rn , ^{215}Po , ^{211}Bi , ^{221}Fr , ^{217}At , ^{255}Fm , ^{86}Y , ^{90}Y , ^{89}Sr , ^{165}Dy , ^{186}Re , ^{188}Re , ^{177}Lu , ^{67}Cu , ^{111}In , ^{67}Ga , ^{51}Cr , ^{58}Co , $^{99\text{m}}\text{Tc}$, $^{103\text{m}}\text{Rh}$, $^{195\text{m}}\text{Pt}$, ^{119}Sb , ^{161}Ho , $^{189\text{m}}\text{Os}$, ^{192}Ir , ^{201}Tl , ^{203}Pb , ^{68}Ga , ^{227}Th , or ^{64}Cu , and optionally comprises an alpha particle-emitting isotope, a beta particle-emitting isotope, or an Auger-emitter.

[0033] In one aspect, the present disclosure provides a method for increasing tumor sensitivity to radiation therapy in a subject diagnosed with a STEAP1-associated cancer comprising (a) administering an effective amount of an anti-DOTA bispecific antibody or antigen binding fragment of the present technology to the subject, wherein the anti-DOTA bispecific antibody or antigen binding fragment is configured to localize to a tumor expressing a STEAP1 target antigen; and (b) administering an effective amount of a radiolabeled-DOTA hapten to the subject, wherein the radiolabeled-DOTA hapten is configured to bind to the anti-DOTA bispecific antibody or antigen binding fragment. In another aspect, the present disclosure provides a method for treating cancer in a subject in need thereof comprising (a) administering an effective amount of an anti-DOTA bispecific antibody or antigen binding fragment of the present technology to the subject, wherein the anti-DOTA bispecific antibody or antigen binding fragment is configured to localize to a tumor expressing a STEAP1 target antigen; and (b) administering an effective amount of a radiolabeled-DOTA hapten to the subject, wherein the radiolabeled-DOTA hapten is configured to bind to the anti-DOTA bispecific antibody or antigen binding fragment. In some embodiments, the methods of the present technology further comprise administering an effective amount of a clearing agent to the subject prior to administration of the radiolabeled-DOTA hapten.

[0034] Additionally or alternatively, in any of the above embodiments of the methods disclosed herein, the radiolabeled-DOTA hapten comprises ^{213}Bi , ^{211}At , ^{225}Ac , ^{152}Dy , ^{212}Bi , ^{223}Ra , ^{219}Rn , ^{215}Po , ^{211}Bi , ^{221}Fr , ^{217}At , ^{255}Fm , ^{86}Y , ^{90}Y , ^{89}Sr , ^{165}Dy , ^{186}Re , ^{188}Re , ^{177}Lu , ^{67}Cu , ^{111}In , ^{67}Ga , ^{51}Cr , ^{58}Co , $^{99\text{m}}\text{Tc}$, $^{103\text{m}}\text{Rh}$, $^{195\text{m}}\text{Pt}$, ^{119}Sb , ^{161}Ho , $^{189\text{m}}\text{Os}$, ^{192}Ir , ^{201}Tl , ^{203}Pb , ^{68}Ga , ^{227}Th , or ^{64}Cu , and optionally comprises an alpha particle-emitting isotope, a beta particle-emitting isotope, or an Auger-emitter. In any of the above embodiments of the methods disclosed herein, the subject is human.

[0035] In one aspect, the present disclosure provides an *ex vivo* armed T cell that is coated or complexed with an effective amount of an anti-STEAP1 multi-specific antibody of the present technology, wherein the anti-STEAP1 multi-specific antibody includes a CD3 binding domain comprising a heavy chain immunoglobulin variable domain (V_H) of SEQ ID NO: 80 and a light chain immunoglobulin variable domain (V_L) of SEQ ID NO: 81, wherein the anti-STEAP1 multi-specific antibody is an immunoglobulin comprising two heavy chains

and two light chains, wherein each of the light chains is fused to a single chain variable fragment (scFv). In some embodiments, at least one scFv of the anti-STEAP1 multi-specific antibody comprises the CD3 binding domain. Additionally or alternatively, in some embodiments, at least one scFv of the anti-STEAP1 multi-specific antibody comprises a DOTA binding domain. In certain embodiments, the DOTA binding domain comprises a V_H sequence and a V_L sequence comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 76 and SEQ ID NO: 77, and SEQ ID NO: 78, and SEQ ID NO: 79. Also disclosed herein are methods for treating a STEAP1-associated cancer in a subject in need thereof comprising administering to the subject an effective amount of the *ex vivo* armed T cell disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] FIG. 1A shows a diagrammatic representation of the EWS-FLI1 Pathway.

[0037] FIG. 1B shows a schematic showing the structure of modular IgG-scFv. CH1 through CH3 are constant domains of the heavy chain of a first antibody. CL is the constant domain of the light chain of the first antibody. The C-terminus of the CL is fused to a single chain Fv fragment (scFv) derived from a second antibody.

[0038] Fig. 1C shows the biochemical purity analysis of the BC261 BsAb of the present technology. Purified BsAbs were subjected to size-exclusion chromatography-high-performance liquid chromatography (SEC-HPLC). The anti-STEAP1-BsAb was passed through a size-exclusion column, and protein in the eluent was detected based on absorbance of ultraviolet light having a wavelength of 280 nm. Fractions were analyzed using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), which showed that the anti-STEAP1-BsAb was eluted in peak 3 at 15.722 minutes of the chromatogram. The peak at 25 minutes corresponds to a citrate buffer peak, or solvent peak.

[0039] FIG. 2A shows the flow cytometry profile of Ewing's sarcoma (ES) cell line immunostained with increasing concentrations of an anti-STEAP1-BsAb BC261. The binding of the anti-STEAP1-BsAb to target cells was assessed by flow cytometry. A control bispecific antibody, which did not bind to TC32 cells, was used as a negative control. These data demonstrate that the anti-STEAP1-BsAb specifically bound to STEAP1(+) Ewing's sarcoma cell lines TC32.

[0040] FIG. 2B shows the FACS staining of anti-STEAP1-BsAb BC261 to the indicated Ewing's sarcoma cell lines as assayed by flow cytometry. As shown in FIG. 2B, all Ewing's sarcoma cell lines, except SKNMC, exhibited significant binding.

[0041] FIGs. 3A-3K show the antibody dependent T cell mediated cytotoxicity (ADTC) of anti-STEAP1-BsAb BC261 on STEAP1(+) ES cells and prostate cancer cells, TC32 cells (FIG. 3A), TC71-Luc cells (FIG. 3B), SKES1 cells (FIG. 3C), A4573 cells (FIG. 3D), SKEAW cells (FIG. 3E), SKELP cells (FIG. 3F), SKERT cells, (FIG. 3G), SKNMC cells (FIG. 3H), LNCaP-AR (FIG. 3I), CWR22(FIG. 3J), and VCaP (FIG. 3K). The indicated cells were tested in a standard 4-hour ^{51}Cr release assays. Substantial killing of all ES cell lines and prostate cancer cell lines in the presence of anti-STEAP1-BsAb BC261 was observed compared to that observed when a control bispecific antibody (BC123, an anti-GPA33 $\times$ CD3 BsAb that does not bind TC32 cells) was present. EC_{50} of 3.6 pM (for TC32 cells, 0.0009 $\mu\text{g/mL}$) was observed and EC_{50} as low as 1.69 pM (for LNCaP-AR cells, 0.000345 $\mu\text{g/mL}$). The control bispecific antibody (BC123) did not kill Ewing's sarcoma cell lines.

[0042] FIG. 4A shows the initial staining of TC32 Ewing's sarcoma cells (STEAP1 positive) with the twenty-four humanized versions of the murine X120 antibody made by pairing 6 humanized V_H with 4 humanized V_L sequences. Chimeric, L1+H1, L2+H2 had consistently superior binding compared to the other clones. Clones with H3, H4, H5 and H6 had poor binding irrespective of whether L1, L2, L3, L4 was used.

[0043] FIG. 4B shows the binding avidity of the humanized IgG1 clones of the murine X120 antibody with TC32 Ewing's sarcoma cells, plus the human-mouse chimeric IgG. Following binding of the primary antibody, cells were washed in PBS with 2 mM EDTA from 1 to 10 times. After each wash cells were stained with the secondary PE-conjugated goat anti-human IgG antibody and washed once with PBS for flow cytometry. Mean fluorescence intensity (MFI) was normalized to time 1 and depicted in FIG. 4B. While chimeric antibody dropped to below 50% after first wash, clones L1+H1, L1+H2, L1+H5 and L2+H2 remained above 50% through wash #8 and therefore scored as slow k_{off} .

[0044] FIG. 4C shows the stability of the twenty-four humanized clones at 40°C over time, from time 0 to day 28. Aggregates formed in some clones leading to decrease in %

monomer content. Clones with %monomer >85% on day14, >80% on d21 and >75% on d28 were scored as stable.

[0045] FIGs. 5A-5E show the ADTC induced by increasing doses of the indicated four bispecific antibodies in STEAP1(+) TC32 cells as measured in standard 4-hour ⁵¹Cr release assays.

[0046] FIG. 6A shows the quantification of tumor volumes from mice harboring TC32 xenografts (Ewing's sarcoma xenograft model) treated with BC261 or BC120 (a HER2 × CD3 control) BsAbs and T cells compared with the tumor only control group. Group 1: tumor only. Group 2: treated with BC120 5μg/dose plus 20 million T cells/dose. Group 3: treated with BC261 50μg/dose plus 20 million T cells/dose. Group 4: treated with BC261 10μg/dose with 20 million T cells/dose. Group 5: treated with BC261 2μg/dose with 20 million T cells/dose. Units are μg/million T cells per injection.

[0047] FIG. 6B shows the quantification of tumor volumes from mice harboring TC32 xenografts treated with BC261 or BC120 (a HER2 × CD3 control) BsAbs and T cells. Top panel shows a longer duration time course and the lower panel shows a seven-week time course. Units are μg/million T cells per injection.

[0048] FIG. 6C shows the survival curve of mice harboring TC32 xenografts (Ewing's sarcoma xenograft model), which were treated with the indicated BsAbs. Units are μg/million T cells per injection.

[0049] FIG. 7A shows the quantification of tumor volumes from mice harboring TC32 xenografts (Ewing's sarcoma xenograft model), which were treated with the indicated BsAbs, and T cells. These data compare the efficacy of the anti-STEAP1-BsAbs (BC259, BC260, BC261, BC262) against human Ewing's sarcoma TC32 xenograft in mice. Group 1: treated with T cells only. Group 2: treated with BC123 (anti-GPA33 × CD3 control) 10μg/dose with 20 million T cells/dose. Group 3: treated with BC259 10μg/dose with 20 million T cells/dose. Group 4: treated with BC260 10μg/dose with 20 million T cells/dose. Group 5: treated with BC261 10μg/dose with 20 million T cells/dose. Group 6: treated with BC262 10μg/dose with 20 million T cells/dose. Group 7: treated with BC120 10μg/dose with 20 million T cells/dose. Group 8: tumor only control.

[0050] FIG. 7B shows the quantification of tumor volumes from mice harboring TC32 xenografts (Ewing's sarcoma xenograft model), which were treated with the indicated BsAbs and T cells. These data demonstrate the efficacy of the anti-STEAP1-BsAb BC261 against large tumors of human Ewing's sarcoma TC32 xenograft in mice. Group 8: tumor only control. Group 9: treated with BC261 10µg/dose with 20 million T cells/dose.

[0051] FIG. 8A shows the quantification of tumor volumes from mice harboring TC71 xenografts treated with BC261 or BC123 (anti-GPA33 × CD3 control) BsAbs and T cells. Group 1: treated with T cells only. Group 2: treated with BC123 (anti-GPA33 × CD3 control) 10µg/dose with 20 million T cells/dose. Group 3: treated with BC261 10µg/dose with 20 million T cells/dose. Group 4: treated with BC261 10µg/dose only.

[0052] FIG. 8B shows the quantification of tumor volumes from mice harboring SKES1 xenografts treated with BC261 or BC123 (anti-GPA33 × CD3 control) BsAbs and T cells. Group 1: treated with T cells only. Group 2: treated with BC123 (anti-GPA33 × CD3 control) 10µg/dose with 20 million T cells/dose. Group 3: treated with BC261 10µg/dose with 20 million T cells/dose. Group 4: treated with BC261 10µg/dose only.

[0053] FIG. 9A (top panel) shows a schematic representation of the structure and organization of STEAP1 protein. The membrane regions are represented by horizontal parallel lines. FIG. 9A (bottom panel) shows the differences in amino acid sequences between human, mouse and canine models in the extracellular domains of STEAP1 protein.

[0054] FIG. 9B (top panel) shows the expression levels of STEAP1 as measured by flow cytometry in HEK293 cells expressing human STEAP1 (STP1h), mouse STEAP1 (STP1m), mouse STEAP1 with human 2nd extracellular domain (ECD) (STP1mH2), and mouse STEAP1 with human 3rd ECD (STP1mH3). FIG. 9B (bottom panel) shows the binding parameters of the flow cytometry profiles shown in FIG. 9B (top panel).

[0055] FIG. 9C (top panel) shows the binding of BC261 BsAb to HEK293 cells expressing human STEAP1 (STP1h), mouse STEAP1 (STP1m), mouse STEAP1 with human 2nd ECD (STP1mH2), and mouse STEAP1 with human 3rd ECD (STP1mH3) as measured by flow cytometry. FIG. 9C (bottom panel) shows the binding parameters of the flow cytometry profiles shown in FIG. 9C (top panel).

[0056] FIG. 10A shows the amino acid sequences of the murine and humanized X120 heavy chain variable domains (SEQ ID NOs: 1, and 5-11, respectively). The Genentech humanized V_H sequence (SEQ ID NO: 5) was disclosed in US Patent No. 8,889,847. X120_VH-1 (SEQ ID NO: 6), X120_VH-2 (SEQ ID NO: 7), X120_VH-3 (SEQ ID NO: 8), X120_VH-4 (SEQ ID NO: 9), X120_VH-5 (SEQ ID NO: 10), and X120_VH-6 (SEQ ID NO: 11) were six variants of the humanized X120 heavy chain variable domain. V_H CDR1 (GYSITSD; SEQ ID NO: 2), V_H CDR2 (NSGS; SEQ ID NO: 3), and V_H CDR3 (ERNYDYDDYYYAMDY; SEQ ID NO: 4) are indicated using boldface, underlined font.

[0057] FIG. 10B shows the amino acid sequences of the murine and humanized X120 light chain variable domains (SEQ ID NOs: 12, and 16-20, respectively). The Genentech humanized V_L sequence (SEQ ID NO: 16) was disclosed in US Patent No. 8,889,847. X120_VL-1 (SEQ ID NO: 17), X120_VL-2 (SEQ ID NO: 18), X120_VL-3 (SEQ ID NO: 19), and X120_VL-4 (SEQ ID NO: 20) were four variants of the humanized X120 light chain variable domain. V_L CDR1 (KSSQSLLYRSNQKNYLA; SEQ ID NO: 13), V_L CDR2 (WASTRES; SEQ ID NO: 14), and V_L CDR3 (QQYYNYPRT; SEQ ID NO: 15) are indicated using boldface, underlined font.

[0058] FIGs. 11A and 11B show the amino acid sequences of the light chain (SEQ ID NO: 21) and heavy chain (SEQ ID NO: 22) of humanized anti-STEAP1 (VH-2/VL-2) antibody, respectively. The variable domains of the humanized anti-STEAP1 antibody are indicated in boldface font, and two mutations, N297A and K322A, introduced in the constant domain of the heavy chain sequence are shown by boldface, underlined font.

[0059] FIGs. 12A and 12B show the nucleotide and amino acid sequences of the light chain (SEQ ID NOs: 23-24) and heavy chain (SEQ ID NOs: 25-26) of BiClone261 (BC261) STEAP1-CD3 BsAb, respectively. The signal peptide is underlined, the variable domains of the bispecific anti-STEAP1 antibody are indicated in boldface font, and linker sequences are italicized and underlined.

[0060] FIGs. 13A and 13B show the amino acid sequences of the light chain (SEQ ID NOs: 27 and 28) comprising the X120_VL-2 humanized anti-STEAP1 light chain with an anti-DOTA scFv based on mouse C825 or humanized C825 antibody. These light chains may be combined with a heavy chains such as those disclosed in **FIGs. 11B** (SEQ ID NO: 22) or **12B** (SEQ ID NO: 26) to generate an anti-STEAP1-DOTA BsAb. The signal peptide is

underlined, the variable domains of the bispecific anti-STEAP1 antibody are indicated in boldface font, and linker sequences are italicized and underlined.

[0061] FIGs. 14A to 14P show the amino acid sequences of the humanized X120 x C825 (anti-DOTA) BsAbs of the single-chain bispecific tandem fragment variable (scBsTaFv) format (SEQ ID NOs: 29-40, and 61-64). The signal peptide is underlined, the variable domains of the humanized anti-STEAP1 antibody are indicated in boldface font, linker and spacer sequences are italicized and underlined, p53-, p63- or p73-tetramerization domains are thick-underlined and histidine₆ tags are indicated in italic fonts.

[0062] FIG. 15A shows the quantification of tumor volumes from mice harboring Prostate cancer patient derived xenografts (PDX: TM00298 from JAX lab) treated with BC261 or BC123 (anti-GPA33 × CD3 control) BsAbs and T cells. Group 1: treated with T cells only. Group 2: treated with BC123 (anti-GPA33 × CD3 control) 10μg/dose with 20 million T cells/dose. Group 3: treated with BC261 10μg/dose with 20 million T cells/dose.

[0063] FIG. 15B (top panel) shows the quantification of tumor volumes for treated with T cells only group and for treated with BC123 group, provided with average and individual mice. FIG. 15B (bottom panel) shows the quantification of tumor volumes for BC261 treated group in average and individual mice.

[0064] FIG. 15C shows the quantification of tumor volumes from DKO (BALB/cA-*Rag2^{tm1Fwa}/Il2rg^{tm1Sug}* (BRG)) mice harboring Prostate cancer patient derived xenografts (PDX: TM00298 from JAX lab) treated with BC261 or BC123 (anti-GPA33 × CD3 negative control) BsAbs and T cells. Group 1: treated with T cells only. Group 2: treated with BC123 (control BsAb) 10μg/dose with 20 million T cells/dose. Group 3: treated with BC261 10μg/dose with 20 million T cells/dose. Group 4: no treatment. The survival curve were relevant because the tumor bearing mice were BRG mice. Diseases associated with IL2RG (Interleukin 2 Receptor Subunit Gamma) include Severe Combined Immunodeficiency, X-Linked and Combined Immunodeficiency, X-Linked. Among its related pathways are Common Cytokine Receptor Gamma-Chain Family Signaling Pathways and RET signaling. Gene Ontology (GO) annotations related to the IL2RG gene include cytokine receptor activity and interleukin-2 binding.

[0065] FIG. 16 shows the staining of canine osteosarcoma cell lines by anti-STEAP1 BsAb BC261. The canine cell lines, D-17 and DSN, exhibited significant binding of BC261, and DSDH and DAN were also positive for anti-STEAP1 BsAb staining. The FACS analysis results demonstrate canine osteosarcoma can be treated by anti-STEAP1 BsAb.

[0066] FIGs. 17A-17D show the antibody dependent T cell mediated cytotoxicity (ADTC) of anti-STEAP1-BsAb BC261 on STEAP1(+) canine osteosarcoma cell lines, specifically on D-17 (FIG. 17A), DSN (FIG. 17B), DSDh (FIG. 17C), and DAN cells (FIG. 17D). The indicated cells were tested in a standard 4-hour ⁵¹Cr release assays. Substantial killing in four canine osteosarcoma cell lines was detected, which was consistent with the observation that STEAP1-BsAb BC261 binds to canine STEAP1 as determined by FACS analysis (FIG. 16) and sequence alignment (FIG. 9). These results demonstrate that STEAP1-BsAbs are useful for treating osteosarcoma in canine subjects.

[0067] FIG. 18 demonstrates that BC261 showed picomolar range EC₅₀ against Ewing sarcoma, prostate cancer and dog osteosarcoma cell lines.

[0068] FIGs. 19A-19D show the amino acid sequences of the humanized X120 x OKT3 (anti-CD3) BsAbs in alternate formats (SEQ ID NOs: 65-75).

[0069] FIGs. 20A-20B show a quantitative summary of the binding affinities of the twenty-four humanized X120 variants of the present disclosure.

[0070] FIG. 21 shows the amino acid sequences of the V_H and V_L domains of the humanized C825 antibody (SEQ ID NOs: 76-77, respectively), murine C825 antibody (SEQ ID NOs: 78-79, respectively) and the OKT3 antibody (SEQ ID NOs: 80-81, respectively).

DETAILED DESCRIPTION

[0071] It is to be appreciated that certain aspects, modes, embodiments, variations and features of the present methods are described below in various levels of detail in order to provide a substantial understanding of the present technology.

[0072] The present disclosure generally provides immunoglobulin-related compositions (*e.g.*, antibodies or antigen binding fragments thereof), which can specifically bind to STEAP1 polypeptides. The immunoglobulin-related compositions of the present technology are useful in methods for detecting or treating STEAP1-associated cancers in a subject in

need thereof. Accordingly, the various aspects of the present methods relate to the preparation, characterization, and manipulation of anti-STEAP1 antibodies. The immunoglobulin-related compositions of the present technology are useful alone or in combination with additional therapeutic agents for treating cancer. In some embodiments, the immunoglobulin-related composition is a humanized antibody, a chimeric antibody, or a bispecific antibody.

[0073] In practicing the present methods, many conventional techniques in molecular biology, protein biochemistry, cell biology, immunology, microbiology and recombinant DNA are used. *See, e.g.*, Sambrook and Russell eds. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edition; the series Ausubel *et al.* eds. (2007) *Current Protocols in Molecular Biology*; the series *Methods in Enzymology* (Academic Press, Inc., N.Y.); MacPherson *et al.* (1991) *PCR 1: A Practical Approach* (IRL Press at Oxford University Press); MacPherson *et al.* (1995) *PCR 2: A Practical Approach*; Harlow and Lane eds. (1999) *Antibodies, A Laboratory Manual*; Freshney (2005) *Culture of Animal Cells: A Manual of Basic Technique*, 5th edition; Gait ed. (1984) *Oligonucleotide Synthesis*; U.S. Patent No. 4,683,195; Hames and Higgins eds. (1984) *Nucleic Acid Hybridization*; Anderson (1999) *Nucleic Acid Hybridization*; Hames and Higgins eds. (1984) *Transcription and Translation; Immobilized Cells and Enzymes* (IRL Press (1986)); Perbal (1984) *A Practical Guide to Molecular Cloning*; Miller and Calos eds. (1987) *Gene Transfer Vectors for Mammalian Cells* (Cold Spring Harbor Laboratory); Makrides ed. (2003) *Gene Transfer and Expression in Mammalian Cells*; Mayer and Walker eds. (1987) *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); and Herzenberg *et al.* eds (1996) *Weir's Handbook of Experimental Immunology*. Methods to detect and measure levels of polypeptide gene expression products (*i.e.*, gene translation level) are well-known in the art and include the use of polypeptide detection methods such as antibody detection and quantification techniques. (*See also*, Strachan & Read, *Human Molecular Genetics*, Second Edition. (John Wiley and Sons, Inc., NY, 1999)).

Definitions

[0074] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs. As used in this specification and the appended claims, the singular

forms “a”, “an” and “the” include plural referents unless the content clearly dictates otherwise. For example, reference to “a cell” includes a combination of two or more cells, and the like. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry, analytical chemistry and nucleic acid chemistry and hybridization described below are those well-known and commonly employed in the art.

[0075] As used herein, the term “about” in reference to a number is generally taken to include numbers that fall within a range of 1%, 5%, or 10% in either direction (greater than or less than) of the number unless otherwise stated or otherwise evident from the context (except where such number would be less than 0% or exceed 100% of a possible value).

[0076] As used herein, the “administration” of an agent or drug to a subject includes any route of introducing or delivering to a subject a compound to perform its intended function. Administration can be carried out by any suitable route, including but not limited to, orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, intrathecally, intratumorally or topically. Administration includes self-administration and the administration by another.

[0077] An “adjuvant” refers to one or more substances that cause stimulation of the immune system. In this context, an adjuvant is used to enhance an immune response to one or more vaccine antigens or antibodies. An adjuvant may be administered to a subject before, in combination with, or after administration of the vaccine. Examples of chemical compounds used as adjuvants include aluminum compounds, oils, block polymers, immune stimulating complexes, vitamins and minerals (*e.g.*, vitamin E, vitamin A, selenium, and vitamin B12), Quil A (saponins), bacterial and fungal cell wall components (*e.g.*, lipopolysaccharides, lipoproteins, and glycoproteins), hormones, cytokines, and co-stimulatory factors.

[0078] As used herein, the term “antibody” collectively refers to immunoglobulins or immunoglobulin-like molecules including by way of example and without limitation, IgA, IgD, IgE, IgG and IgM, combinations thereof, and similar molecules produced during an immune response in any vertebrate, for example, in mammals such as humans, goats, rabbits and mice, as well as non-mammalian species, such as shark immunoglobulins. As used

herein, “antibodies” (includes intact immunoglobulins) and “antigen binding fragments” specifically bind to a molecule of interest (or a group of highly similar molecules of interest) to the substantial exclusion of binding to other molecules (for example, antibodies and antibody fragments that have a binding constant for the molecule of interest that is at least 10^3 M^{-1} greater, at least 10^4 M^{-1} greater or at least 10^5 M^{-1} greater than a binding constant for other molecules in a biological sample). The term “antibody” also includes genetically engineered forms such as chimeric antibodies (for example, humanized murine antibodies), heteroconjugate antibodies (such as, bispecific antibodies). See also, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, Ill.); Kuby, J., *Immunology*, 3rd Ed., W.H. Freeman & Co., New York, 1997.

[0079] More particularly, antibody refers to a polypeptide ligand comprising at least a light chain immunoglobulin variable region or heavy chain immunoglobulin variable region which specifically recognizes and binds an epitope of an antigen. Antibodies are composed of a heavy and a light chain, each of which has a variable region, termed the variable heavy (V_H) region and the variable light (V_L) region. Together, the V_H region and the V_L region are responsible for binding the antigen recognized by the antibody. Typically, an immunoglobulin has heavy (H) chains and light (L) chains interconnected by disulfide bonds. There are two types of light chain, lambda (λ) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each heavy and light chain contains a constant region and a variable region, (the regions are also known as “domains”). In combination, the heavy and the light chain variable regions specifically bind the antigen. Light and heavy chain variable regions contain a “framework” region interrupted by three hypervariable regions, also called “complementarity-determining regions” or “CDRs”. The extent of the framework region and CDRs have been defined (see, Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, 1991, which is hereby incorporated by reference). The Kabat database is now maintained online. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, largely adopt a β -sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β -sheet structure. Thus, framework regions act

to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions.

[0080] The CDRs are primarily responsible for binding to an epitope of an antigen. The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus, and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is located in the variable domain of the heavy chain of the antibody in which it is found, whereas a V_L CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found. An antibody that binds STEAP1 protein will have a specific V_H region and the V_L region sequence, and thus specific CDR sequences. Antibodies with different specificities (*i.e.* different combining sites for different antigens) have different CDRs. Although it is the CDRs that vary from antibody to antibody, only a limited number of amino acid positions within the CDRs are directly involved in antigen binding. These positions within the CDRs are called specificity determining residues (SDRs). “Immunoglobulin-related compositions” as used herein, refers to antibodies (including monoclonal antibodies, polyclonal antibodies, humanized antibodies, chimeric antibodies, recombinant antibodies, multispecific antibodies, bispecific antibodies, *etc.*) as well as antibody fragments. An antibody or antigen binding fragment thereof specifically binds to an antigen.

[0081] As used herein, the term “antibody-related polypeptide” means antigen-binding antibody fragments, including single-chain antibodies, that can comprise the variable region(s) alone, or in combination, with all or part of the following polypeptide elements: hinge region, CH₁, CH₂, and CH₃ domains of an antibody molecule. Also included in the technology are any combinations of variable region(s) and hinge region, CH₁, CH₂, and CH₃ domains. Antibody-related molecules useful in the present methods, *e.g.*, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. Examples include: (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and CH₁ domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and CH₁ domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, *Nature* 341: 544-546, 1989), which consists of a V_H domain; and (vi)

an isolated complementarity determining region (CDR). As such “antibody fragments” or “antigen binding fragments” can comprise a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments or antigen binding fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0082] “Bispecific antibody” or “BsAb”, as used herein, refers to an antibody that can bind simultaneously to two targets that have a distinct structure, *e.g.*, two different target antigens, two different epitopes on the same target antigen, or a hapten and a target antigen or epitope on a target antigen. A variety of different bispecific antibody structures are known in the art. In some embodiments, each antigen binding moiety in a bispecific antibody includes V_H and/or V_L regions; in some such embodiments, the V_H and/or V_L regions are those found in a particular monoclonal antibody. In some embodiments, the bispecific antibody contains two antigen binding moieties, each including V_H and/or V_L regions from different monoclonal antibodies. In some embodiments, the bispecific antibody contains two antigen binding moieties, wherein one of the two antigen binding moieties includes an immunoglobulin molecule having V_H and/or V_L regions that contain CDRs from a first monoclonal antibody, and the other antigen binding moiety includes an antibody fragment (*e.g.*, Fab, F(ab'), F(ab')₂, Fd, Fv, dAB, scFv, *etc.*) having V_H and/or V_L regions that contain CDRs from a second monoclonal antibody.

[0083] As used herein, a “clearing agent” is an agent that binds to excess bispecific antibody that is present in the blood compartment of a subject to facilitate rapid clearance *via* kidneys. The use of the clearing agent prior to hapten administration (*e.g.*, DOTA) facilitates better tumor-to-background ratios in pretargeted radioimmunotherapy (PRIT) systems. Examples of clearing agents include 500 kD-dextran-DOTA-Bn(Y) (Orcutt *et al.*, *Mol Cancer Ther.* 11(6): 1365–1372 (2012)), 500 kD aminodextran-DOTA conjugate, antibodies against the pretargeting antibody, *etc.*

[0084] As used herein, the term “conjugated” refers to the association of two molecules by any method known to those in the art. Suitable types of associations include chemical bonds and physical bonds. Chemical bonds include, for example, covalent bonds and coordinate bonds. Physical bonds include, for instance, hydrogen bonds, dipolar interactions,

van der Waal forces, electrostatic interactions, hydrophobic interactions and aromatic stacking.

[0085] As used herein, the term “diabodies” refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain ($V_H V_L$). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen binding sites. Diabodies are described more fully in, *e.g.*, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90: 6444-6448 (1993).

[0086] As used herein, the terms “single-chain antibodies” or “single-chain Fv (scFv)” refer to an antibody fusion molecule of the two domains of the Fv fragment, V_L and V_H . Single-chain antibody molecules may comprise a polymer with a number of individual molecules, for example, dimer, trimer or other polymers. Furthermore, although the two domains of the Fv fragment, V_L and V_H , are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single-chain Fv (scFv)). Bird *et al.* (1988) *Science* 242:423-426 and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883. Such single-chain antibodies can be prepared by recombinant techniques or enzymatic or chemical cleavage of intact antibodies.

[0087] Any of the above-noted antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for binding specificity and neutralization activity in the same manner as are intact antibodies.

[0088] As used herein, an “antigen” refers to a molecule to which an antibody (or antigen binding fragment thereof) can selectively bind. The target antigen may be a protein, carbohydrate, nucleic acid, lipid, hapten, or other naturally occurring or synthetic compound. In some embodiments, the target antigen may be a polypeptide (*e.g.*, a STEAP1 polypeptide). An antigen may also be administered to an animal to generate an immune response in the animal.

[0089] The term “antigen binding fragment” refers to a fragment of the whole immunoglobulin structure which possesses a part of a polypeptide responsible for binding to

antigen. Examples of the antigen binding fragment useful in the present technology include scFv, (scFv)₂, scFvFc, Fab, Fab' and F(ab')₂, but are not limited thereto.

[0090] By “binding affinity” is meant the strength of the total noncovalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen or antigenic peptide). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured by standard methods known in the art, including those described herein. A low-affinity complex contains an antibody that generally tends to dissociate readily from the antigen, whereas a high-affinity complex contains an antibody that generally tends to remain bound to the antigen for a longer duration.

[0091] As used herein, the term “biological sample” means sample material derived from living cells. Biological samples may include tissues, cells, protein or membrane extracts of cells, and biological fluids (*e.g.*, ascites fluid or cerebrospinal fluid (CSF)) isolated from a subject, as well as tissues, cells and fluids present within a subject. Biological samples of the present technology include, but are not limited to, samples taken from breast tissue, renal tissue, the uterine cervix, the endometrium, the head or neck, the gallbladder, parotid tissue, the prostate, the brain, the pituitary gland, kidney tissue, muscle, the esophagus, the stomach, the small intestine, the colon, the liver, the spleen, the pancreas, thyroid tissue, heart tissue, lung tissue, the bladder, adipose tissue, lymph node tissue, the uterus, ovarian tissue, adrenal tissue, testis tissue, the tonsils, thymus, blood, hair, buccal, skin, serum, plasma, CSF, semen, prostate fluid, seminal fluid, urine, feces, sweat, saliva, sputum, mucus, bone marrow, lymph, and tears. Biological samples can also be obtained from biopsies of internal organs or from cancers. Biological samples can be obtained from subjects for diagnosis or research or can be obtained from non-diseased individuals, as controls or for basic research. Samples may be obtained by standard methods including, *e.g.*, venous puncture and surgical biopsy. In certain embodiments, the biological sample is a tissue sample obtained by needle biopsy.

[0092] As used herein, the term “CDR-grafted antibody” means an antibody in which at least one CDR of an “acceptor” antibody is replaced by a CDR “graft” from a “donor” antibody possessing a desirable antigen specificity.

[0093] As used herein, the term “chimeric antibody” means an antibody in which the Fc constant region of a monoclonal antibody from one species (*e.g.*, a mouse Fc constant region) is replaced, using recombinant DNA techniques, with an Fc constant region from an antibody of another species (*e.g.*, a human Fc constant region). *See generally*, Robinson *et al.*, PCT/US86/02269; Akira *et al.*, European Patent Application 184,187; Taniguchi, European Patent Application 171,496; Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 0125,023; Better *et al.*, *Science* 240: 1041-1043, 1988; Liu *et al.*, *Proc. Natl. Acad. Sci. USA* 84: 3439-3443, 1987; Liu *et al.*, *J. Immunol* 139: 3521-3526, 1987; Sun *et al.*, *Proc. Natl. Acad. Sci. USA* 84: 214-218, 1987; Nishimura *et al.*, *Cancer Res* 47: 999-1005, 1987; Wood *et al.*, *Nature* 314: 446-449, 1985; and Shaw *et al.*, *J. Natl. Cancer Inst.* 80: 1553-1559, 1988.

[0094] As used herein, the term “consensus FR” means a framework (FR) antibody region in a consensus immunoglobulin sequence. The FR regions of an antibody do not contact the antigen.

[0095] As used herein, a “control” is an alternative sample used in an experiment for comparison purpose. A control can be “positive” or “negative.” For example, where the purpose of the experiment is to determine a correlation of the efficacy of a therapeutic agent for the treatment for a particular type of disease, a positive control (a compound or composition known to exhibit the desired therapeutic effect) and a negative control (a subject or a sample that does not receive the therapy or receives a placebo) are typically employed.

[0096] As used herein, the term “effective amount” refers to a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, *e.g.*, an amount which results in the prevention of, or a decrease in a disease or condition described herein or one or more signs or symptoms associated with a disease or condition described herein. In the context of therapeutic or prophylactic applications, the amount of a composition administered to the subject will vary depending on the composition, the degree, type, and severity of the disease and on the characteristics of the individual, such as general health, age, sex, body weight and tolerance to drugs. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. The compositions can also be administered in combination with one or more additional therapeutic compounds. In the methods described

herein, the therapeutic compositions may be administered to a subject having one or more signs or symptoms of a disease or condition described herein. As used herein, a "therapeutically effective amount" of a composition refers to composition levels in which the physiological effects of a disease or condition are ameliorated or eliminated. A therapeutically effective amount can be given in one or more administrations.

[0097] As used herein, the term "effector cell" means an immune cell which is involved in the effector phase of an immune response, as opposed to the cognitive and activation phases of an immune response. Exemplary immune cells include a cell of a myeloid or lymphoid origin, *e.g.*, lymphocytes (*e.g.*, B cells and T cells including cytolytic T cells (CTLs)), killer cells, natural killer cells, macrophages, monocytes, eosinophils, neutrophils, polymorphonuclear cells, granulocytes, mast cells, and basophils. Effector cells express specific Fc receptors and carry out specific immune functions. An effector cell can induce antibody-dependent cell-mediated cytotoxicity (ADCC), *e.g.*, a neutrophil capable of inducing ADCC. For example, monocytes, macrophages, neutrophils, eosinophils, and lymphocytes which express Fc α R are involved in specific killing of target cells and presenting antigens to other components of the immune system, or binding to cells that present antigens.

[0098] As used herein, the term "epitope" means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents. In some embodiments, an "epitope" of the STEAP1 protein is a region of the protein to which the anti-STEAP1 antibodies of the present technology specifically bind. In some embodiments, the epitope is a conformational epitope or a non-conformational epitope. To screen for anti-STEAP1 antibodies which bind to an epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. This assay can be used to determine if an anti-STEAP1 antibody binds the same site or epitope as an anti-STEAP1 antibody of the present technology. Alternatively, or additionally, epitope mapping can be performed by methods

known in the art. For example, the antibody sequence can be mutagenized such as by alanine scanning, to identify contact residues. In a different method, peptides corresponding to different regions of STEAP1 protein can be used in competition assays with the test antibodies or with a test antibody and an antibody with a characterized or known epitope.

[0099] As used herein, “expression” includes one or more of the following: transcription of the gene into precursor mRNA; splicing and other processing of the precursor mRNA to produce mature mRNA; mRNA stability; translation of the mature mRNA into protein (including codon usage and tRNA availability); and glycosylation and/or other modifications of the translation product, if required for proper expression and function.

[00100] As used herein, the term “gene” means a segment of DNA that contains all the information for the regulated biosynthesis of an RNA product, including promoters, exons, introns, and other untranslated regions that control expression.

[00101] “Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) of “sequence identity” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art. In some embodiments, default parameters are used for alignment. One alignment program is BLAST, using default parameters. In particular, programs are BLASTN and BLASTP, using the following default parameters: Genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by =HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+SwissProtein+SPupdate+PIR. Details of these programs can be found at the National Center for Biotechnology Information. Biologically equivalent polynucleotides are those having the specified percent homology and encoding a polypeptide having the same

or similar biological activity. Two sequences are deemed “unrelated” or “non-homologous” if they share less than 40% identity, or less than 25% identity, with each other.

[00102] As used herein, “humanized” forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some embodiments, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance such as binding affinity. Generally, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains (*e.g.*, Fab, Fab', F(ab')₂, or Fv), in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus FR sequence although the FR regions may include one or more amino acid substitutions that improve binding affinity. The number of these amino acid substitutions in the FR are typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally may also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature* 321:522-525 (1986); Reichmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See *e.g.*, Ahmed & Cheung, *FEBS Letters* 588(2):288-297 (2014).

[00103] As used herein, the term “hypervariable region” refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a “complementarity determining region” or “CDR” (*e.g.*, around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and around about 31-35B (H1), 50-65 (H2) and 95-102 (H3) in the V_H (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a “hypervariable loop” (*e.g.*, residues 26-

32 (L1), 50-52 (L2) and 91-96 (L3) in the V_L, and 26-32 (H1), 52A-55 (H2) and 96-101 (H3) in the V_H (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)).

[00104] As used herein, the terms “identical” or percent “identity”, when used in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region (*e.g.*, nucleotide sequence encoding an antibody described herein or amino acid sequence of an antibody described herein)), when compared and aligned for maximum correspondence over a comparison window or designated region as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (*e.g.*, NCBI web site). Such sequences are then said to be “substantially identical.” This term also refers to, or can be applied to, the complement of a test sequence. The term also includes sequences that have deletions and/or additions, as well as those that have substitutions. In some embodiments, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or 50-100 amino acids or nucleotides in length.

[00105] As used herein, the term “intact antibody” or “intact immunoglobulin” means an antibody that has at least two heavy (H) chain polypeptides and two light (L) chain polypeptides interconnected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH₁, CH₂ and CH₃. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR₁, CDR₁, FR₂, CDR₂, FR₃, CDR₃, FR₄. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies can mediate the binding of the immunoglobulin to host tissues or

factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

[00106] As used herein, the terms “individual”, “patient”, or “subject” can be an individual organism, a vertebrate, a mammal, or a human. In some embodiments, the individual, patient or subject is a human.

[00107] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. For example, a monoclonal antibody can be an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including, *e.g.*, but not limited to, hybridoma, recombinant, and phage display technologies. For example, the monoclonal antibodies to be used in accordance with the present methods may be made by the hybridoma method first described by Kohler *et al.*, *Nature* 256:495 (1975), or may be made by recombinant DNA methods (*See, e.g.*, U.S. Patent No. 4,816,567). The “monoclonal antibodies” may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), for example.

[00108] As used herein, the term “pharmaceutically-acceptable carrier” is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal compounds, isotonic and absorption delaying compounds, and the like, compatible with pharmaceutical administration. Pharmaceutically-acceptable carriers and their formulations are known to one skilled in the art and are described, for example, in Remington's

Pharmaceutical Sciences (20th edition, ed. A. Gennaro, 2000, Lippincott, Williams & Wilkins, Philadelphia, Pa.).

[00109] As used herein, the term “polyclonal antibody” means a preparation of antibodies derived from at least two (2) different antibody-producing cell lines. The use of this term includes preparations of at least two (2) antibodies that contain antibodies that specifically bind to different epitopes or regions of an antigen.

[00110] As used herein, the term “polynucleotide” or “nucleic acid” means any RNA or DNA, which may be unmodified or modified RNA or DNA. Polynucleotides include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, RNA that is mixture of single- and double-stranded regions, and hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, polynucleotide refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons.

[00111] As used herein, the terms “polypeptide,” “peptide” and “protein” are used interchangeably herein to mean a polymer comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, *i.e.*, peptide isosteres. Polypeptide refers to both short chains, commonly referred to as peptides, glycopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. Polypeptides include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques that are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature.

[00112] As used herein, “PRIT” or “pretargeted radioimmunotherapy” refers to a multistep process that resolves the slow blood clearance of tumor targeting antibodies, which contributes to undesirable toxicity to normal tissues such as bone marrow. In pre-targeting, a radionuclide or other diagnostic or therapeutic agent is attached to a small hapten. A pre-

targeting bispecific antibody, which has binding sites for the hapten as well as a target antigen, is administered first. Unbound antibody is then allowed to clear from circulation and the hapten is subsequently administered.

[00113] As used herein, the term “recombinant” when used with reference, *e.g.*, to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the material is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[00114] As used herein, the term “separate” therapeutic use refers to an administration of at least two active ingredients at the same time or at substantially the same time by different routes.

[00115] As used herein, the term “sequential” therapeutic use refers to administration of at least two active ingredients at different times, the administration route being identical or different. More particularly, sequential use refers to the whole administration of one of the active ingredients before administration of the other or others commences. It is thus possible to administer one of the active ingredients over several minutes, hours, or days before administering the other active ingredient or ingredients. There is no simultaneous treatment in this case.

[00116] As used herein, “specifically binds” refers to a molecule (*e.g.*, an antibody or antigen binding fragment thereof) which recognizes and binds another molecule (*e.g.*, an antigen), but that does not substantially recognize and bind other molecules. The terms “specific binding,” “specifically binds to,” or is “specific for” a particular molecule (*e.g.*, a polypeptide, or an epitope on a polypeptide), as used herein, can be exhibited, for example, by a molecule having a K_D for the molecule to which it binds to of about 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M. The term “specifically binds” may also refer to binding where a molecule (*e.g.*, an antibody or antigen binding fragment thereof) binds to a particular polypeptide (*e.g.*, a STEAP1 polypeptide), or an epitope on a

particular polypeptide, without substantially binding to any other polypeptide, or polypeptide epitope.

[00117] As used herein, the term “simultaneous” therapeutic use refers to the administration of at least two active ingredients by the same route and at the same time or at substantially the same time.

[00118] As used herein, the term “therapeutic agent” is intended to mean a compound that, when present in an effective amount, produces a desired therapeutic effect on a subject in need thereof.

[00119] “Treating” or “treatment” as used herein covers the treatment of a disease or disorder described herein, in a subject, such as a human, and includes: (i) inhibiting a disease or disorder, *i.e.*, arresting its development; (ii) relieving a disease or disorder, *i.e.*, causing regression of the disorder; (iii) slowing progression of the disorder; and/or (iv) inhibiting, relieving, or slowing progression of one or more symptoms of the disease or disorder. In some embodiments, treatment means that the symptoms associated with the disease are, *e.g.*, alleviated, reduced, cured, or placed in a state of remission.

[00120] It is also to be appreciated that the various modes of treatment of disorders as described herein are intended to mean “substantial,” which includes total but also less than total treatment, and wherein some biologically or medically relevant result is achieved. The treatment may be a continuous prolonged treatment for a chronic disease or a single, or few time administrations for the treatment of an acute condition.

[00121] Amino acid sequence modification(s) of the anti-STEAP1 antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an anti-STEAP1 antibody are prepared by introducing appropriate nucleotide changes into the antibody nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to obtain the antibody of interest, as long as the obtained antibody possesses the desired properties. The modification also includes the change of the pattern of glycosylation of the protein. The sites of greatest interest for substitutional mutagenesis include the hypervariable

regions, but FR alterations are also contemplated. “Conservative substitutions” are shown in the Table below.

Table 1. Amino Acid Substitutions		
Original Residue	Exemplary Substitutions	Conservative Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr

Table 1. Amino Acid Substitutions		
Original Residue	Exemplary Substitutions	Conservative Substitutions
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

[00122] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Specifically, several hypervariable region sites (*e.g.*, 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.*, binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and the antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with similar or superior properties in one or more relevant assays may be selected for further development.

STEAP1

[00123] STEAP1, also known as PRSS24, STEAP, six transmembrane epithelial antigen of the prostate 1, or STEAP family member 1, is a 339-amino-acid protein named for its 6 transmembrane spanning regions, and is upregulated in a variety of tumors, including

prostate, bladder, ovarian, rhabdomyosarcoma, and Ewing family of tumors (EFT). Hubert *et al.*, *Proc Natl Acad Sci U S A* 96(25): 14523-8 (1999); Rodeberg *et al.*, *Clin Cancer Res* 11(12): 4545-52 (2005). Transcriptome and proteome analyses as well as functional studies show that STEAP1 expression correlates with oxidative stress responses and elevated levels of reactive oxygen species. This in turn regulates redox-sensitive and pro-invasive genes, suggesting that STEAP1 may be associated with an invasive phenotype of EFT. Grunewald *et al.*, *Mol Cancer Res* 10(1): 52-65 (2012). STEAP1 can serve as an immunohistological marker for patients with EFT; 71 of 114 (62.3%) EFT samples displayed detectable membranous STEAP1 immunoreactivity, making STEAP1 a potential therapeutic target. Grunewald *et al.*, *Ann Oncol*, 23(8): p. 2185-90 (2012). Another genetic profiling study done in EFT patients showed that the absence of STEAP1 transcript in the bone marrow was strongly correlated with patient overall survival and survival without new metastases. Given the expression of STEAP1 in >60% of EFT tumors but with limited expression in normal tissue (secretory tissue of the bladder and prostate), STEAP1 may serve as a useful target for antibody-based and T-cell based strategies.

[00124] Human STEAP1 (NCBI Reference Sequence: NP_036581.1) has the following amino acid sequence (SEQ ID NO: 41):

[00125] MESRKDITNQEELWKMKPRRNLEEDDYLHKDTGETSMLKRPVLLHLHQT
 AHAEFDCPSELQHTQELFPQWHLPIKIAAIIASLTFLYTLLREVTHPLATSHQQYFYKI
 PILVINKVLPMVSITLLALVYLPGVIAAIVQLHNGTKYKKFPHWLDKWMLTRKQFGL
 LSFFFAVLHAIYSLSYPMRRSYRYKLLNWAYQQVQQNKEDAWIEHDVWRMEIYVSL
 GIVGLAILALLAVTSIPSVSDSLTWREFHYIQSKLGIVSLLLGTIHALIFAWNKWIDIKQ
 FVWYTPPTFMIAVFLPIVVLIFKSILFLPCLRKKILKIRHGWEDVTKINKTEICSQL

[00126] Mouse STEAP1 (NCBI Reference Sequence: NP_081675.2) has the following amino acid sequence (SEQ ID NO: 42):

[00127] MEISDDVTNPEQLWKMKPKGNLEDDSYSTKDSGETSMLKRPGLSHLQHA
 VHVDAFDCPSELQHTQEFPNWRLPVKVAIISSLTFLYTLLREIHYPLVTSREQYFYKI
 PILVINKVLPMVAITLLALVYLPGELAAVVQLRNGTKYKKFPPWLDRWMLARKQFG
 LLSFFFAVLHAVYSLSYPMRRSYRYKLLNWAYKQVQQNKEDAWVEHDVWRMEIY
 VSLGIVGLAILALLAVTSIPSVSDSLTWREFHYIQSKLGIVSLLLGTVHALVFAWNKW

VDVSQFVWYMPPTFMIAVFLPTLV LICKIALCLPCLRKKILKIRCGWEDVSKINRTEM
ASRL

[00128] Canine STEAP1 (NCBI Reference Sequence: XP_013974694.1) has the following amino acid sequence (SEQ ID NO: 60):

[00129] MESRQDITSQEELWTMKPRRNLEEDDYLDKDSGDTRVLKRPVLLHMHQT
THFDEFDCPAELKHKQELFPMWRWPVKIAAVISSLTFLYTLLREIHPFVTSHQQYFY
KIPILVINKVLPMSITLLALVYLPGVIAAVVQLHNGTKYKKFPHWLDRWMLTRKQF
GLLSFFFAVLHAIYSLSPMRRSYRYKLLNWAYQQVQQNKEDAWIEHDVWRMEIY
VSLGIVTLAILALLAVTSIPSVSDSLTWREFHYIQSKLGMVSLLLGTIHALIFAWNKW
VDIKQFVWYTPPTFMIAVFLPIVV LICKAILFLPCLRKKILKIRHWEDVTKINKTEMS
SQL

EWS-FLI1 Pathway

[00130] The EWS-FLI1 fusion protein results in the production of a unique tumor driver only found in tumor cells. Tumorigenesis in EFT is dependent on EWS-FLI1 fusion protein expression. **FIG. 1A** shows a diagrammatic representation of the EWS-FLI1 pathway, including some approaches for molecular therapies.

[00131] Studies in the 1960s and 1970s utilizing various peptides and natural products to target the EWS-FLI1 fusion protein showed activity in the preclinical setting, although their translation into the clinical setting was limited by toxicity. For example, mithramycin is a natural product known to repress the EWS-FLI1 protein *in vitro*. A phase I/II study including 8 patients with refractory EFT treated with mithramycin showed no clinical responses with an inability to safely achieved the desired dose secondary to hepatotoxicity. See Grohar *et al.*, *Cancer Chemother Pharmacol* 80(3): 645-652 (2017).

Immunoglobulin-related Compositions of the Present Technology

[00132] The present technology describes methods and compositions for the generation and use of anti-STEAP1 immunoglobulin-related compositions (*e.g.*, anti-STEAP1 antibodies or antigen binding fragments thereof). The anti-STEAP1 immunoglobulin-related compositions of the present disclosure may be useful in the diagnosis, or treatment of STEAP1-associated cancers. Anti-STEAP1 immunoglobulin-related compositions within the scope of the present technology include, *e.g.*, but are not limited to, monoclonal, chimeric,

humanized, bispecific antibodies and diabodies that specifically bind the target polypeptide, a homolog, derivative or a fragment thereof. The present disclosure also provides antigen binding fragments of any of the anti-STEAP1 antibodies disclosed herein, wherein the antigen binding fragment is selected from the group consisting of Fab, F(ab)'2, Fab', scFv, and Fv. In one aspect, the present technology provides chimeric and humanized variants of X120, including multispecific immunoglobulin-related compositions (*e.g.*, bispecific antibody agents). The Table below provides CDR sequences of the antibodies of present technology:

Variable Domain	CDR1	CDR2	CDR3
V_H	GYSITSD (SEQ ID NO: 2)	NSGS (SEQ ID NO: 3)	ERNYDYDDYYYAMDY (SEQ ID NO: 4)
V_L	KSSQSLLYRSNQKNYLA (SEQ ID NO: 13)	WASTRES (SEQ ID NO: 14)	QQYYNYPRT (SEQ ID NO: 15)

[00133] In one aspect, the present technology provides an antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein (a) the V_H comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11; and/or (b) the V_L comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

[00134] In any of the above embodiments, the antibody further comprises a Fc domain of any isotype, *e.g.*, but are not limited to, IgG (including IgG1, IgG2, IgG3, and IgG4), IgA (including IgA₁ and IgA₂), IgD, IgE, or IgM, and IgY. Non-limiting examples of constant region sequences include:

[00135] Human IgD constant region, Uniprot: P01880 (SEQ ID NO: 43)

APTKAPDVFPIISGCRHPKDNSPVVLACLITGYHPTSVTVTWYMGTSQSPQRTFPEIQ
RRDSYYMTSSQLSTPLQQWRQGEYKCVVQHTASKSKKEIFRWPESPKAQASSVPTA
QPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQEERETKTPECPSHTQPLGVY

LLTPAVQDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGVEEGLLERHSNG
 SQSQHSRLTLPRSLWNAGTSVTCTLNHPSSLPPQRLMALREPAAQAPVKLSLNLLASS
 DPPEAASWLLCEVSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFWAWSVL
 RVPAPPSPQPATYTCVVSHEDSRLLNASRSLEVSYVTDHGPMK

[00136] Human IgG1 constant region, Uniprot: P01857 (SEQ ID NO: 44)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
 QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP
 ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
 EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
 GSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

[00137] Human IgG2 constant region, Uniprot: P01859 (SEQ ID NO: 45)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCAPPVA
 GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPR
 EEQFNSTFRVVSIVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVY
 TLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFL
 YSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

[00138] Human IgG3 constant region, Uniprot: P01860 (SEQ ID NO: 46)

ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
 QSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRC
 PEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGPSVFLFPP
 KPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFR
 VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEM
 TKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKS
 RWQQGNIFCFSVMHEALHNRFQKSLSLSPGK

[00139] Human IgM constant region, Uniprot: P01871 (SEQ ID NO: 47)

GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITLSWKYKNNSDISSTRGFPSV
 LRGGKYAATSQVLLPSKDV MQGTDEHV VCKVQHPNGNKEKNVPLPVIAELPPKVS

FVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTDDQVQAEAKESG
 PTTYKVTSTLTIKESDWLGQSMFTCRVDHRGLTFQQNASSMCVPDQDPAIRVFAIPPS
 FASIFLTKSTKLTCLVTDLT TYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEAS
 ICEDDWNNGERFTCTVTHTDLPSPKQKTISRPKGVALHRPDVYLLPPAREQLNLRESA
 TITCLVTGFSPADV FVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEE
 WNTGETYTCVAHEALPNRV TERTVDKSTGKPTLYNVSLVMSDTAGTCY

[00140] Human IgG4 constant region, Uniprot: P01861 (SEQ ID NO: 48)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
 SSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLG
 GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
 EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
 TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
 YSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLGK

[00141] Human IgA1 constant region, Uniprot: P01876 (SEQ ID NO: 49)

ASPTSPKVFPLSLCSTQPDGNVVIACL VQGFFPQEPLSVTWSESGQGV TARNFPPSQD
 ASGDLYTTSSQLTLPATQCLAGKSVTCHVKHYTNPSQDVTVP CPVPSTPPTPSPSTPP
 TPSPSCCHPRLSLHRPALEDLLL GSEANLTCTLTGLRDASGVTFTWTPSSGKSAVQGP
 PERDLCGCYSVSSVLP GCAEPWNH GKTFCTAAYPESKTPLTATLSKSGNTFRPEVH
 LLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQG
 TTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVV
 MAEVDGTCY

[00142] Human IgA2 constant region, Uniprot: P01877 (SEQ ID NO: 50)

ASPTSPKVFPLSLDSTPQDGNVVVACL VQGFFPQEPLSVTWSESGQNV TARNFPPSQD
 ASGDLYTTSSQLTLPATQCPDGKSVTCHVKHYTNPSQDVTVP CPVPPPPPCCHPRLSL
 HRPALEDLLL GSEANLTCTLTGLRDASGATFTWTPSSGKSAVQGPPERDLCGCYSVS
 SVLP GCAQPWNHGETFTCTAAHPELKTPLTANITKSGNTFRPEVHLLPPPSEELALNE
 LVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQGT TTTFAVTSILRVA
 AEDWKKGDTFSCMVGHEALPLAFTQKTIDRMAGKPTHVNVSVVMAEVDGTCY

[00143] Human Ig kappa constant region, Uniprot: P01834 (SEQ ID NO: 51)

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE
QDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[00144] In some embodiments, the immunoglobulin-related compositions of the present technology comprise a heavy chain constant region that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or is 100% identical to SEQ ID NOS: 43-50. Additionally or alternatively, in some embodiments, the immunoglobulin-related compositions of the present technology comprise a light chain constant region that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or is 100% identical to SEQ ID NO: 51. In some embodiments, the immunoglobulin-related compositions of the present technology bind to the second ECD of a STEAP1 polypeptide, STEAP1B1 polypeptide and/or STEAP1B2 polypeptide. In some embodiments, the epitope is a conformational epitope or non-conformational epitope.

[00145] In another aspect, the present disclosure provides an isolated immunoglobulin-related composition (*e.g.*, an antibody or antigen binding fragment thereof) comprising a heavy chain (HC) amino acid sequence comprising a heavy chain (HC) amino acid sequence comprising SEQ ID NO: 22, SEQ ID NO: 26, or a variant thereof having one or more conservative amino acid substitutions.

[00146] Additionally or alternatively, in some embodiments, the immunoglobulin-related compositions of the present technology comprise a light chain (LC) amino acid sequence comprising SEQ ID NO: 21, SEQ ID NO: 24, SEQ ID NO: 27, SEQ ID NO: 28, or a variant thereof having one or more conservative amino acid substitutions.

[00147] In some embodiments, the immunoglobulin-related compositions of the present technology comprise a HC amino acid sequence and a LC amino acid sequence selected from the group consisting of: SEQ ID NO: 22 and SEQ ID NO: 21; SEQ ID NO: 22 and SEQ ID NO: 24; SEQ ID NO: 22 and SEQ ID NO: 27; SEQ ID NO: 22 and SEQ ID NO: 28; SEQ ID NO: 26 and SEQ ID NO: 21; SEQ ID NO: 26 and SEQ ID NO: 24; SEQ ID NO: 26 and SEQ ID NO: 27; and SEQ ID NO: 26 and SEQ ID NO: 28, respectively.

[00148] In any of the above embodiments of the immunoglobulin-related compositions, the HC and LC immunoglobulin variable domain sequences form an antigen binding site that binds to the second ECD of a STEAP1 polypeptide, STEAP1B1 polypeptide and/or

STEAP1B2 polypeptide. In some embodiments, the epitope is a conformational epitope or a non-conformational epitope.

[00149] In some embodiments, the HC and LC immunoglobulin variable domain sequences are components of the same polypeptide chain. In other embodiments, the HC and LC immunoglobulin variable domain sequences are components of different polypeptide chains. In certain embodiments, the antibody is a full-length antibody.

[00150] In some embodiments, the immunoglobulin-related compositions of the present technology bind specifically to at least one STEAP1 polypeptide. In some embodiments, the immunoglobulin-related compositions of the present technology bind at least one STEAP1 polypeptide with a dissociation constant (K_D) of about 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M. In certain embodiments, the immunoglobulin-related compositions are monoclonal antibodies, chimeric antibodies, humanized antibodies, or bispecific antibodies. In some embodiments, the antibodies comprise a human antibody framework region.

[00151] In certain embodiments, the immunoglobulin-related composition includes one or more of the following characteristics: (a) a light chain immunoglobulin variable domain sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the light chain immunoglobulin variable domain sequence present in any one of SEQ ID NOs: 17, 18, 19, or 20; and/or (b) a heavy chain immunoglobulin variable domain sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the heavy chain immunoglobulin variable domain sequence present in any one of SEQ ID NOs: 6, 7, 8, 9, 10, or 11. In another aspect, one or more amino acid residues in the immunoglobulin-related compositions provided herein are substituted with another amino acid. The substitution may be a “conservative substitution” as defined herein.

[00152] In one aspect, the present disclosure provides an immunoglobulin-related composition comprising an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to an amino acid sequence selected from SEQ ID NOs: 29-40 or 61-64.

[00153] In another aspect, the present disclosure provides an antibody comprising (a) a LC sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%

identical to the LC sequence present in any one of SEQ ID NO: 21, SEQ ID NO: 24, SEQ ID NO: 27, or SEQ ID NO: 28; and/or (b) a HC sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the HC sequence present in SEQ ID NO: 22 or SEQ ID NO: 26.

[00154] In one aspect, the present disclosure provides a bispecific antigen binding fragment comprising a first polypeptide chain, wherein: the first polypeptide chain comprises in the N-terminal to C-terminal direction: (i) a heavy chain variable domain of a first immunoglobulin that is capable of specifically binding to a first epitope; (ii) a flexible peptide linker comprising the amino acid sequence (GGGGS)₆; (iii) a light chain variable domain of the first immunoglobulin; (iv) a flexible peptide linker comprising the amino acid sequence (GGGGS)₄; (v) a heavy chain variable domain of a second immunoglobulin that is capable of specifically binding to a second epitope; (vi) a flexible peptide linker comprising the amino acid sequence (GGGGS)₆; (vii) a light chain variable domain of the second immunoglobulin; (viii) a flexible peptide linker sequence comprising the amino acid sequence TPLGDTTHT; and (ix) a self-assembly disassembly (SADA) polypeptide; wherein the heavy chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and/or the light chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20.

[00155] In another aspect, the present disclosure provides a bispecific antigen binding fragment comprising a first polypeptide chain, wherein: the first polypeptide chain comprises in the N-terminal to C-terminal direction: (i) a light chain variable domain of a first immunoglobulin that is capable of specifically binding to a first epitope; (ii) a flexible peptide linker comprising the amino acid sequence (GGGGS)₆; (iii) a heavy chain variable domain of the first immunoglobulin; (iv) a flexible peptide linker comprising the amino acid sequence (GGGGS)₄; (v) a heavy chain variable domain of a second immunoglobulin that is capable of specifically binding to a second epitope; (vi) a flexible peptide linker comprising the amino acid sequence (GGGGS)₆; (vii) a light chain variable domain of the second immunoglobulin; (viii) a flexible peptide linker sequence comprising the amino acid sequence TPLGDTTHT; and (ix) a self-assembly disassembly (SADA) polypeptide; wherein the heavy chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 6,

SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and/or the light chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20.

[00156] In certain embodiments of the bispecific antigen binding fragments disclosed herein, the SADA polypeptide comprises a tetramerization, pentamerization, or hexamerization domain. In some embodiments, the SADA polypeptide comprises a tetramerization domain of any one of p53, p63, p73, hnRNPC, SNA-23, Stefin B, KCNQ4, and CBFA2T1. Additionally or alternatively, in some embodiments, the bispecific antigen binding fragment comprises an amino acid sequence selected from SEQ ID NOs: 29-40 or 61-64.

[00157] In one aspect, the present disclosure provides a bispecific antibody comprising a first polypeptide chain, a second polypeptide chain, a third polypeptide chain and a fourth polypeptide chain, wherein the first and second polypeptide chains are covalently bonded to one another, the second and third polypeptide chains are covalently bonded to one another, and the third and fourth polypeptide chain are covalently bonded to one another, and wherein: (a) each of the first polypeptide chain and the fourth polypeptide chain comprises in the N-terminal to C-terminal direction: (i) a light chain variable domain of a first immunoglobulin that is capable of specifically binding to a first epitope; (ii) a light chain constant domain of the first immunoglobulin; (iii) a flexible peptide linker comprising the amino acid sequence (GGGGS)₃; and (iv) a light chain variable domain of a second immunoglobulin that is linked to a complementary heavy chain variable domain of the second immunoglobulin, or a heavy chain variable domain of a second immunoglobulin that is linked to a complementary light chain variable domain of the second immunoglobulin, wherein the light chain and heavy chain variable domains of the second immunoglobulin are capable of specifically binding to a second epitope, and are linked together via a flexible peptide linker comprising the amino acid sequence (GGGGS)₆ to form a single-chain variable fragment; and (b) each of the second polypeptide chain and the third polypeptide chain comprises in the N-terminal to C-terminal direction: (i) a heavy chain variable domain of the first immunoglobulin that is capable of specifically binding to the first epitope; and (ii) a heavy chain constant domain of the first immunoglobulin; and wherein the heavy chain variable domain of the first

immunoglobulin is selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and/or the light chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In certain embodiments, the second immunoglobulin binds to CD3, CD4, CD8, CD20, CD19, CD21, CD23, CD46, CD80, HLA-DR, CD74, CD22, CD14, CD15, CD16, CD123, TCR gamma/delta, NKp46, KIR, or a small molecule DOTA hapten.

[00158] In certain embodiments, the immunoglobulin-related compositions contain an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A and K322A. Additionally or alternatively, in some embodiments, the immunoglobulin-related compositions contain an IgG4 constant region comprising a S228P mutation.

[00159] In some aspects, the anti-STEAP1 immunoglobulin-related compositions described herein contain structural modifications to facilitate rapid binding and cell uptake and/or slow release. In some aspects, the anti-STEAP1 immunoglobulin-related composition of the present technology (*e.g.*, an antibody) may contain a deletion in the CH2 constant heavy chain region to facilitate rapid binding and cell uptake and/or slow release. In some aspects, a Fab fragment is used to facilitate rapid binding and cell uptake and/or slow release. In some aspects, a F(ab)₂ fragment is used to facilitate rapid binding and cell uptake and/or slow release.

[00160] In one aspect, the present technology provides a nucleic acid sequence encoding any of the immunoglobulin-related compositions described herein. Also disclosed herein are recombinant nucleic acid sequences encoding any of the antibodies described herein. In some embodiments, the nucleic acid sequence is selected from the group consisting of SEQ ID NOs: 23, and 25.

[00161] In another aspect, the present technology provides a host cell expressing any nucleic acid sequence encoding any of the immunoglobulin-related compositions described herein.

[00162] The immunoglobulin-related compositions of the present technology (*e.g.*, an anti-STEAP1 antibody) can be monospecific, bispecific, trispecific or of greater multispecificity.

Multispecific antibodies can be specific for different epitopes of one or more STEAP1 polypeptides or can be specific for both the STEAP1 polypeptide(s) as well as for heterologous compositions, such as a heterologous polypeptide or solid support material. *See, e.g.*, WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt *et al.*, *J. Immunol.* 147: 60-69 (1991); U.S. Pat. Nos. 5,573,920, 4,474,893, 5,601,819, 4,714,681, 4,925,648; 6,106,835; Kostelny *et al.*, *J. Immunol.* 148: 1547-1553 (1992). In some embodiments, the immunoglobulin-related compositions are chimeric. In certain embodiments, the immunoglobulin-related compositions are humanized.

[00163] The immunoglobulin-related compositions of the present technology can further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, the immunoglobulin-related compositions of the present technology can be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, or toxins. *See, e.g.*, WO 92/08495; WO 91/14438; WO 89/12624; U.S. Pat. No. 5,314,995; and EP 0 396 387.

[00164] In any of the above embodiments of the immunoglobulin-related compositions of the present technology, the antibody or antigen binding fragment may be optionally conjugated to an agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof. For a chemical bond or physical bond, a functional group on the immunoglobulin-related composition typically associates with a functional group on the agent. Alternatively, a functional group on the agent associates with a functional group on the immunoglobulin-related composition.

[00165] The functional groups on the agent and immunoglobulin-related composition can associate directly. For example, a functional group (*e.g.*, a sulfhydryl group) on an agent can associate with a functional group (*e.g.*, sulfhydryl group) on an immunoglobulin-related composition to form a disulfide. Alternatively, the functional groups can associate through a cross-linking agent (*i.e.*, linker). Some examples of cross-linking agents are described below. The cross-linker can be attached to either the agent or the immunoglobulin-related

composition. The number of agents or immunoglobulin-related compositions in a conjugate is also limited by the number of functional groups present on the other. For example, the maximum number of agents associated with a conjugate depends on the number of functional groups present on the immunoglobulin-related composition. Alternatively, the maximum number of immunoglobulin-related compositions associated with an agent depends on the number of functional groups present on the agent.

[00166] In yet another embodiment, the conjugate comprises one immunoglobulin-related composition associated to one agent. In one embodiment, a conjugate comprises at least one agent chemically bonded (*e.g.*, conjugated) to at least one immunoglobulin-related composition. The agent can be chemically bonded to an immunoglobulin-related composition by any method known to those in the art. For example, a functional group on the agent may be directly attached to a functional group on the immunoglobulin-related composition. Some examples of suitable functional groups include, for example, amino, carboxyl, sulfhydryl, maleimide, isocyanate, isothiocyanate and hydroxyl.

[00167] The agent may also be chemically bonded to the immunoglobulin-related composition by means of cross-linking agents, such as dialdehydes, carbodiimides, dimaleimides, and the like. Cross-linking agents can, for example, be obtained from Pierce Biotechnology, Inc., Rockford, Ill. The Pierce Biotechnology, Inc. web-site can provide assistance. Additional cross-linking agents include the platinum cross-linking agents described in U.S. Pat. Nos. 5,580,990; 5,985,566; and 6,133,038 of Kretech Biotechnology, B.V., Amsterdam, The Netherlands.

[00168] Alternatively, the functional group on the agent and immunoglobulin-related composition can be the same. Homobifunctional cross-linkers are typically used to cross-link identical functional groups. Examples of homobifunctional cross-linkers include EGS (*i.e.*, ethylene glycol bis[succinimidylsuccinate]), DSS (*i.e.*, disuccinimidyl suberate), DMA (*i.e.*, dimethyl adipimidate.2HCl), DTSSP (*i.e.*, 3,3'-dithiobis[sulfosuccinimidylpropionate]), DPDPB (*i.e.*, 1,4-di-[3'-(2'-pyridyldithio)-propionamido]butane), and BMH (*i.e.*, bis-maleimido-hexane). Such homobifunctional cross-linkers are also available from Pierce Biotechnology, Inc.

[00169] In other instances, it may be beneficial to cleave the agent from the immunoglobulin-related composition. The web-site of Pierce Biotechnology, Inc. described above can also provide assistance to one skilled in the art in choosing suitable cross-linkers which can be cleaved by, for example, enzymes in the cell. Thus the agent can be separated from the immunoglobulin-related composition. Examples of cleavable linkers include SMPT (*i.e.*, 4-succinimidylloxycarbonyl-methyl-a-[2-pyridyldithio]toluene), Sulfo-LC-SPDP (*i.e.*, sulfosuccinimidyl 6-(3-[2-pyridyldithio]-propionamido)hexanoate), LC-SPDP (*i.e.*, succinimidyl 6-(3-[2-pyridyldithio]-propionamido)hexanoate), Sulfo-LC-SPDP (*i.e.*, sulfosuccinimidyl 6-(3-[2-pyridyldithio]-propionamido)hexanoate), SPDP (*i.e.*, N-succinimidyl 3-[2-pyridyldithio]-propionamidohexanoate), and AEDP (*i.e.*, 3-[(2-aminoethyl)dithio]propionic acid HCl).

[00170] In another embodiment, a conjugate comprises at least one agent physically bonded with at least one immunoglobulin-related composition. Any method known to those in the art can be employed to physically bond the agents with the immunoglobulin-related compositions. For example, the immunoglobulin-related compositions and agents can be mixed together by any method known to those in the art. The order of mixing is not important. For instance, agents can be physically mixed with immunoglobulin-related compositions by any method known to those in the art. For example, the immunoglobulin-related compositions and agents can be placed in a container and agitated, by for example, shaking the container, to mix the immunoglobulin-related compositions and agents.

[00171] The immunoglobulin-related compositions can be modified by any method known to those in the art. For instance, the immunoglobulin-related composition may be modified by means of cross-linking agents or functional groups, as described above.

A. Methods of Preparing Anti-STEAP1 Antibodies of the Present Technology

[00172] *General Overview.* Initially, a target polypeptide is chosen to which an antibody of the present technology can be raised. For example, an antibody may be raised against the full-length STEAP1 protein, or to a portion of the extracellular domain of the STEAP1 protein (*e.g.*, the second ECD of STEAP1 protein). Techniques for generating antibodies directed to such target polypeptides are well known to those skilled in the art. Examples of such techniques include, for example, but are not limited to, those involving display libraries, xeno or human mice, hybridomas, and the like. Target polypeptides within the scope of the

present technology include any polypeptide derived from STEAP1 protein containing the extracellular domain which is capable of eliciting an immune response (*e.g.*, the second ECD of STEAP1 protein).

[00173] It should be understood that recombinantly engineered antibodies and antibody fragments, *e.g.*, antibody-related polypeptides, which are directed to STEAP1 protein and fragments thereof are suitable for use in accordance with the present disclosure.

[00174] Anti-STEAP1 antibodies that can be subjected to the techniques set forth herein include monoclonal and polyclonal antibodies, and antibody fragments such as Fab, Fab', F(ab')₂, Fd, scFv, diabodies, antibody light chains, antibody heavy chains and/or antibody fragments. Methods useful for the high yield production of antibody Fv-containing polypeptides, *e.g.*, Fab' and F(ab')₂ antibody fragments have been described. *See* U.S. Pat. No. 5,648,237.

[00175] Generally, an antibody is obtained from an originating species. More particularly, the nucleic acid or amino acid sequence of the variable portion of the light chain, heavy chain or both, of an originating species antibody having specificity for a target polypeptide antigen is obtained. An originating species is any species which was useful to generate the antibody of the present technology or library of antibodies, *e.g.*, rat, mouse, rabbit, chicken, monkey, human, and the like.

[00176] Phage or phagemid display technologies are useful techniques to derive the antibodies of the present technology. Techniques for generating and cloning monoclonal antibodies are well known to those skilled in the art. Expression of sequences encoding antibodies of the present technology, can be carried out in *E. coli*.

[00177] Due to the degeneracy of nucleic acid coding sequences, other sequences which encode substantially the same amino acid sequences as those of the naturally occurring proteins may be used in the practice of the present technology. These include, but are not limited to, nucleic acid sequences including all or portions of the nucleic acid sequences encoding the above polypeptides, which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. It is appreciated that the nucleotide sequence of an immunoglobulin according to the present technology tolerates sequence homology variations of up to 25% as

calculated by standard methods ("Current Methods in Sequence Comparison and Analysis," *Macromolecule Sequencing and Synthesis, Selected Methods and Applications*, pp. 127-149, 1998, Alan R. Liss, Inc.) so long as such a variant forms an operative antibody which recognizes STEAP1 proteins. For example, one or more amino acid residues within a polypeptide sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Also included within the scope of the present technology are proteins or fragments or derivatives thereof which are differentially modified during or after translation, *e.g.*, by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligands, *etc.* Additionally, an immunoglobulin encoding nucleic acid sequence can be mutated *in vitro* or *in vivo* to create and/or destroy translation, initiation, and/or termination sequences or to create variations in coding regions and/or form new restriction endonuclease sites or destroy pre-existing ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to *in vitro* site directed mutagenesis, *J. Biol. Chem.* 253:6551, use of Tab linkers (Pharmacia), and the like.

[00178] *Preparation of Polyclonal Antisera and Immunogens.* Methods of generating antibodies or antibody fragments of the present technology typically include immunizing a subject (generally a non-human subject such as a mouse or rabbit) with a purified STEAP1 protein or fragment thereof or with a cell expressing the STEAP1 protein or fragment thereof. An appropriate immunogenic preparation can contain, *e.g.*, a recombinantly-expressed STEAP1 protein or a chemically-synthesized STEAP1 peptide. The extracellular domain of the STEAP1 protein, or a portion or fragment thereof (*e.g.*, the second ECD of STEAP1 protein), can be used as an immunogen to generate an anti-STEAP1 antibody that binds to the STEAP1 protein, or a portion or fragment thereof using standard techniques for polyclonal and monoclonal antibody preparation.

[00179] The full-length STEAP1 protein or fragments thereof, are useful as fragments as immunogens. In some embodiments, a STEAP1 fragment comprises the second ECD of the STEAP1 protein such that an antibody raised against the peptide forms a specific immune complex with STEAP1 protein. In some embodiments, an antibody raised against the peptide forms a specific immune complex with STEAP1B1 and/or STEAP1B2 proteins.

[00180] The second ECD of STEAP1 protein of STEAP1 spans amino acids 185-216 of the full length protein. In some embodiments, the antigenic STEAP1 peptide comprises at least 5, 8, 10, 15, 20, 30, 40, 50, or 60 amino acid residues. Longer antigenic peptides are sometimes desirable over shorter antigenic peptides, depending on use and according to methods well known to those skilled in the art. Multimers of a given epitope are sometimes more effective than a monomer.

[00181] If needed, the immunogenicity of the STEAP1 protein (or fragment thereof) can be increased by fusion or conjugation to a carrier protein such as keyhole limpet hemocyanin (KLH) or ovalbumin (OVA). Many such carrier proteins are known in the art. One can also combine the STEAP1 protein with a conventional adjuvant such as Freund's complete or incomplete adjuvant to increase the subject's immune reaction to the polypeptide. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, *etc.*), human adjuvants such as Bacille Calmette-Guerin and *Corynebacterium parvum*, or similar immunostimulatory compounds. These techniques are standard in the art.

[00182] In describing the present technology, immune responses may be described as either "primary" or "secondary" immune responses. A primary immune response, which is also described as a "protective" immune response, refers to an immune response produced in an individual as a result of some initial exposure (*e.g.*, the initial "immunization") to a particular antigen, *e.g.*, STEAP1 protein. In some embodiments, the immunization can occur as a result of vaccinating the individual with a vaccine containing the antigen. For example, the vaccine can be a STEAP1 vaccine comprising one or more STEAP1 protein-derived antigens. A primary immune response can become weakened or attenuated over time and can even disappear or at least become so attenuated that it cannot be detected. Accordingly, the present technology also relates to a "secondary" immune response, which is also described

here as a “memory immune response.” The term secondary immune response refers to an immune response elicited in an individual after a primary immune response has already been produced.

[00183] Thus, a secondary immune response can be elicited, *e.g.*, to enhance an existing immune response that has become weakened or attenuated, or to recreate a previous immune response that has either disappeared or can no longer be detected. The secondary or memory immune response can be either a humoral (antibody) response or a cellular response. A secondary or memory humoral response occurs upon stimulation of memory B cells that were generated at the first presentation of the antigen. Delayed type hypersensitivity (DTH) reactions are a type of cellular secondary or memory immune response that are mediated by CD4⁺ T cells. A first exposure to an antigen primes the immune system and additional exposure(s) results in a DTH.

[00184] Following appropriate immunization, the anti-STEAP1 antibody can be prepared from the subject's serum. If desired, the antibody molecules directed against the STEAP1 protein can be isolated from the mammal (*e.g.*, from the blood) and further purified by well-known techniques, such as polypeptide A chromatography to obtain the IgG fraction.

[00185] *Monoclonal Antibody.* In one embodiment of the present technology, the antibody is an anti-STEAP1 monoclonal antibody. For example, in some embodiments, the anti-STEAP1 monoclonal antibody may be a human or a mouse anti-STEAP1 monoclonal antibody. For preparation of monoclonal antibodies directed towards the STEAP1 protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture can be utilized. Such techniques include, but are not limited to, the hybridoma technique (*See, e.g.*, Kohler & Milstein, 1975. *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (*See, e.g.*, Kozbor, *et al.*, 1983. *Immunol. Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (*See, e.g.*, Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies can be utilized in the practice of the present technology and can be produced by using human hybridomas (*See, e.g.*, Cote, *et al.*, 1983. *Proc. Natl. Acad. Sci. USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (*See, e.g.*, Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER

THERAPY, Alan R. Liss, Inc., pp. 77-96). For example, a population of nucleic acids that encode regions of antibodies can be isolated. PCR utilizing primers derived from sequences encoding conserved regions of antibodies is used to amplify sequences encoding portions of antibodies from the population and then DNAs encoding antibodies or fragments thereof, such as variable domains, are reconstructed from the amplified sequences. Such amplified sequences also can be fused to DNAs encoding other proteins – *e.g.*, a bacteriophage coat, or a bacterial cell surface protein – for expression and display of the fusion polypeptides on phage or bacteria. Amplified sequences can then be expressed and further selected or isolated based, *e.g.*, on the affinity of the expressed antibody or fragment thereof for an antigen or epitope present on the STEAP1 protein. Alternatively, hybridomas expressing anti-STEAP1 monoclonal antibodies can be prepared by immunizing a subject and then isolating hybridomas from the subject's spleen using routine methods. *See, e.g.*, Milstein *et al.*, (Galfre and Milstein, *Methods Enzymol* (1981) 73: 3-46). Screening the hybridomas using standard methods will produce monoclonal antibodies of varying specificity (*i.e.*, for different epitopes) and affinity. A selected monoclonal antibody with the desired properties, *e.g.*, STEAP1 binding, can be used as expressed by the hybridoma, it can be bound to a molecule such as polyethylene glycol (PEG) to alter its properties, or a cDNA encoding it can be isolated, sequenced and manipulated in various ways. Synthetic dendromeric trees can be added to reactive amino acid side chains, *e.g.*, lysine, to enhance the immunogenic properties of STEAP1 protein. Also, CPG-dinucleotide techniques can be used to enhance the immunogenic properties of the STEAP1 protein. Other manipulations include substituting or deleting particular amino acyl residues that contribute to instability of the antibody during storage or after administration to a subject, and affinity maturation techniques to improve affinity of the antibody of the STEAP1 protein.

[00186] *Hybridoma Technique.* In some embodiments, the antibody of the present technology is an anti-STEAP1 monoclonal antibody produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell. Hybridoma techniques include those known in the art and taught in Harlow *et al.*, *Antibodies: A Laboratory Manual* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 349 (1988); Hammerling *et al.*, *Monoclonal Antibodies And T-Cell*

Hybridomas, 563-681 (1981). Other methods for producing hybridomas and monoclonal antibodies are well known to those of skill in the art.

[00187] *Phage Display Technique.* As noted above, the antibodies of the present technology can be produced through the application of recombinant DNA and phage display technology. For example, anti-STEAP1 antibodies, can be prepared using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of a phage particle which carries polynucleotide sequences encoding them. Phages with a desired binding property are selected from a repertoire or combinatorial antibody library (*e.g.*, human or murine) by selecting directly with an antigen, typically an antigen bound or captured to a solid surface or bead. Phages used in these methods are typically filamentous phage including fd and M13 with Fab, Fv or disulfide stabilized Fv antibody domains that are recombinantly fused to either the phage gene III or gene VIII protein. In addition, methods can be adapted for the construction of Fab expression libraries (*See, e.g.*, Huse, *et al.*, *Science* 246: 1275-1281, 1989) to allow rapid and effective identification of monoclonal Fab fragments with the desired specificity for a STEAP1 polypeptide, *e.g.*, a polypeptide or derivatives, fragments, analogs or homologs thereof. Other examples of phage display methods that can be used to make the antibodies of the present technology include those disclosed in Huston *et al.*, *Proc. Natl. Acad. Sci U.S.A.*, 85: 5879-5883, 1988; Chaudhary *et al.*, *Proc. Natl. Acad. Sci U.S.A.*, 87: 1066-1070, 1990; Brinkman *et al.*, *J. Immunol. Methods* 182: 41-50, 1995; Ames *et al.*, *J. Immunol. Methods* 184: 177-186, 1995; Kettleborough *et al.*, *Eur. J. Immunol.* 24: 952-958, 1994; Persic *et al.*, *Gene* 187: 9-18, 1997; Burton *et al.*, *Advances in Immunology* 57: 191-280, 1994; PCT/GB91/01134; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; WO 96/06213; WO 92/01047 (Medical Research Council *et al.*); WO 97/08320 (Morphosys); WO 92/01047 (CAT/MRC); WO 91/17271 (Affymax); and U.S. Pat. Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727 and 5,733,743. Methods useful for displaying polypeptides on the surface of bacteriophage particles by attaching the polypeptides *via* disulfide bonds have been described by Lohning, U.S. Pat. No. 6,753,136. As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole

antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax *et al.*, *BioTechniques* 12: 864-869, 1992; and Sawai *et al.*, *AJRI* 34: 26-34, 1995; and Better *et al.*, *Science* 240: 1041-1043, 1988.

[00188] Generally, hybrid antibodies or hybrid antibody fragments that are cloned into a display vector can be selected against the appropriate antigen in order to identify variants that maintain good binding activity, because the antibody or antibody fragment will be present on the surface of the phage or phagemid particle. *See, e.g.,* Barbas III *et al.*, *Phage Display, A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001). However, other vector formats could be used for this process, such as cloning the antibody fragment library into a lytic phage vector (modified T7 or Lambda Zap systems) for selection and/or screening.

[00189] *Expression of Recombinant Anti-STEAP1 Antibodies.* As noted above, the antibodies of the present technology can be produced through the application of recombinant DNA technology. Recombinant polynucleotide constructs encoding an anti-STEAP1 antibody of the present technology typically include an expression control sequence operably-linked to the coding sequences of anti-STEAP1 antibody chains, including naturally-associated or heterologous promoter regions. As such, another aspect of the technology includes vectors containing one or more nucleic acid sequences encoding an anti-STEAP1 antibody of the present technology. For recombinant expression of one or more of the polypeptides of the present technology, the nucleic acid containing all or a portion of the nucleotide sequence encoding the anti-STEAP1 antibody is inserted into an appropriate cloning vector, or an expression vector (*i.e.*, a vector that contains the necessary elements for the transcription and translation of the inserted polypeptide coding sequence) by recombinant DNA techniques well known in the art and as detailed below. Methods for producing diverse populations of vectors have been described by Lerner *et al.*, U.S. Pat. Nos. 6,291,160 and 6,680,192.

[00190] In general, expression vectors useful in recombinant DNA techniques are often in the form of plasmids. In the present disclosure, “plasmid” and “vector” can be used

interchangeably as the plasmid is the most commonly used form of vector. However, the present technology is intended to include such other forms of expression vectors that are not technically plasmids, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions. Such viral vectors permit infection of a subject and expression of a construct in that subject. In some embodiments, the expression control sequences are eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences encoding the anti-STEAP1 antibody, and the collection and purification of the anti-STEAP1 antibody, *e.g.*, cross-reacting anti-STEAP1 antibodies. *See generally*, U.S. 2002/0199213. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors contain selection markers, *e.g.*, ampicillin-resistance or hygromycin-resistance, to permit detection of those cells transformed with the desired DNA sequences. Vectors can also encode signal peptide, *e.g.*, pectate lyase, useful to direct the secretion of extracellular antibody fragments. *See* U.S. Pat. No. 5,576,195.

[00191] The recombinant expression vectors of the present technology comprise a nucleic acid encoding a protein with STEAP1 binding properties in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression that is operably-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, “operably-linked” is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term “regulatory sequence” is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, *e.g.*, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct

expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of polypeptide desired, *etc.* Typical regulatory sequences useful as promoters of recombinant polypeptide expression (*e.g.*, anti-STEAP1 antibody), include, *e.g.*, but are not limited to, promoters of 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization. In one embodiment, a polynucleotide encoding an anti-STEAP1 antibody of the present technology is operably-linked to an *ara B* promoter and expressible in a host cell. *See* U.S. Pat. 5,028,530. The expression vectors of the present technology can be introduced into host cells to thereby produce polypeptides or peptides, including fusion polypeptides, encoded by nucleic acids as described herein (*e.g.*, anti-STEAP1 antibody, *etc.*).

[00192] Another aspect of the present technology pertains to anti-STEAP1 antibody-expressing host cells, which contain a nucleic acid encoding one or more anti-STEAP1 antibodies. The recombinant expression vectors of the present technology can be designed for expression of an anti-STEAP1 antibody in prokaryotic or eukaryotic cells. For example, an anti-STEAP1 antibody can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors), fungal cells, *e.g.*, yeast, yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, *e.g.*, using T7 promoter regulatory sequences and T7 polymerase. Methods useful for the preparation and screening of polypeptides having a predetermined property, *e.g.*, anti-STEAP1 antibody, *via* expression of stochastically generated polynucleotide sequences has been previously described. *See* U.S. Pat. Nos. 5,763,192; 5,723,323; 5,814,476; 5,817,483; 5,824,514; 5,976,862; 6,492,107; 6,569,641.

[00193] Expression of polypeptides in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion polypeptides. Fusion vectors add a number of amino acids to a polypeptide encoded therein, usually to the amino terminus of the recombinant polypeptide.

Such fusion vectors typically serve three purposes: (i) to increase expression of recombinant polypeptide; (ii) to increase the solubility of the recombinant polypeptide; and (iii) to aid in the purification of the recombinant polypeptide by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant polypeptide to enable separation of the recombinant polypeptide from the fusion moiety subsequent to purification of the fusion polypeptide. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding polypeptide, or polypeptide A, respectively, to the target recombinant polypeptide.

[00194] Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69: 301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89). Methods for targeted assembly of distinct active peptide or protein domains to yield multifunctional polypeptides *via* polypeptide fusion has been described by Pack *et al.*, U.S. Pat. Nos. 6,294,353; 6,692,935. One strategy to maximize recombinant polypeptide expression, *e.g.*, an anti-STEAP1 antibody, in *E. coli* is to express the polypeptide in host bacteria with an impaired capacity to proteolytically cleave the recombinant polypeptide. *See, e.g.*, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in the expression host, *e.g.*, *E. coli* (*See, e.g.*, Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the present technology can be carried out by standard DNA synthesis techniques.

[00195] In another embodiment, the anti-STEAP1 antibody expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerevisiae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, *Cell* 30: 933-943, 1982), pJRY88 (Schultz *et al.*, *Gene* 54: 113-123, 1987), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego,

Calif.). Alternatively, an anti-STEAP1 antibody can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of polypeptides, *e.g.*, anti-STEAP1 antibody, in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith, *et al.*, *Mol. Cell. Biol.* 3: 2156-2165, 1983) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

[00196] In yet another embodiment, a nucleic acid encoding an anti-STEAP1 antibody of the present technology is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include, *e.g.*, but are not limited to, pCDM8 (Seed, *Nature* 329: 840, 1987) and pMT2PC (Kaufman, *et al.*, *EMBO J.* 6: 187-195, 1987). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells that are useful for expression of the anti-STEAP1 antibody of the present technology, see, *e.g.*, Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

[00197] In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid in a particular cell type (*e.g.*, tissue-specific regulatory elements). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, *Genes Dev.* 1: 268-277, 1987), lymphoid-specific promoters (Calame and Eaton, *Adv. Immunol.* 43: 235-275, 1988), promoters of T cell receptors (Winoto and Baltimore, *EMBO J.* 8: 729-733, 1989) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and Baltimore, *Cell* 33: 741-748, 1983.), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle, *Proc. Natl. Acad. Sci. USA* 86: 5473-5477, 1989), pancreas-specific promoters (Edlund, *et al.*, 1985. *Science* 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, *Science* 249: 374-379, 1990) and the α -fetoprotein promoter (Campes and Tilghman, *Genes Dev.* 3: 537-546, 1989).

[00198] Another aspect of the present methods pertains to host cells into which a recombinant expression vector of the present technology has been introduced. The terms “host cell” and “recombinant host cell” are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[00199] A host cell can be any prokaryotic or eukaryotic cell. For example, an anti-STEAP1 antibody can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells. Mammalian cells are a suitable host for expressing nucleotide segments encoding immunoglobulins or fragments thereof. See Winnacker, *From Genes To Clones*, (VCH Publishers, NY, 1987). A number of suitable host cell lines capable of secreting intact heterologous proteins have been developed in the art, and include Chinese hamster ovary (CHO) cell lines, various COS cell lines, HeLa cells, L cells and myeloma cell lines. In some embodiments, the cells are non-human. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer, and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Queen *et al.*, *Immunol. Rev.* 89: 49, 1986. Illustrative expression control sequences are promoters derived from endogenous genes, cytomegalovirus, SV40, adenovirus, bovine papillomavirus, and the like. Co *et al.*, *J Immunol.* 148: 1149, 1992. Other suitable host cells are known to those skilled in the art.

[00200] Vector DNA can be introduced into prokaryotic or eukaryotic cells *via* conventional transformation or transfection techniques. As used herein, the terms “transformation” and “transfection” are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, biolistics or viral-based transfection. Other methods used to transform mammalian cells include the use of polybrene, protoplast fusion, liposomes, electroporation, and microinjection (*See generally*, Sambrook *et al.*, *Molecular Cloning*). Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.*

(MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals. The vectors containing the DNA segments of interest can be transferred into the host cell by well-known methods, depending on the type of cellular host.

[00201] For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding the anti-STEAP1 antibody or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

[00202] A host cell that includes an anti-STEAP1 antibody of the present technology, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) recombinant anti-STEAP1 antibody. In one embodiment, the method comprises culturing the host cell (into which a recombinant expression vector encoding the anti-STEAP1 antibody has been introduced) in a suitable medium such that the anti-STEAP1 antibody is produced. In another embodiment, the method further comprises the step of isolating the anti-STEAP1 antibody from the medium or the host cell. Once expressed, collections of the anti-STEAP1 antibody, *e.g.*, the anti-STEAP1 antibodies or the anti-STEAP1 antibody-related polypeptides are purified from culture media and host cells. The anti-STEAP1 antibody can be purified according to standard procedures of the art, including HPLC purification, column chromatography, gel electrophoresis and the like. In one embodiment, the anti-STEAP1 antibody is produced in a host organism by the method of Boss *et al.*, U.S. Pat. No. 4,816,397. Usually, anti-STEAP1 antibody chains are expressed with signal sequences and are thus released to the culture media. However, if the anti-STEAP1 antibody chains are not naturally secreted by host cells, the anti-STEAP1 antibody chains can be released by treatment with mild detergent. Purification of recombinant polypeptides is well known in the art and includes ammonium sulfate precipitation, affinity chromatography purification

technique, column chromatography, ion exchange purification technique, gel electrophoresis and the like (*See generally* Scopes, *Protein Purification* (Springer-Verlag, N.Y., 1982).

[00203] Polynucleotides encoding anti-STEAP1 antibodies, *e.g.*, the anti-STEAP1 antibody coding sequences, can be incorporated in transgenes for introduction into the genome of a transgenic animal and subsequent expression in the milk of the transgenic animal. *See, e.g.*, U.S. Pat. Nos. 5,741,957, 5,304,489, and 5,849,992. Suitable transgenes include coding sequences for light and/or heavy chains in operable linkage with a promoter and enhancer from a mammary gland specific gene, such as casein or β -lactoglobulin. For production of transgenic animals, transgenes can be microinjected into fertilized oocytes, or can be incorporated into the genome of embryonic stem cells, and the nuclei of such cells transferred into enucleated oocytes.

[00204] *Single-Chain Antibodies.* In one embodiment, the anti-STEAP1 antibody of the present technology is a single-chain anti-STEAP1 antibody. According to the present technology, techniques can be adapted for the production of single-chain antibodies specific to a STEAP1 protein (*See, e.g.*, U.S. Pat. No. 4,946,778). Examples of techniques which can be used to produce single-chain Fvs and antibodies of the present technology include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston *et al.*, *Methods in Enzymology*, 203: 46-88, 1991; Shu, L. *et al.*, *Proc. Natl. Acad. Sci. USA*, 90: 7995-7999, 1993; and Skerra *et al.*, *Science* 240: 1038-1040, 1988.

[00205] *Chimeric and Humanized Antibodies.* In one embodiment, the anti-STEAP1 antibody of the present technology is a chimeric anti-STEAP1 antibody. In one embodiment, the anti-STEAP1 antibody of the present technology is a humanized anti-STEAP1 antibody. In one embodiment of the present technology, the donor and acceptor antibodies are monoclonal antibodies from different species. For example, the acceptor antibody is a human antibody (to minimize its antigenicity in a human), in which case the resulting CDR-grafted antibody is termed a “humanized” antibody.

[00206] Recombinant anti-STEAP1 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, can be made using standard recombinant DNA techniques, and are within the scope of the present technology. For some uses, including *in vivo* use of the anti-STEAP1 antibody of the present technology

in humans as well as use of these agents in *in vitro* detection assays, it is possible to use chimeric or humanized anti-STEAP1 antibodies. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art. Such useful methods include, *e.g.*, but are not limited to, methods described in International Application No. PCT/US86/02269; U.S. Pat. No. 5,225,539; European Patent No. 184187; European Patent No. 171496; European Patent No. 173494; PCT International Publication No. WO 86/01533; U.S. Pat. Nos. 4,816,567; 5,225,539; European Patent No. 125023; Better, *et al.*, 1988. *Science* 240: 1041-1043; Liu, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 3439-3443; Liu, *et al.*, 1987. *J. Immunol.* 139: 3521-3526; Sun, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 214-218; Nishimura, *et al.*, 1987. *Cancer Res.* 47: 999-1005; Wood, *et al.*, 1985. *Nature* 314: 446-449; Shaw, *et al.*, 1988. *J. Natl. Cancer Inst.* 80: 1553-1559; Morrison (1985) *Science* 229: 1202-1207; Oi, *et al.* (1986) *BioTechniques* 4: 214; Jones, *et al.*, 1986. *Nature* 321: 552-525; Verhoeyan, *et al.*, 1988. *Science* 239: 1534; Morrison, *Science* 229: 1202, 1985; Oi *et al.*, *BioTechniques* 4: 214, 1986; Gillies *et al.*, *J. Immunol. Methods*, 125: 191-202, 1989; U.S. Pat. No. 5,807,715; and Beidler, *et al.*, 1988. *J. Immunol.* 141: 4053-4060. For example, antibodies can be humanized using a variety of techniques including CDR-grafting (EP 0 239 400; WO 91/09967; U.S. Pat. No. 5,530,101; 5,585,089; 5,859,205; 6,248,516; EP460167), veneering or resurfacing (EP 0 592 106; EP 0 519 596; Padlan E. A., *Molecular Immunology*, 28: 489-498, 1991; Studnicka *et al.*, *Protein Engineering* 7: 805-814, 1994; Roguska *et al.*, *PNAS* 91: 969-973, 1994), and chain shuffling (U.S. Pat. No. 5,565,332). In one embodiment, a cDNA encoding a murine anti-STEAP1 monoclonal antibody is digested with a restriction enzyme selected specifically to remove the sequence encoding the Fc constant region, and the equivalent portion of a cDNA encoding a human Fc constant region is substituted (See Robinson *et al.*, PCT/US86/02269; Akira *et al.*, European Patent Application 184,187; Taniguchi, European Patent Application 171,496; Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 125,023; Better *et al.* (1988) *Science* 240: 1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84: 3439-3443; Liu *et al.* (1987) *J Immunol* 139: 3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84: 214-218; Nishimura *et al.* (1987) *Cancer Res* 47: 999-1005; Wood *et al.* (1985) *Nature* 314:

446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80: 1553-1559; U.S. Pat. No. 6,180,370; U.S. Pat. Nos. 6,300,064; 6,696,248; 6,706,484; 6,828,422.

[00207] In one embodiment, the present technology provides the construction of humanized anti-STEAP1 antibodies that are unlikely to induce a human anti-mouse antibody (hereinafter referred to as “HAMA”) response, while still having an effective antibody effector function. As used herein, the terms “human” and “humanized”, in relation to antibodies, relate to any antibody which is expected to elicit a therapeutically tolerable weak immunogenic response in a human subject. In one embodiment, the present technology provides for a humanized anti-STEAP1 antibodies, heavy and light chain immunoglobulins.

[00208] *CDR Antibodies.* In some embodiments, the anti-STEAP1 antibody of the present technology is an anti-STEAP1 CDR antibody. Generally the donor and acceptor antibodies used to generate the anti-STEAP1 CDR antibody are monoclonal antibodies from different species; typically the acceptor antibody is a human antibody (to minimize its antigenicity in a human), in which case the resulting CDR-grafted antibody is termed a “humanized” antibody. The graft may be of a single CDR (or even a portion of a single CDR) within a single V_H or V_L of the acceptor antibody, or can be of multiple CDRs (or portions thereof) within one or both of the V_H and V_L. Frequently, all three CDRs in all variable domains of the acceptor antibody will be replaced with the corresponding donor CDRs, though one needs to replace only as many as necessary to permit adequate binding of the resulting CDR-grafted antibody to STEAP1 protein. Methods for generating CDR-grafted and humanized antibodies are taught by Queen *et al.* U.S. Pat. No. 5,585,089; U.S. Pat. No. 5,693,761; U.S. Pat. No. 5,693,762; and Winter U.S. 5,225,539; and EP 0682040. Methods useful to prepare V_H and V_L polypeptides are taught by Winter *et al.*, U.S. Pat. Nos. 4,816,397; 6,291,158; 6,291,159; 6,291,161; 6,545,142; EP 0368684; EP0451216; and EP0120694.

[00209] After selecting suitable framework region candidates from the same family and/or the same family member, either or both the heavy and light chain variable regions are produced by grafting the CDRs from the originating species into the hybrid framework regions. Assembly of hybrid antibodies or hybrid antibody fragments having hybrid variable chain regions with regard to either of the above aspects can be accomplished using conventional methods known to those skilled in the art. For example, DNA sequences encoding the hybrid variable domains described herein (*i.e.*, frameworks based on the target

species and CDRs from the originating species) can be produced by oligonucleotide synthesis and/or PCR. The nucleic acid encoding CDR regions can also be isolated from the originating species antibodies using suitable restriction enzymes and ligated into the target species framework by ligating with suitable ligation enzymes. Alternatively, the framework regions of the variable chains of the originating species antibody can be changed by site-directed mutagenesis.

[00210] Since the hybrids are constructed from choices among multiple candidates corresponding to each framework region, there exist many combinations of sequences which are amenable to construction in accordance with the principles described herein. Accordingly, libraries of hybrids can be assembled having members with different combinations of individual framework regions. Such libraries can be electronic database collections of sequences or physical collections of hybrids.

[00211] This process typically does not alter the acceptor antibody's FRs flanking the grafted CDRs. However, one skilled in the art can sometimes improve antigen binding affinity of the resulting anti-STEAP1 CDR-grafted antibody by replacing certain residues of a given FR to make the FR more similar to the corresponding FR of the donor antibody. Suitable locations of the substitutions include amino acid residues adjacent to the CDR, or which are capable of interacting with a CDR (*See, e.g.*, US 5,585,089, especially columns 12-16). Or one skilled in the art can start with the donor FR and modify it to be more similar to the acceptor FR or a human consensus FR. Techniques for making these modifications are known in the art. Particularly if the resulting FR fits a human consensus FR for that position, or is at least 90% or more identical to such a consensus FR, doing so may not increase the antigenicity of the resulting modified anti-STEAP1 CDR-grafted antibody significantly compared to the same antibody with a fully human FR.

[00212] *Bispecific Antibodies (BsAbs).* A bispecific antibody is an antibody that can bind simultaneously to two targets that have a distinct structure, *e.g.*, two different target antigens, two different epitopes on the same target antigen, or a hapten and a target antigen or epitope on a target antigen. BsAbs can be made, for example, by combining heavy chains and/or light chains that recognize different epitopes of the same or different antigen. In some embodiments, by molecular function, a bispecific binding agent binds one antigen (or epitope) on one of its two binding arms (one VH/VL pair), and binds a different antigen (or

epitope) on its second arm (a different VH/VL pair). By this definition, a bispecific binding agent has two distinct antigen binding arms (in both specificity and CDR sequences), and is monovalent for each antigen to which it binds.

[00213] Bispecific antibodies (BsAb) and bispecific antibody fragments (BsFab) of the present technology have at least one arm that specifically binds to, for example, STEAP1 and at least one other arm that specifically binds to a second target antigen. In some embodiments, the second target antigen is an antigen or epitope of a B-cell, a T-cell, a myeloid cell, a plasma cell, or a mast-cell. Additionally or alternatively, in certain embodiments, the second target antigen is selected from the group consisting of CD3, CD4, CD8, CD20, CD19, CD21, CD23, CD46, CD80, HLA-DR, CD74, CD22, CD14, CD15, CD16, CD123, TCR gamma/delta, NKp46 and KIR. In certain embodiments, the BsAbs are capable of binding to tumor cells that express STEAP1 antigen on the cell surface. In some embodiments, the BsAbs have been engineered to facilitate killing of tumor cells by directing (or recruiting) cytotoxic T cells to a tumor site. Other exemplary BsAbs include those with a first antigen binding site specific for STEAP1 and a second antigen binding site specific for a small molecule hapten (*e.g.*, DTP A, IMP288, DOTA, DOTA-Bn, DOTA-desferrioxamine, other DOTA-chelates described herein, Biotin, fluorescein, or those disclosed in Goodwin, D A. *et al*, 1994, *Cancer Res.* 54(22):5937-5946). Additionally or alternatively, in certain embodiments, the bispecific antibody (or antigen binding fragment thereof) of the present technology comprises an additional V_H and/or V_L comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, and SEQ ID NO: 79. In some embodiments, the bispecific antibody (or antigen binding fragment thereof) of the present technology comprises an additional V_H sequence and an additional V_L sequence comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 76 and SEQ ID NO: 77, and SEQ ID NO: 78, and SEQ ID NO: 79.

[00214] A variety of bispecific fusion proteins can be produced using molecular engineering. For example, BsAbs have been constructed that either utilize the full immunoglobulin framework (*e.g.*, IgG), single chain variable fragment (scFv), or combinations thereof. In some embodiments, the bispecific fusion protein is divalent, comprising, for example, a scFv with a single binding site for one antigen and a Fab fragment with a single binding site for a second antigen. In some embodiments, the bispecific fusion

protein is divalent, comprising, for example, an scFv with a single binding site for one antigen and another scFv fragment with a single binding site for a second antigen. In other embodiments, the bispecific fusion protein is tetravalent, comprising, for example, an immunoglobulin (*e.g.*, IgG) with two binding sites for one antigen and two identical scFvs for a second antigen. BsAbs composed of two scFv units in tandem have been shown to be a clinically successful bispecific antibody format. In some embodiments, BsAbs comprise two single chain variable fragments (scFvs) in tandem have been designed such that an scFv that binds a tumor antigen (*e.g.*, STEAP1) is linked with an scFv that engages T cells (*e.g.*, by binding CD3). In this way, T cells are recruited to a tumor site such that they can mediate cytotoxic killing of the tumor cells. See *e.g.*, Dreier *et al.*, *J. Immunol.* 170:4397-4402 (2003); Bargou *et al.*, *Science* 321 :974- 977 (2008)). In some embodiments, BsAbs of the present technology comprise two single chain variable fragments (scFvs) in tandem have been designed such that an scFv that binds a tumor antigen (*e.g.*, STEAP1) is linked with an scFv that engages a small molecule DOTA hapten.

[00215] Recent methods for producing BsAbs include engineered recombinant monoclonal antibodies which have additional cysteine residues so that they crosslink more strongly than the more common immunoglobulin isotypes. See, *e.g.*, FitzGerald *et al.*, *Protein Eng.* 10(10):1221-1225 (1997). Another approach is to engineer recombinant fusion proteins linking two or more different single-chain antibody or antibody fragment segments with the needed dual specificities. See, *e.g.*, Coloma *et al.*, *Nature Biotech.* 15:159-163 (1997). A variety of bispecific fusion proteins can be produced using molecular engineering.

[00216] Bispecific fusion proteins linking two or more different single-chain antibodies or antibody fragments are produced in a similar manner. Recombinant methods can be used to produce a variety of fusion proteins. In some certain embodiments, a BsAb according to the present technology comprises an immunoglobulin, which immunoglobulin comprises a heavy chain and a light chain, and an scFv. In some certain embodiments, the scFv is linked to the C-terminal end of the heavy chain of any STEAP1 immunoglobulin disclosed herein. In some certain embodiments, scFvs are linked to the C-terminal end of the light chain of any STEAP1 immunoglobulin disclosed herein. In various embodiments, scFvs are linked to heavy or light chains *via* a linker sequence. Appropriate linker sequences necessary for the in-frame connection of the heavy chain Fd to the scFv are introduced into the VL and V_{kappa}

domains through PCR reactions. The DNA fragment encoding the scFv is then ligated into a staging vector containing a DNA sequence encoding the CH1 domain. The resulting scFv-CH1 construct is excised and ligated into a vector containing a DNA sequence encoding the V_H region of a STEAP1 antibody. The resulting vector can be used to transfect an appropriate host cell, such as a mammalian cell for the expression of the bispecific fusion protein.

[00217] In some embodiments, a linker is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acids in length. In some embodiments, a linker is characterized in that it tends not to adopt a rigid three-dimensional structure, but rather provides flexibility to the polypeptide (*e.g.*, first and/or second antigen binding sites). In some embodiments, a linker is employed in a BsAb described herein based on specific properties imparted to the BsAb such as, for example, an increase in stability. In some embodiments, a BsAb of the present technology comprises a G₄S linker. In some certain embodiments, a BsAb of the present technology comprises a (G₄S)_n linker, wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more.

[00218] *Self assembly disassembly (SADA) Conjugates.* In some embodiments, the anti-STEAP1 antibodies of the present technology comprise one or more SADA domains. SADA domains can be designed and/or tailored to achieve environmentally-dependent multimerization with beneficial kinetic, thermodynamic, and/or pharmacologic properties. For example, it is recognized that SADA domains may be part of a conjugate that permit effective delivery of a payload to a target site of interest while minimizing the risk off-target interactions. The anti-STEAP1 antibodies of the present technology may comprise a SADA domain linked to one or more binding domains. In some embodiments, such conjugates are characterized in that they multimerize to form a complex of a desired size under relevant conditions (*e.g.*, in a solution in which the conjugate is present above a threshold concentration or pH and/or when present at a target site characterized by a relevant level or density of receptors for the payload), and disassemble to a smaller form under other conditions (*e.g.*, absent the relevant environmental multimerization trigger).

[00219] A SADA conjugate may have improved characteristics compared to a conjugate without a SADA domain. In some embodiments, improved characteristics of a multimeric

conjugate include: increased avidity /binding to a target, increased specificity for target cells or tissues, and/or extended initial serum half-life. In some embodiments, improved characteristics include that through dissociation to smaller states (*e.g.*, dimeric or monomeric), a SADA conjugate exhibits reduced non-specific binding, decreased toxicity, and/or improved renal clearance. In some embodiments, a SADA conjugate comprises a SADA polypeptide having an amino acid sequence that shows at least 75% identity with that of a human homo-multimerizing polypeptide and is characterized by one or more multimerization dissociation constants (K_D).

[00220] In some embodiments, a SADA conjugate is constructed and arranged so that it adopts a first multimerization state and one or more higher-order multimerization states. In some embodiments, a first multimerization state is less than about ~70 kDa in size. In some embodiments, a first multimerization state is an unmultimerized state (*e.g.*, a monomer or a dimer). In some embodiments, a first multimerization state is a monomer. In some embodiments, a first multimerization state is a dimer. In some embodiments, a first multimerization state is a multimerized state (*e.g.*, a trimer or a tetramer). In some embodiments, a higher-order multimerization states is a homo-tetramer or higher-order homo-multimer greater than 150 kDa in size. In some embodiments, a higher-order homo-multimerized conjugate is stable in aqueous solution when the conjugate is present at a concentration above the SADA polypeptide K_D . In some embodiments, a SADA conjugate transitions from a higher-order multimerization state(s) to a first multimerization state under physiological conditions when the concentration of the conjugate is below the SADA polypeptide K_D .

[00221] In some embodiments, a SADA polypeptide is covalently linked to a binding domain via a linker. Any suitable linker known in the art can be used. In some embodiments, a SADA polypeptide is linked to a binding domain via a polypeptide linker. In some embodiments, a polypeptide linker is a Gly-Ser linker. In some embodiments, a polypeptide linker is or comprises a sequence of (GGGGS) n , where n represents the number of repeating GGGGS units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments, a binding domain is directly fused to a SADA polypeptide.

[00222] In some embodiments, a SADA domain is a human polypeptide or a fragment and/or derivative thereof. In some embodiments, a SADA domain is substantially non-immunogenic in a human. In some embodiments, a SADA polypeptide is stable as a multimer. In some embodiments, a SADA polypeptide lacks unpaired cysteine residues. In some embodiments, a SADA polypeptide does not have large exposed hydrophobic surfaces. In some embodiments, a SADA domain has or is predicted to have a structure comprising helical bundles that can associate in a parallel or anti-parallel orientation. In some embodiments, a SADA polypeptide is capable of reversible multimerization. In some embodiments, a SADA domain is a tetramerization domain, a heptamerization domain, a hexamerization domain or an octamerization domain. In certain embodiments, a SADA domain is a tetramerization domain. In some embodiments, a SADA domain is composed of a multimerization domains which are each composed of helical bundles that associate in a parallel or anti-parallel orientation. In some embodiments, a SADA domain is selected from the group of one of the following human proteins: p53, p63, p73, heterogeneous nuclear Ribonucleoprotein C (hnRNPC), N-terminal domain of Synaptosomal-associated protein 23 (SNAP-23), Stefin B (Cystatin B), Potassium voltage-gated channel subfamily KQT member 4 (KCNQ4), or Cyclin-D-related protein (CBFA2T1). Examples of suitable SADA domains are described in PCT/US2018/031235, which is hereby incorporated by reference in its entirety. Provided below are polypeptide sequences for exemplary SADA domains.

[00223] Human p53 tetramerization domain amino acid sequence (321-359)
KPLDGEYFTLQIRGRERFEMFRELNEALELKDAQAGKEP (SEQ ID NO: 52)

[00224] Human p63 tetramerization domain amino acid sequence (396-450)
RSPDDELLYLPVRGRETYEMLLKIKESLELMQYLPQHTIETYRQQQQQQHQHLLQKQ
(SEQ ID NO: 53)

[00225] Human p73 tetramerization domain amino acid sequence (348-399)
RHGDEDTYYLQVRGRENFEILMKLKESLELMELVPQPLVDSYRQQQQQLLQRP (SEQ
ID NO: 54).

[00226] Human HNRNPC tetramerization domain amino acid sequence (194-220)
QAIKKELTQIKQKVDSLLENLEKIEKE (SEQ ID NO: 55)

[00227] Human SNAP-23 tetramerization domain amino acid sequence (23-76)
STRRLGLAIESQDAGIKTITMLDEQKEQLNRIEEGLDQINKDMRETEKTLTEL (SEQ ID NO: 56)

[00228] Human Stefin B tetramerization domain amino acid sequence (2-98)
MCGAPSATQPATAETQHIADQVRSQLEEKENKKFPVFKAVSFKSQVVAGTNYFIKV
HVGDEDFVHLRVFQSLPHENKPLTLSNYQTNKAKHDELTYP (SEQ ID NO: 57)

[00229] KCNQ4 tetramerization domain amino acid sequence (611-640)
DEISMMGRVVKVEKQVQSIEHKLDLLLLGFY (SEQ ID NO: 58)

[00230] CBFA2T1 tetramerization domain amino acid sequence (462-521)
TVAEAKRQAAEDALAVINQQEDSSWSCWNCGRKASETCSGCNTARYCGSFCQHKD
WEKHH (SEQ ID NO: 59)

[00231] In some embodiments, a SADA polypeptide is or comprises a tetramerization domain of p53, p63, p73, heterogeneous nuclear Ribonucleoprotein C (hnRNP C), N-terminal domain of Synaptosomal-associated protein 23 (SNAP-23), Stefin B (Cystatin B), Potassium voltage-gated channel subfamily KQT member 4 (KCNQ4), or Cyclin-D-related protein (CBFA2T1). In some embodiments, a SADA polypeptide is or comprises a sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence as set forth in any one of SEQ ID NOs: 52-59.

[00232] *Fc Modifications.* In some embodiments, the anti-STEAP1 antibodies of the present technology comprise a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild-type Fc region (or the parental Fc region), such that said molecule has an altered affinity for an Fc receptor (*e.g.*, an FcγR), provided that said variant Fc region does not have a substitution at positions that make a direct contact with Fc receptor based on crystallographic and structural analysis of Fc-Fc receptor interactions such as those disclosed by Sondermann *et al.*, *Nature*, 406:267-273 (2000). Examples of positions within the Fc region that make a direct contact with an Fc receptor such as an FcγR, include amino acids 234-239 (hinge region), amino acids 265-269 (B/C loop), amino acids 297-299 (C7E loop), and amino acids 327-332 (F/G) loop.

[00233] In some embodiments, an anti-STEAP1 antibody of the present technology has an altered affinity for activating and/or inhibitory receptors, having a variant Fc region with one

or more amino acid modifications, wherein said one or more amino acid modification is a N297 substitution with alanine, or a K322 substitution with alanine.

[00234] *Glycosylation Modifications.* In some embodiments, anti-STEAP1 antibodies of the present technology have an Fc region with variant glycosylation as compared to a parent Fc region. In some embodiments, variant glycosylation includes the absence of fucose; in some embodiments, variant glycosylation results from expression in GnT1-deficient CHO cells.

[00235] In some embodiments, the antibodies of the present technology, may have a modified glycosylation site relative to an appropriate reference antibody that binds to an antigen of interest (*e.g.*, STEAP1), without altering the functionality of the antibody, *e.g.*, binding activity to the antigen. As used herein, "glycosylation sites" include any specific amino acid sequence in an antibody to which an oligosaccharide (*i.e.*, carbohydrates containing two or more simple sugars linked together) will specifically and covalently attach.

[00236] Oligosaccharide side chains are typically linked to the backbone of an antibody *via* either N-or O-linkages. N-linked glycosylation refers to the attachment of an oligosaccharide moiety to the side chain of an asparagine residue. O-linked glycosylation refers to the attachment of an oligosaccharide moiety to a hydroxyamino acid, *e.g.*, serine, threonine. For example, an Fc-glycoform (hSTEAP1-IgGln) that lacks certain oligosaccharides including fucose and terminal N- acetylglucosamine may be produced in special CHO cells and exhibit enhanced ADCC effector function.

[00237] In some embodiments, the carbohydrate content of an immunoglobulin-related composition disclosed herein is modified by adding or deleting a glycosylation site. Methods for modifying the carbohydrate content of antibodies are well known in the art and are included within the present technology, see, *e.g.*, U.S. Patent No. 6,218,149; EP 0359096B1; U.S. Patent Publication No. US 2002/0028486; International Patent Application Publication WO 03/035835; U.S. Patent Publication No. 2003/0115614; U.S. Patent No. 6,218,149; U.S. Patent No. 6,472,511; all of which are incorporated herein by reference in their entirety. In some embodiments, the carbohydrate content of an antibody (or relevant portion or component thereof) is modified by deleting one or more endogenous carbohydrate moieties of the antibody. In some certain embodiments, the present technology includes deleting the

glycosylation site of the Fc region of an antibody, by modifying position 297 from asparagine to alanine.

[00238] Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. Engineered glycoforms may be generated by any method known to one skilled in the art, for example by using engineered or variant expression strains, by co-expression with one or more enzymes, for example N-acetylglucosaminyltransferase III (GnTIII), by expressing a molecule comprising an Fc region in various organisms or cell lines from various organisms, or by modifying carbohydrate(s) after the molecule comprising Fc region has been expressed. Methods for generating engineered glycoforms are known in the art, and include but are not limited to those described in Umana *et al.*, 1999, *Nat. Biotechnol.* 17: 176-180; Davies *et al.*, 2001, *Biotechnol. Bioeng.* 74:288-294; Shields *et al.*, 2002, *J. Biol. Chem.* 277:26733-26740; Shinkawa *et al.*, 2003, *J. Biol. Chem.* 278:3466-3473; U.S. Patent No. 6,602,684; U.S. Patent Application Serial No. 10/277,370; U.S. Patent Application Serial No. 10/113,929; International Patent Application Publications WO 00/61739A1 ; WO 01/292246A1; WO 02/311140A1; WO 02/30954A1; POTILLEGENT™ technology (Biowa, Inc. Princeton, N.J.); GLYCOMAB™ glycosylation engineering technology (GLYCART biotechnology AG, Zurich, Switzerland); each of which is incorporated herein by reference in its entirety. See, *e.g.*, International Patent Application Publication WO 00/061739; U.S. Patent Application Publication No. 2003/0115614; Okazaki *et al.*, 2004, *JMB*, 336: 1239-49.

[00239] *Fusion Proteins.* In one embodiment, the anti-STEAP1 antibody of the present technology is a fusion protein. The anti-STEAP1 antibodies of the present technology, when fused to a second protein, can be used as an antigenic tag. Examples of domains that can be fused to polypeptides include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but can occur through linker sequences. Moreover, fusion proteins of the present technology can also be engineered to improve characteristics of the anti-STEAP1 antibodies. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of the anti-STEAP1 antibody to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties can be added to an anti-STEAP1 antibody to facilitate purification. Such regions can be

removed prior to final preparation of the anti-STEAP1 antibody. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art. The anti-STEAP1 antibody of the present technology can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In select embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., Chatsworth, Calif), among others, many of which are commercially available. As described in Gentz *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 821-824, 1989, for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. Wilson *et al.*, *Cell* 37: 767, 1984.

[00240] Thus, any of these above fusion proteins can be engineered using the polynucleotides or the polypeptides of the present technology. Also, in some embodiments, the fusion proteins described herein show an increased half-life *in vivo*.

[00241] Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can be more efficient in binding and neutralizing other molecules compared to the monomeric secreted protein or protein fragment alone. Fountoulakis *et al.*, *J. Biochem.* 270: 3958-3964, 1995.

[00242] Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or a fragment thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, *e.g.*, improved pharmacokinetic properties. See EP-A 0232 262. Alternatively, deleting or modifying the Fc part after the fusion protein has been expressed, detected, and purified, may be desired. For example, the Fc portion can hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, *e.g.*, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. Bennett *et al.*, *J. Molecular Recognition* 8: 52-58, 1995; Johanson *et al.*, *J. Biol. Chem.*, 270: 9459-9471, 1995.

[00243] *Labeled Anti-STEAP1 antibodies.* In one embodiment, the anti-STEAP1 antibody of the present technology is coupled with a label moiety, *i.e.*, detectable group. The

particular label or detectable group conjugated to the anti-STEAP1 antibody is not a critical aspect of the technology, so long as it does not significantly interfere with the specific binding of the anti-STEAP1 antibody of the present technology to the STEAP1 protein. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well-developed in the field of immunoassays and imaging. In general, almost any label useful in such methods can be applied to the present technology. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Labels useful in the practice of the present technology include magnetic beads (*e.g.*, Dynabeads™), fluorescent dyes (*e.g.*, fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (*e.g.*, ^3H , ^{14}C , ^{35}S , ^{125}I , ^{121}I , ^{131}I , ^{112}In , $^{99\text{m}}\text{Tc}$), other imaging agents such as microbubbles (for ultrasound imaging), ^{18}F , ^{11}C , ^{15}O , (for Positron emission tomography), $^{99\text{m}}\text{Tc}$, ^{111}In (for Single photon emission tomography), enzymes (*e.g.*, horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and calorimetric labels such as colloidal gold or colored glass or plastic (*e.g.*, polystyrene, polypropylene, latex, and the like) beads. Patents that describe the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241, each incorporated herein by reference in their entirety and for all purposes. *See also* Handbook of Fluorescent Probes and Research Chemicals (6th Ed., Molecular Probes, Inc., Eugene OR.).

[00244] The label can be coupled directly or indirectly to the desired component of an assay according to methods well known in the art. As indicated above, a wide variety of labels can be used, with the choice of label depending on factors such as required sensitivity, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

[00245] Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (*e.g.*, biotin) is covalently bound to the molecule. The ligand then binds to an anti-ligand (*e.g.*, streptavidin) molecule which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. A number of ligands and anti-ligands can be used. Where a ligand has a natural anti-ligand, *e.g.*, biotin, thyroxine, and cortisol, it can be used in conjunction with the labeled, naturally-occurring anti-ligands. Alternatively, any haptenic or

antigenic compound can be used in combination with an antibody, *e.g.*, an anti-STEAP1 antibody.

[00246] The molecules can also be conjugated directly to signal generating compounds, *e.g.*, by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds useful as labeling moieties, include, but are not limited to, *e.g.*, fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, and the like. Chemiluminescent compounds useful as labeling moieties, include, but are not limited to, *e.g.*, luciferin, and 2,3-dihydrophthalazinediones, *e.g.*, luminol. For a review of various labeling or signal-producing systems which can be used, see U.S. Pat. No. 4,391,904.

[00247] Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it can be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence can be detected visually, by means of photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels can be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Finally, simple colorimetric labels can be detected simply by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

[00248] Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies, *e.g.*, the anti-STEAP1 antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of the target antibody is detected by simple visual inspection.

B. Identifying and Characterizing the Anti-STEAP1 Antibodies of the Present Technology

[00249] *Methods for identifying and/or screening the anti-STEAP1 antibodies of the present technology.* Methods useful to identify and screen antibodies against STEAP1

polypeptides for those that possess the desired specificity to STEAP1 protein (*e.g.*, those that bind to the second ECD of STEAP1 protein) include any immunologically-mediated techniques known within the art. Components of an immune response can be detected *in vitro* by various methods that are well known to those of ordinary skill in the art. For example, (1) cytotoxic T lymphocytes can be incubated with radioactively labeled target cells and the lysis of these target cells detected by the release of radioactivity; (2) helper T lymphocytes can be incubated with antigens and antigen presenting cells and the synthesis and secretion of cytokines measured by standard methods (Windhagen A *et al.*, *Immunity*, 2: 373-80, 1995); (3) antigen presenting cells can be incubated with whole protein antigen and the presentation of that antigen on MHC detected by either T lymphocyte activation assays or biophysical methods (Harding *et al.*, *Proc. Natl. Acad. Sci.*, 86: 4230-4, 1989); (4) mast cells can be incubated with reagents that cross-link their Fc-epsilon receptors and histamine release measured by enzyme immunoassay (Siraganian *et al.*, *TIPS*, 4: 432-437, 1983); and (5) enzyme-linked immunosorbent assay (ELISA).

[00250] Similarly, products of an immune response in either a model organism (*e.g.*, mouse) or a human subject can also be detected by various methods that are well known to those of ordinary skill in the art. For example, (1) the production of antibodies in response to vaccination can be readily detected by standard methods currently used in clinical laboratories, *e.g.*, an ELISA; (2) the migration of immune cells to sites of inflammation can be detected by scratching the surface of skin and placing a sterile container to capture the migrating cells over scratch site (Peters *et al.*, *Blood*, 72: 1310-5, 1988); (3) the proliferation of peripheral blood mononuclear cells (PBMCs) in response to mitogens or mixed lymphocyte reaction can be measured using ³H-thymidine; (4) the phagocytic capacity of granulocytes, macrophages, and other phagocytes in PBMCs can be measured by placing PBMCs in wells together with labeled particles (Peters *et al.*, *Blood*, 72: 1310-5, 1988); and (5) the differentiation of immune system cells can be measured by labeling PBMCs with antibodies to CD molecules such as CD4 and CD8 and measuring the fraction of the PBMCs expressing these markers.

[00251] In one embodiment, anti-STEAP1 antibodies of the present technology are selected using display of STEAP1 peptides on the surface of replicable genetic packages. *See, e.g.*, U.S. Pat. Nos. 5,514,548; 5,837,500; 5,871,907; 5,885,793; 5,969,108; 6,225,447;

6,291,650; 6,492,160; EP 585 287; EP 605522; EP 616640; EP 1024191; EP 589 877; EP 774 511; EP 844 306. Methods useful for producing/selecting a filamentous bacteriophage particle containing a phagemid genome encoding for a binding molecule with a desired specificity has been described. *See, e.g.*, EP 774 511; US 5871907; US 5969108; US 6225447; US 6291650; US 6492160.

[00252] In some embodiments, anti-STEAP1 antibodies of the present technology are selected using display of STEAP1 peptides on the surface of a yeast host cell. Methods useful for the isolation of scFv polypeptides by yeast surface display have been described by Kieke *et al.*, *Protein Eng.* 1997 Nov; 10(11): 1303-10.

[00253] In some embodiments, anti-STEAP1 antibodies of the present technology are selected using ribosome display. Methods useful for identifying ligands in peptide libraries using ribosome display have been described by Mattheakis *et al.*, *Proc. Natl. Acad. Sci. USA* 91: 9022-26, 1994; and Hanes *et al.*, *Proc. Natl. Acad. Sci. USA* 94: 4937-42, 1997.

[00254] In certain embodiments, anti-STEAP1 antibodies of the present technology are selected using tRNA display of STEAP1 peptides. Methods useful for *in vitro* selection of ligands using tRNA display have been described by Merryman *et al.*, *Chem. Biol.*, 9: 741-46, 2002.

[00255] In one embodiment, anti-STEAP1 antibodies of the present technology are selected using RNA display. Methods useful for selecting peptides and proteins using RNA display libraries have been described by Roberts *et al.* *Proc. Natl. Acad. Sci. USA*, 94: 12297-302, 1997; and Nemoto *et al.*, *FEBS Lett.*, 414: 405-8, 1997. Methods useful for selecting peptides and proteins using unnatural RNA display libraries have been described by Frankel *et al.*, *Curr. Opin. Struct. Biol.*, 13: 506-12, 2003.

[00256] In some embodiments, anti-STEAP1 antibodies of the present technology are expressed in the periplasm of gram negative bacteria and mixed with labeled STEAP1 protein. *See* WO 02/34886. In clones expressing recombinant polypeptides with affinity for STEAP1 protein, the concentration of the labeled STEAP1 protein bound to the anti-STEAP1 antibodies is increased and allows the cells to be isolated from the rest of the library as described in Harvey *et al.*, *Proc. Natl. Acad. Sci.* 22: 9193-98 2004 and U.S. Pat. Publication No. 2004/0058403.

[00257] After selection of the desired anti-STEAP1 antibodies, it is contemplated that said antibodies can be produced in large volume by any technique known to those skilled in the art, *e.g.*, prokaryotic or eukaryotic cell expression and the like. The anti-STEAP1 antibodies which are, *e.g.*, but not limited to, anti-STEAP1 hybrid antibodies or fragments can be produced by using conventional techniques to construct an expression vector that encodes an antibody heavy chain in which the CDRs and, if necessary, a minimal portion of the variable region framework, that are required to retain original species antibody binding specificity (as engineered according to the techniques described herein) are derived from the originating species antibody and the remainder of the antibody is derived from a target species immunoglobulin which can be manipulated as described herein, thereby producing a vector for the expression of a hybrid antibody heavy chain.

[00258] *Measurement of STEAP1 Binding.* In some embodiments, a STEAP1 binding assay refers to an assay format wherein STEAP1 protein and an anti-STEAP1 antibody are mixed under conditions suitable for binding between the STEAP1 protein and the anti-STEAP1 antibody and assessing the amount of binding between the STEAP1 protein and the anti-STEAP1 antibody. The amount of binding is compared with a suitable control, which can be the amount of binding in the absence of the STEAP1 protein, the amount of the binding in the presence of a non-specific immunoglobulin composition, or both. The amount of binding can be assessed by any suitable method. Binding assay methods include, *e.g.*, ELISA, radioimmunoassays, scintillation proximity assays, fluorescence energy transfer assays, liquid chromatography, membrane filtration assays, and the like. Biophysical assays for the direct measurement of STEAP1 protein binding to anti-STEAP1 antibody are, *e.g.*, nuclear magnetic resonance, fluorescence, fluorescence polarization, surface plasmon resonance (BIAcore chips) and the like. Specific binding is determined by standard assays known in the art, *e.g.*, radioligand binding assays, ELISA, FRET, immunoprecipitation, SPR, NMR (2D-NMR), mass spectroscopy and the like. If the specific binding of a candidate anti-STEAP1 antibody is at least 1 percent greater than the binding observed in the absence of the candidate anti-STEAP1 antibody, the candidate anti-STEAP1 antibody is useful as an anti-STEAP1 antibody of the present technology.

Uses of the Anti-STEAP1 Antibodies of the Present Technology

[00259] *General.* The anti-STEAP1 antibodies of the present technology are useful in methods known in the art relating to the localization and/or quantitation of STEAP1 protein (*e.g.*, for use in measuring levels of the STEAP1 protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the polypeptide, and the like). Antibodies of the present technology are useful to isolate a STEAP1 protein by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-STEAP1 antibody of the present technology can facilitate the purification of natural immunoreactive STEAP1 proteins from biological samples, *e.g.*, mammalian sera or cells as well as recombinantly-produced immunoreactive STEAP1 proteins expressed in a host system. Moreover, anti-STEAP1 antibodies can be used to detect an immunoreactive STEAP1 protein (*e.g.*, in plasma, a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the immunoreactive polypeptide. The anti-STEAP1 antibodies of the present technology can be used diagnostically to monitor immunoreactive STEAP1 protein levels in tissue as part of a clinical testing procedure, *e.g.*, to determine the efficacy of a given treatment regimen. As noted above, the detection can be facilitated by coupling (*i.e.*, physically linking) the anti-STEAP1 antibodies of the present technology to a detectable substance.

[00260] *Detection of STEAP1 protein.* An exemplary method for detecting the presence or absence of an immunoreactive STEAP1 protein in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with an anti-STEAP1 antibody of the present technology capable of detecting an immunoreactive STEAP1 protein such that the presence of an immunoreactive STEAP1 protein is detected in the biological sample. Detection may be accomplished by means of a detectable label attached to the antibody.

[00261] The term “labeled” with regard to the anti-STEAP1 antibody is intended to encompass direct labeling of the antibody by coupling (*i.e.*, physically linking) a detectable substance to the antibody, as well as indirect labeling of the antibody by reactivity with another compound that is directly labeled, such as a secondary antibody. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled

secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin.

[00262] In some embodiments, the anti-STEAP1 antibodies disclosed herein are conjugated to one or more detectable labels. For such uses, anti-STEAP1 antibodies may be detectably labeled by covalent or non-covalent attachment of a chromogenic, enzymatic, radioisotopic, isotopic, fluorescent, toxic, chemiluminescent, nuclear magnetic resonance contrast agent or other label.

[00263] Examples of suitable chromogenic labels include diaminobenzidine and 4-hydroxyazo-benzene-2-carboxylic acid. Examples of suitable enzyme labels include malate dehydrogenase, staphylococcal nuclease, Δ -5-steroid isomerase, yeast-alcohol dehydrogenase, α -glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, β -galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholine esterase.

[00264] Examples of suitable radioisotopic labels include ^3H , ^{111}In , ^{125}I , ^{131}I , ^{32}P , ^{35}S , ^{14}C , ^{51}Cr , ^{57}To , ^{58}Co , ^{59}Fe , ^{75}Se , ^{152}Eu , ^{90}Y , ^{67}Cu , ^{217}Ci , ^{211}At , ^{212}Pb , ^{47}Sc , ^{109}Pd , *etc.* ^{111}In is an exemplary isotope where *in vivo* imaging is used since it avoids the problem of dehalogenation of the ^{125}I or ^{131}I -labeled STEAP1-binding antibodies by the liver. In addition, this isotope has a more favorable gamma emission energy for imaging (Perkins *et al*, *Eur. J. Nucl. Med.* 70:296-301 (1985); Carasquillo *et al.*, *J. Nucl. Med.* 25:281-287 (1987)). For example, ^{111}In coupled to monoclonal antibodies with 1-(P-isothiocyanatobenzyl)-DPTA exhibits little uptake in non-tumorous tissues, particularly the liver, and enhances specificity of tumor localization (Esteban *et al.*, *J. Nucl. Med.* 28:861-870 (1987)). Examples of suitable non-radioactive isotopic labels include ^{157}Gd , ^{55}Mn , ^{162}Dy , ^{52}Tr , and ^{56}Fe .

[00265] Examples of suitable fluorescent labels include an ^{152}Eu label, a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, a Green Fluorescent Protein (GFP) label, an o-phthaldehyde label, and a fluorescamine label. Examples of suitable toxin labels include diphtheria toxin, ricin, and cholera toxin.

[00266] Examples of chemiluminescent labels include a luminol label, an isoluminol label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, and an aequorin label. Examples of nuclear magnetic resonance contrasting agents include heavy metal nuclei such as Gd, Mn, and iron.

[00267] The detection method of the present technology can be used to detect an immunoreactive STEAP1 protein in a biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of an immunoreactive STEAP1 protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, radioimmunoassay, and immunofluorescence. Furthermore, *in vivo* techniques for detection of an immunoreactive STEAP1 protein include introducing into a subject a labeled anti-STEAP1 antibody. For example, the anti-STEAP1 antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques. In one embodiment, the biological sample contains STEAP1 protein molecules from the test subject.

[00268] *Immunoassay and Imaging.* An anti-STEAP1 antibody of the present technology can be used to assay immunoreactive STEAP1 protein levels in a biological sample (*e.g.*, human plasma) using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. Jalkanen, M. *et al.*, *J. Cell. Biol.* 101: 976-985, 1985; Jalkanen, M. *et al.*, *J. Cell. Biol.* 105: 3087-3096, 1987. Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes or other radioactive agent, such as iodine (^{125}I , ^{121}I , ^{131}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{112}In), and technetium ($^{99\text{m}}\text{Tc}$), and fluorescent labels, such as fluorescein, rhodamine, and green fluorescent protein (GFP), as well as biotin.

[00269] In addition to assaying immunoreactive STEAP1 protein levels in a biological sample, anti-STEAP1 antibodies of the present technology may be used for *in vivo* imaging of STEAP1. Antibodies useful for this method include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable

markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which can be incorporated into the anti-STEAP1 antibodies by labeling of nutrients for the relevant scFv clone.

[00270] An anti-STEAP1 antibody which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (*e.g.*, ^{131}I , ^{112}In , $^{99\text{m}}\text{Tc}$), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (*e.g.*, parenterally, subcutaneously, or intraperitoneally) into the subject. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of $^{99\text{m}}\text{Tc}$. The labeled anti-STEAP1 antibody will then accumulate at the location of cells which contain the specific target polypeptide. For example, labeled anti-STEAP1 antibodies of the present technology will accumulate within the subject in cells and tissues in which the STEAP1 protein has localized.

[00271] Thus, the present technology provides a diagnostic method of a medical condition, which involves: (a) assaying the expression of immunoreactive STEAP1 protein by measuring binding of an anti-STEAP1 antibody of the present technology in cells or body fluid of an individual; (b) comparing the amount of immunoreactive STEAP1 protein present in the sample with a standard reference, wherein an increase or decrease in immunoreactive STEAP1 protein levels compared to the standard is indicative of a medical condition.

[00272] *Affinity Purification.* The anti-STEAP1 antibodies of the present technology may be used to purify immunoreactive STEAP1 protein from a sample. In some embodiments, the antibodies are immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir *et al.*, "Handbook of Experimental Immunology" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10 (1986); Jacoby *et al.*, *Meth. Enzym.* 34 Academic Press, N.Y. (1974)).

[00273] The simplest method to bind the antigen to the antibody-support matrix is to collect the beads in a column and pass the antigen solution down the column. The efficiency

of this method depends on the contact time between the immobilized antibody and the antigen, which can be extended by using low flow rates. The immobilized antibody captures the antigen as it flows past. Alternatively, an antigen can be contacted with the antibody-support matrix by mixing the antigen solution with the support (*e.g.*, beads) and rotating or rocking the slurry, allowing maximum contact between the antigen and the immobilized antibody. After the binding reaction has been completed, the slurry is passed into a column for collection of the beads. The beads are washed using a suitable washing buffer and then the pure or substantially pure antigen is eluted.

[00274] An antibody or polypeptide of interest can be conjugated to a solid support, such as a bead. In addition, a first solid support such as a bead can also be conjugated, if desired, to a second solid support, which can be a second bead or other support, by any suitable means, including those disclosed herein for conjugation of a polypeptide to a support. Accordingly, any of the conjugation methods and means disclosed herein with reference to conjugation of a polypeptide to a solid support can also be applied for conjugation of a first support to a second support, where the first and second solid support can be the same or different.

[00275] Appropriate linkers, which can be cross-linking agents, for use for conjugating a polypeptide to a solid support include a variety of agents that can react with a functional group present on a surface of the support, or with the polypeptide, or both. Reagents useful as cross-linking agents include homo-bi-functional and, in particular, hetero-bi-functional reagents. Useful bi-functional cross-linking agents include, but are not limited to, *N*-SIAB, dimaleimide, DTNB, *N*-SATA, *N*-SPDP, SMCC and 6-HYNIC. A cross-linking agent can be selected to provide a selectively cleavable bond between a polypeptide and the solid support. For example, a photolabile cross-linker, such as 3-amino-(2-nitrophenyl)propionic acid can be employed as a means for cleaving a polypeptide from a solid support. (Brown *et al.*, *Mol. Divers.*, pp, 4-12 (1995); Rothschild *et al.*, *Nucl. Acids Res.*, 24:351-66 (1996); and US. Pat. No. 5,643,722). Other cross-linking reagents are well-known in the art. (*See, e.g.*, Wong (1991), *supra*, and Hermanson (1996), *supra*).

[00276] An antibody or polypeptide can be immobilized on a solid support, such as a bead, through a covalent amide bond formed between a carboxyl group functionalized bead and the amino terminus of the polypeptide or, conversely, through a covalent amide bond formed

between an amino group functionalized bead and the carboxyl terminus of the polypeptide. In addition, a bi-functional trityl linker can be attached to the support, *e.g.*, to the 4-nitrophenyl active ester on a resin, such as a Wang resin, through an amino group or a carboxyl group on the resin *via* an amino resin. Using a bi-functional trityl approach, the solid support can require treatment with a volatile acid, such as formic acid or trifluoroacetic acid to ensure that the polypeptide is cleaved and can be removed. In such a case, the polypeptide can be deposited as a beadless patch at the bottom of a well of a solid support or on the flat surface of a solid support. After addition of a matrix solution, the polypeptide can be desorbed into a MS.

[00277] Hydrophobic trityl linkers can also be exploited as acid-labile linkers by using a volatile acid or an appropriate matrix solution, *e.g.*, a matrix solution containing 3-HPA, to cleave an amino linked trityl group from the polypeptide. Acid lability can also be changed. For example, trityl, monomethoxytrityl, dimethoxytrityl or trimethoxytrityl can be changed to the appropriate *p*-substituted, or more acid-labile tritylamine derivatives, of the polypeptide, *i.e.*, trityl ether and tritylamine bonds can be made to the polypeptide. Accordingly, a polypeptide can be removed from a hydrophobic linker, *e.g.*, by disrupting the hydrophobic attraction or by cleaving tritylether or tritylamine bonds under acidic conditions, including, if desired, under typical MS conditions, where a matrix, such as 3-HPA acts as an acid.

[00278] Orthogonally cleavable linkers can also be useful for binding a first solid support, *e.g.*, a bead to a second solid support, or for binding a polypeptide of interest to a solid support. Using such linkers, a first solid support, *e.g.*, a bead, can be selectively cleaved from a second solid support, without cleaving the polypeptide from the support; the polypeptide then can be cleaved from the bead at a later time. For example, a disulfide linker, which can be cleaved using a reducing agent, such as DTT, can be employed to bind a bead to a second solid support, and an acid cleavable bi-functional trityl group could be used to immobilize a polypeptide to the support. As desired, the linkage of the polypeptide to the solid support can be cleaved first, *e.g.*, leaving the linkage between the first and second support intact. Trityl linkers can provide a covalent or hydrophobic conjugation and, regardless of the nature of the conjugation, the trityl group is readily cleaved in acidic conditions.

[00279] For example, a bead can be bound to a second support through a linking group which can be selected to have a length and a chemical nature such that high density binding

of the beads to the solid support, or high density binding of the polypeptides to the beads, is promoted. Such a linking group can have, *e.g.*, “tree-like” structure, thereby providing a multiplicity of functional groups per attachment site on a solid support. Examples of such linking group; include polylysine, polyglutamic acid, penta-erythrole and *tris*-hydroxy-aminomethane.

[00280] *Noncovalent Binding Association.* An antibody or polypeptide can be conjugated to a solid support, or a first solid support can also be conjugated to a second solid support, through a noncovalent interaction. For example, a magnetic bead made of a ferromagnetic material, which is capable of being magnetized, can be attracted to a magnetic solid support, and can be released from the support by removal of the magnetic field. Alternatively, the solid support can be provided with an ionic or hydrophobic moiety, which can allow the interaction of an ionic or hydrophobic moiety, respectively, with a polypeptide, *e.g.*, a polypeptide containing an attached trityl group or with a second solid support having hydrophobic character.

[00281] A solid support can also be provided with a member of a specific binding pair and, therefore, can be conjugated to a polypeptide or a second solid support containing a complementary binding moiety. For example, a bead coated with avidin or with streptavidin can be bound to a polypeptide having a biotin moiety incorporated therein, or to a second solid support coated with biotin or derivative of biotin, such as iminobiotin.

[00282] It should be recognized that any of the binding members disclosed herein or otherwise known in the art can be reversed. Thus, biotin, *e.g.*, can be incorporated into either a polypeptide or a solid support and, conversely, avidin or other biotin binding moiety would be incorporated into the support or the polypeptide, respectively. Other specific binding pairs contemplated for use herein include, but are not limited to, hormones and their receptors, enzyme, and their substrates, a nucleotide sequence and its complementary sequence, an antibody and the antigen to which it interacts specifically, and other such pairs known to those skilled in the art.

A. Diagnostic Uses of Anti-STEAP1 Antibodies of the Present Technology

[00283] *General.* The anti-STEAP1 antibodies of the present technology are useful in diagnostic methods. As such, the present technology provides methods using the antibodies

in the diagnosis of STEAP1 activity in a subject. Anti-STEAP1 antibodies of the present technology may be selected such that they have any level of epitope binding specificity and very high binding affinity to a STEAP1 protein. In general, the higher the binding affinity of an antibody the more stringent wash conditions can be performed in an immunoassay to remove nonspecifically bound material without removing target polypeptide. Accordingly, anti-STEAP1 antibodies of the present technology useful in diagnostic assays usually have binding affinities of about 10^8 M^{-1} , 10^9 M^{-1} , 10^{10} M^{-1} , 10^{11} M^{-1} or 10^{12} M^{-1} . Further, it is desirable that anti-STEAP1 antibodies used as diagnostic reagents have a sufficient kinetic on-rate to reach equilibrium under standard conditions in at least 12 h, at least five (5) h, or at least one (1) hour.

[00284] Anti-STEAP1 antibodies can be used to detect an immunoreactive STEAP1 protein in a variety of standard assay formats. Such formats include immunoprecipitation, Western blotting, ELISA, radioimmunoassay, and immunometric assays. See Harlow & Lane, *Antibodies, A Laboratory Manual* (Cold Spring Harbor Publications, New York, 1988); U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,879,262; 4,034,074, 3,791,932; 3,817,837; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; and 4,098,876. Biological samples can be obtained from any tissue or body fluid of a subject. In certain embodiments, the subject is at an early stage of cancer. In one embodiment, the early stage of cancer is determined by the level or expression pattern of STEAP1 protein in a sample obtained from the subject. In certain embodiments, the sample is selected from the group consisting of urine, blood, serum, plasma, saliva, amniotic fluid, cerebrospinal fluid (CSF), and biopsied body tissue.

[00285] Immunometric or sandwich assays are one format for the diagnostic methods of the present technology. See U.S. Pat. No. 4,376,110, 4,486,530, 5,914,241, and 5,965,375. Such assays use one antibody, e.g., an anti-STEAP1 antibody or a population of anti-STEAP1 antibodies immobilized to a solid phase, and another anti-STEAP1 antibody or a population of anti-STEAP1 antibodies in solution. Typically, the solution anti-STEAP1 antibody or population of anti-STEAP1 antibodies is labeled. If an antibody population is used, the population can contain antibodies binding to different epitope specificities within the target polypeptide. Accordingly, the same population can be used for both solid phase and solution antibody. If anti-STEAP1 monoclonal antibodies are used, first and second

STEAP1 monoclonal antibodies having different binding specificities are used for the solid and solution phase. Solid phase (also referred to as “capture”) and solution (also referred to as “detection”) antibodies can be contacted with target antigen in either order or simultaneously. If the solid phase antibody is contacted first, the assay is referred to as being a forward assay. Conversely, if the solution antibody is contacted first, the assay is referred to as being a reverse assay. If the target is contacted with both antibodies simultaneously, the assay is referred to as a simultaneous assay. After contacting the STEAP1 protein with the anti-STEAP1 antibody, a sample is incubated for a period that usually varies from about 10 min to about 24 hr and is usually about 1 hr. A wash step is then performed to remove components of the sample not specifically bound to the anti-STEAP1 antibody being used as a diagnostic reagent. When solid phase and solution antibodies are bound in separate steps, a wash can be performed after either or both binding steps. After washing, binding is quantified, typically by detecting a label linked to the solid phase through binding of labeled solution antibody. Usually for a given pair of antibodies or populations of antibodies and given reaction conditions, a calibration curve is prepared from samples containing known concentrations of target antigen. Concentrations of the immunoreactive STEAP1 protein in samples being tested are then read by interpolation from the calibration curve (*i.e.*, standard curve). Analyte can be measured either from the amount of labeled solution antibody bound at equilibrium or by kinetic measurements of bound labeled solution antibody at a series of time points before equilibrium is reached. The slope of such a curve is a measure of the concentration of the STEAP1 protein in a sample.

[00286] Suitable supports for use in the above methods include, *e.g.*, nitrocellulose membranes, nylon membranes, and derivatized nylon membranes, and also particles, such as agarose, a dextran-based gel, dipsticks, particulates, microspheres, magnetic particles, test tubes, microtiter wells, SEPHADEX™ (Amersham Pharmacia Biotech, Piscataway N.J.), and the like. Immobilization can be by absorption or by covalent attachment. Optionally, anti-STEAP1 antibodies can be joined to a linker molecule, such as biotin for attachment to a surface bound linker, such as avidin.

[00287] In some embodiments, the present disclosure provides an anti-STEAP1 antibody of the present technology conjugated to a diagnostic agent. The diagnostic agent may comprise a radioactive or non-radioactive label, a contrast agent (such as for magnetic

resonance imaging, computed tomography or ultrasound), and the radioactive label can be a gamma-, beta-, alpha-, Auger electron-, or positron-emitting isotope. A diagnostic agent is a molecule which is administered conjugated to an antibody moiety, *i.e.*, antibody or antibody fragment, or subfragment, and is useful in diagnosing or detecting a disease by locating the cells containing the antigen.

[00288] Useful diagnostic agents include, but are not limited to, radioisotopes, dyes (such as with the biotin-streptavidin complex), contrast agents, fluorescent compounds or molecules and enhancing agents (*e.g.*, paramagnetic ions) for magnetic resonance imaging (MRI). U.S. Pat. No. 6,331,175 describes MRI technique and the preparation of antibodies conjugated to a MRI enhancing agent and is incorporated in its entirety by reference. In some embodiments, the diagnostic agents are selected from the group consisting of radioisotopes, enhancing agents for use in magnetic resonance imaging, and fluorescent compounds. In order to load an antibody component with radioactive metals or paramagnetic ions, it may be necessary to react it with a reagent having a long tail to which are attached a multiplicity of chelating groups for binding the ions. Such a tail can be a polymer such as a polylysine, polysaccharide, or other derivatized or derivatizable chain having pendant groups to which can be bound chelating groups such as, *e.g.*, ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, polyoximes, and like groups known to be useful for this purpose. Chelates may be coupled to the antibodies of the present technology using standard chemistries. The chelate is normally linked to the antibody by a group which enables formation of a bond to the molecule with minimal loss of immunoreactivity and minimal aggregation and/or internal cross-linking. Other methods and reagents for conjugating chelates to antibodies are disclosed in U.S. Pat. No. 4,824,659. Particularly useful metal-chelate combinations include 2-benzyl-DTPA and its monomethyl and cyclohexyl analogs, used with diagnostic isotopes for radio-imaging. The same chelates, when complexed with non-radioactive metals, such as manganese, iron and gadolinium are useful for MRI, when used along with the STEAP1 antibodies of the present technology.

[00289] Macrocyclic chelates such as NOTA (1,4,7-triaza-cyclononane-N,N',N''-triacetic acid), DOTA, and TETA (p-bromoacetamido-benzyl-tetraethylaminetetraacetic acid) are of use with a variety of metals and radiometals, such as radionuclides of gallium, yttrium and

copper, respectively. Such metal-chelate complexes can be stabilized by tailoring the ring size to the metal of interest. Examples of other DOTA chelates include (i) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH₂; (ii) Ac-Lys(HSG)-D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH₂; (iii) DOTA-D-Asp-D-Lys(HSG)-D-Asp-D-Lys(HSG)-NH₂; (iv) DOTA-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (v) DOTA-D-Tyr-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (vi) DOTA-D-Ala-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (vii) DOTA-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-NH₂; (viii) Ac-D-Phe-D-Lys(DOTA)-D-Tyr-D-Lys(DOTA)-NH₂; (ix) Ac-D-Phe-D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-NH₂; (x) Ac-D-Phe-D-Lys(Bz-DTPA)-D-Tyr-D-Lys(Bz-DTPA)-NH₂; (xi) Ac-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(Tscg-Cys)-NH₂; (xii) DOTA-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(Tscg-Cys)-NH₂; (xiii) (Tscg-Cys)-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(DOTA)-NH₂; (xiv) Tscg-D-Cys-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (xv) (Tscg-Cys)-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (xvi) Ac-D-Cys-D-Lys(DOTA)-D-Tyr-D-Ala-D-Lys(DOTA)-D-Cys-NH₂; (xvii) Ac-D-Cys-D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-NH₂; (xviii) Ac-D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-D-Lys(Tscg-Cys)-NH₂; and (xix) Ac-D-Lys(DOTA)-D-Tyr-D-Lys(DOTA)-D-Lys(Tscg-Cys)-NH₂.

[00290] Other ring-type chelates such as macrocyclic polyethers, which are of interest for stably binding nuclides, such as ²²³Ra for RAIT are also contemplated.

B. Therapeutic Use of Anti-STEAP1 Antibodies of the Present Technology

[00291] The immunoglobulin-related compositions (*e.g.*, antibodies or antigen binding fragments thereof) of the present technology are useful for the treatment of STEAP1-associated cancers, such as Ewing family of tumors (including Ewing's sarcoma), prostate cancer, bladder cancer, breast cancer, ovary cancer, colon cancer, lung cancer, and kidney cancer. Such treatment can be used in patients identified as having pathologically high levels of the STEAP1 (*e.g.*, those diagnosed by the methods described herein) or in patients diagnosed with a disease known to be associated with such pathological levels. In one aspect, the present disclosure provides a method for treating a STEAP1-associated cancer in a subject in need thereof, comprising administering to the subject an effective amount of an antibody (or antigen binding fragment thereof) of the present technology. Examples of cancers that can be treated by the antibodies of the present technology include, but are not

limited to: Ewing's sarcoma, prostate cancer, osteosarcoma, bladder cancer, breast cancer, ovary cancer, colon cancer, lung cancer, and kidney cancer.

[00292] The compositions of the present technology may be employed in conjunction with other therapeutic agents useful in the treatment of STEAP1-associated cancers. For example, the antibodies of the present technology may be separately, sequentially or simultaneously administered with at least one additional therapeutic agent-selected from the group consisting of alkylating agents, platinum agents, taxanes, vinca agents, anti-estrogen drugs, aromatase inhibitors, ovarian suppression agents, VEGF/VEGFR inhibitors, EGF/EGFR inhibitors, PARP inhibitors, cytostatic alkaloids, cytotoxic antibiotics, antimetabolites, endocrine/hormonal agents, bisphosphonate therapy agents and targeted biological therapy agents (*e.g.*, therapeutic peptides described in US 6306832, WO 2012007137, WO 2005000889, WO 2010096603 *etc.*). In some embodiments, the at least one additional therapeutic agent is a chemotherapeutic agent. Specific chemotherapeutic agents include, but are not limited to, cyclophosphamide, fluorouracil (or 5-fluorouracil or 5-FU), methotrexate, edatrexate (10-ethyl-10-deaza-aminopterin), thiotepa, carboplatin, cisplatin, taxanes, paclitaxel, protein-bound paclitaxel, docetaxel, vinorelbine, tamoxifen, raloxifene, toremifene, fulvestrant, gemcitabine, irinotecan, ixabepilone, temozolamide, topotecan, vincristine, vinblastine, eribulin, mutamycin, capecitabine, anastrozole, exemestane, letrozole, leuprolide, abarelix, buserlin, goserelin, megestrol acetate, risendronate, pamidronate, ibandronate, alendronate, denosumab, zoledronate, trastuzumab, tykerb, anthracyclines (*e.g.*, daunorubicin and doxorubicin), bevacizumab, oxaliplatin, melphalan, etoposide, mechlorethamine, bleomycin, microtubule poisons, annonaceous acetogenins, or combinations thereof.

[00293] The compositions of the present technology may optionally be administered as a single bolus to a subject in need thereof. Alternatively, the dosing regimen may comprise multiple administrations performed at various times after the appearance of tumors.

[00294] Administration can be carried out by any suitable route, including orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, intracranially, intratumorally, intrathecally, or topically. Administration includes self-administration and the administration by another. It is also to be appreciated that the various modes of treatment of medical conditions as described are

intended to mean “substantial”, which includes total but also less than total treatment, and wherein some biologically or medically relevant result is achieved.

[00295] In some embodiments, the antibodies of the present technology comprise pharmaceutical formulations which may be administered to subjects in need thereof in one or more doses. Dosage regimens can be adjusted to provide the desired response (*e.g.*, a therapeutic response).

[00296] Typically, an effective amount of the antibody compositions of the present technology, sufficient for achieving a therapeutic effect, range from about 0.000001 mg per kilogram body weight per day to about 10,000 mg per kilogram body weight per day. Typically, the dosage ranges are from about 0.0001 mg per kilogram body weight per day to about 100 mg per kilogram body weight per day. For administration of anti-STEAP1 antibodies, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg every week, every two weeks or every three weeks, of the subject body weight. For example, dosages can be 1 mg/kg body weight or 10 mg/kg body weight every week, every two weeks or every three weeks or within the range of 1-10 mg/kg every week, every two weeks or every three weeks. In one embodiment, a single dosage of antibody ranges from 0.1-10,000 micrograms per kg body weight. In one embodiment, antibody concentrations in a carrier range from 0.2 to 2000 micrograms per delivered milliliter. An exemplary treatment regime entails administration once per every two weeks or once a month or once every 3 to 6 months. Anti-STEAP1 antibodies may be administered on multiple occasions. Intervals between single dosages can be hourly, daily, weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of the antibody in the subject. In some methods, dosage is adjusted to achieve a serum antibody concentration in the subject of from about 75 µg/mL to about 125 µg/mL, 100 µg/mL to about 150 µg/mL, from about 125 µg/mL to about 175 µg/mL, or from about 150 µg/mL to about 200 µg/mL. Alternatively, anti-STEAP1 antibodies can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the subject. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. In therapeutic applications, a relatively high dosage at relatively short intervals is

sometimes required until progression of the disease is reduced or terminated, or until the subject shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

[00297] In another aspect, the present disclosure provides a method for detecting a tumor in a subject *in vivo* comprising (a) administering to the subject an effective amount of an antibody (or antigen binding fragment thereof) of the present technology, wherein the antibody is configured to localize to a tumor expressing STEAP1 and is labeled with a radioisotope; and (b) detecting the presence of a tumor in the subject by detecting radioactive levels emitted by the antibody that are higher than a reference value. In some embodiments, the reference value is expressed as injected dose per gram (%ID/g). The reference value may be calculated by measuring the radioactive levels present in non-tumor (normal) tissues, and computing the average radioactive levels present in non-tumor (normal) tissues $\pm$ standard deviation. In some embodiments, the ratio of radioactive levels between a tumor and normal tissue is about 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 90:1, 95:1 or 100:1.

[00298] In some embodiments, the subject is diagnosed with or is suspected of having cancer. Radioactive levels emitted by the antibody may be detected using positron emission tomography or single photon emission computed tomography.

[00299] Additionally or alternatively, in some embodiments, the method further comprises administering to the subject an effective amount of an immunoconjugate comprising an antibody of the present technology conjugated to a radionuclide. In some embodiments, the radionuclide is an alpha particle-emitting isotope, a beta particle-emitting isotope, an Auger-emitter, or any combination thereof. Examples of beta particle-emitting isotopes include ^{86}Y , ^{90}Y , ^{89}Sr , ^{165}Dy , ^{186}Re , ^{188}Re , ^{177}Lu , and ^{67}Cu . Examples of alpha particle-emitting isotopes include ^{213}Bi , ^{211}At , ^{225}Ac , ^{152}Dy , ^{212}Bi , ^{223}Ra , ^{219}Rn , ^{215}Po , ^{211}Bi , ^{221}Fr , ^{217}At , and ^{255}Fm . Examples of Auger-emitters include ^{111}In , ^{67}Ga , ^{51}Cr , ^{58}Co , $^{99\text{m}}\text{Tc}$, $^{103\text{m}}\text{Rh}$, $^{195\text{m}}\text{Pt}$, ^{119}Sb , ^{161}Ho , $^{189\text{m}}\text{Os}$, ^{192}Ir , ^{201}Tl , and ^{203}Pb . In some embodiments of the method, nonspecific FcR-dependent binding in normal tissues is eliminated or reduced (*e.g.*, *via* N297A mutation in Fc region, which results in aglycosylation). The therapeutic effectiveness of such an immunoconjugate may be determined by computing the area under the curve (AUC) tumor: AUC normal tissue ratio. In some embodiments, the immunoconjugate has a AUC tumor:

AUC normal tissue ratio of about 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 90:1, 95:1 or 100:1.

[00300] *PRIT*. In one aspect, the present disclosure provides a method for detecting tumors in a subject in need thereof comprising (a) administering to the subject an effective amount of a complex comprising a radiolabeled DOTA hapten and a bispecific antibody of the present technology that binds to the radiolabeled DOTA hapten and a STEAP1 antigen, wherein the complex is configured to localize to a tumor expressing the STEAP1 antigen recognized by the bispecific antibody of the complex; and (b) detecting the presence of solid tumors in the subject by detecting radioactive levels emitted by the complex that are higher than a reference value. In some embodiments, the subject is human.

[00301] In another aspect, the present disclosure provides a method for selecting a subject for pretargeted radioimmunotherapy comprising (a) administering to the subject an effective amount of a complex comprising a radiolabeled DOTA hapten and a bispecific antibody of the present technology that binds to the radiolabeled DOTA hapten and a STEAP1 antigen, wherein the complex is configured to localize to a tumor expressing the STEAP1 antigen recognized by the bispecific antibody of the complex; (b) detecting radioactive levels emitted by the complex; and (c) selecting the subject for pretargeted radioimmunotherapy when the radioactive levels emitted by the complex are higher than a reference value. In some embodiments, the subject is human.

[00302] Examples of DOTA haptens include (i) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH₂; (ii) Ac-Lys(HSG)-D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH₂; (iii) DOTA-D-Asp-D-Lys(HSG)-D-Asp-D-Lys(HSG)-NH₂; (iv) DOTA-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (v) DOTA-D-Tyr-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (vi) DOTA-D-Ala-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (vii) DOTA-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-NH₂; (viii) Ac-D-Phe-D-Lys(DOTA)-D-Tyr-D-Lys(DOTA)-NH₂; (ix) Ac-D-Phe-D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-NH₂; (x) Ac-D-Phe-D-Lys(Bz-DTPA)-D-Tyr-D-Lys(Bz-DTPA)-NH₂; (xi) Ac-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(Tscg-Cys)-NH₂; (xii) DOTA-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(Tscg-Cys)-NH₂; (xiii) (Tscg-Cys)-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(DOTA)-NH₂; (xiv) Tscg-D-Cys-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (xv) (Tscg-Cys)-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (xvi) Ac-D-Cys-D-Lys(DOTA)-D-Tyr-D-Ala-D-Lys(DOTA)-D-Cys-NH₂; (xvii) Ac-D-Cys-

D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-NH₂; (xviii) Ac-D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-D-Lys(Tscg-Cys)-NH₂; (xix) Ac-D-Lys(DOTA)-D-Tyr-D-Lys(DOTA)-D-Lys(Tscg-Cys)-NH₂ and (xx) DOTA. The radiolabel may be an alpha particle-emitting isotope, a beta particle-emitting isotope, or an Auger-emitter. Examples of radiolabels include ²¹³Bi, ²¹¹At, ²²⁵Ac, ¹⁵²Dy, ²¹²Bi, ²²³Ra, ²¹⁹Rn, ²¹⁵Po, ²¹¹Bi, ²²¹Fr, ²¹⁷At, ²⁵⁵Fm, ⁸⁶Y, ⁹⁰Y, ⁸⁹Sr, ¹⁶⁵Dy, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁷⁷Lu, ⁶⁷Cu, ¹¹¹In, ⁶⁷Ga, ⁵¹Cr, ⁵⁸Co, ^{99m}Tc, ^{103m}Rh, ^{195m}Pt, ¹¹⁹Sb, ¹⁶¹Ho, ^{189m}Os, ¹⁹²Ir, ²⁰¹Tl, ²⁰³Pb, ⁶⁸Ga, ²²⁷Th, or ⁶⁴Cu.

[00303] In some embodiments of the methods disclosed herein, the radioactive levels emitted by the complex are detected using positron emission tomography or single photon emission computed tomography. Additionally or alternatively, in some embodiments of the methods disclosed herein, the subject is diagnosed with, or is suspected of having a STEAP1-associated cancer such as Ewing's sarcoma, prostate cancer, osteosarcoma, bladder cancer, breast cancer, ovary cancer, colon cancer, lung cancer, or kidney cancer.

[00304] Additionally or alternatively, in some embodiments of the methods disclosed herein, the complex is administered intravenously, intramuscularly, intraarterially, intrathecally, intracapsularly, intraorbitally, intradermally, intraperitoneally, transtracheally, subcutaneously, intracerebroventricularly, orally, intratumorally, or intranasally. In certain embodiments, the complex is administered into the cerebral spinal fluid or blood of the subject.

[00305] In some embodiments of the methods disclosed herein, the radioactive levels emitted by the complex are detected between 2 to 120 hours after the complex is administered. In certain embodiments of the methods disclosed herein, the radioactive levels emitted by the complex are expressed as the percentage injected dose per gram tissue (%ID/g). The reference value may be calculated by measuring the radioactive levels present in non-tumor (normal) tissues, and computing the average radioactive levels present in non-tumor (normal) tissues $\pm$ standard deviation. In some embodiments, the reference value is the standard uptake value (SUV). See Thie JA, *J Nucl Med.* 45(9):1431-4 (2004). In some embodiments, the ratio of radioactive levels between a tumor and normal tissue is about 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 90:1, 95:1 or 100:1.

[00306] In another aspect, the present disclosure provides a method for increasing tumor sensitivity to radiation therapy in a subject diagnosed with a STEAP1-associated cancer comprising (a) administering an effective amount of an anti-DOTA bispecific antibody of the present technology to the subject, wherein the anti-DOTA bispecific antibody is configured to localize to a tumor expressing a STEAP1 antigen target; and (b) administering an effective amount of a radiolabeled-DOTA hapten to the subject, wherein the radiolabeled-DOTA hapten is configured to bind to the anti-DOTA bispecific antibody. In some embodiments, the subject is human.

[00307] The anti-DOTA bispecific antibody is administered under conditions and for a period of time (*e.g.*, according to a dosing regimen) sufficient for it to saturate tumor cells. In some embodiments, unbound anti-DOTA bispecific antibody is removed from the blood stream after administration of the anti-DOTA bispecific antibody. In some embodiments, the radiolabeled-DOTA hapten is administered after a time period that may be sufficient to permit clearance of unbound anti-DOTA bispecific antibody.

[00308] The radiolabeled-DOTA hapten may be administered at any time between 1 minute to 4 or more days following administration of the anti-DOTA bispecific antibody. For example, in some embodiments, the radiolabeled-DOTA hapten is administered 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 1 hour, 1.25 hours, 1.5 hours, 1.75 hours, 2 hours, 2.5 hours, 3 hours, 3.5 hours, 4 hours, 4.5 hours, 5 hours, 5.5 hours, 6 hours, 6.5 hours, 7 hours, 7.5 hours, 8 hours, 8.5 hours, 9 hours, 9.5 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 48 hours, 72 hours, 96 hours, or any range therein, following administration of the anti-DOTA bispecific antibody. Alternatively, the radiolabeled-DOTA hapten may be administered at any time after 4 or more days following administration of the anti-DOTA bispecific antibody.

[00309] Additionally or alternatively, in some embodiments, the method further comprises administering an effective amount of a clearing agent to the subject prior to administration of the radiolabeled-DOTA hapten. A clearing agent can be any molecule (dextran or dendrimer or polymer) that can be conjugated with C825-hapten. In some embodiments, the clearing agent is no more than 2000 kD, 1500 kD, 1000 kD, 900 kD, 800 kD, 700 kD, 600 kD, 500

kD, 400 kD, 300 kD, 200 kD, 100 kD, 90 kD, 80 kD, 70 kD, 60 kD, 50 kD, 40 kD, 30 kD, 20 kD, 10 kD, or 5kD. In some embodiments, the clearing agent is a 500 kD aminodextran-DOTA conjugate (*e.g.*, 500 kD dextran-DOTA-Bn (Y), 500 kD dextran-DOTA-Bn (Lu), or 500 kD dextran-DOTA-Bn (In) *etc.*).

[00310] In some embodiments, the clearing agent and the radiolabeled-DOTA hapten are administered without further administration of the anti-DOTA bispecific antibody of the present technology. For example, in some embodiments, an anti-DOTA bispecific antibody of the present technology is administered according to a regimen that includes at least one cycle of: (i) administration of the anti-DOTA bispecific antibody of the present technology (optionally so that relevant tumor cells are saturated); (ii) administration of a radiolabeled-DOTA hapten and, optionally a clearing agent; (iii) optional additional administration of the radiolabeled-DOTA hapten and/or the clearing agent, without additional administration of the anti-DOTA bispecific antibody. In some embodiments, the method may comprise multiple such cycles (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more cycles).

[00311] Additionally or alternatively, in some embodiments of the method, the anti-DOTA bispecific antibody and/or the radiolabeled-DOTA hapten is administered intravenously, intramuscularly, intraarterially, intrathecally, intracapsularly, intraorbitally, intradermally, intraperitoneally, transtracheally, subcutaneously, intracerebroventricularly, intratumorally, orally or intranasally.

[00312] In one aspect, the present disclosure provides a method for increasing tumor sensitivity to radiation therapy in a subject diagnosed with a STEAP1-associated cancer comprising administering to the subject an effective amount of a complex comprising a radiolabeled-DOTA hapten and a bispecific antibody of the present technology that recognizes and binds to the radiolabeled-DOTA hapten and a STEAP1 antigen target, wherein the complex is configured to localize to a tumor expressing the STEAP1 antigen target recognized by the bispecific antibody of the complex. The complex may be administered intravenously, intramuscularly, intraarterially, intrathecally, intracapsularly, intraorbitally, intradermally, intraperitoneally, transtracheally, subcutaneously, intracerebroventricularly, orally, intratumorally, or intranasally. In some embodiments, the subject is human.

[00313] In another aspect, the present disclosure provides a method for treating cancer in a subject in need thereof comprising (a) administering an effective amount of an anti-DOTA bispecific antibody of the present technology to the subject, wherein the anti-DOTA bispecific antibody is configured to localize to a tumor expressing a STEAP1 antigen target; and (b) administering an effective amount of a radiolabeled-DOTA hapten to the subject, wherein the radiolabeled-DOTA hapten is configured to bind to the anti-DOTA bispecific antibody. The anti-DOTA bispecific antibody is administered under conditions and for a period of time (*e.g.*, according to a dosing regimen) sufficient for it to saturate tumor cells. In some embodiments, unbound anti-DOTA bispecific antibody is removed from the blood stream after administration of the anti-DOTA bispecific antibody. In some embodiments, the radiolabeled-DOTA hapten is administered after a time period that may be sufficient to permit clearance of unbound anti-DOTA bispecific antibody. In some embodiments, the subject is human.

[00314] Accordingly, in some embodiments, the method further comprises administering an effective amount of a clearing agent to the subject prior to administration of the radiolabeled-DOTA hapten. The radiolabeled-DOTA hapten may be administered at any time between 1 minute to 4 or more days following administration of the anti-DOTA bispecific antibody. For example, in some embodiments, the radiolabeled-DOTA hapten is administered 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 1 hour, 1.25 hours, 1.5 hours, 1.75 hours, 2 hours, 2.5 hours, 3 hours, 3.5 hours, 4 hours, 4.5 hours, 5 hours, 5.5 hours, 6 hours, 6.5 hours, 7 hours, 7.5 hours, 8 hours, 8.5 hours, 9 hours, 9.5 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 48 hours, 72 hours, 96 hours, or any range therein, following administration of the anti-DOTA bispecific antibody. Alternatively, the radiolabeled-DOTA hapten may be administered at any time after 4 or more days following administration of the anti-DOTA bispecific antibody.

[00315] The clearing agent may be a 500 kD aminodextran-DOTA conjugate (*e.g.*, 500 kD dextran-DOTA-Bn (Y), 500 kD dextran-DOTA-Bn (Lu), or 500 kD dextran-DOTA-Bn (In) *etc.*). In some embodiments, the clearing agent and the radiolabeled-DOTA hapten are administered without further administration of the anti-DOTA bispecific antibody. For

example, in some embodiments, an anti-DOTA bispecific antibody is administered according to a regimen that includes at least one cycle of: (i) administration of the an anti-DOTA bispecific antibody of the present technology (optionally so that relevant tumor cells are saturated); (ii) administration of a radiolabeled-DOTA hapten and, optionally a clearing agent; (iii) optional additional administration of the radiolabeled-DOTA hapten and/or the clearing agent, without additional administration of the anti-DOTA bispecific antibody. In some embodiments, the method may comprise multiple such cycles (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more cycles).

[00316] Also provided herein are methods for treating cancer in a subject in need thereof comprising administering to the subject an effective amount of a complex comprising a radiolabeled-DOTA hapten and a bispecific antibody of the present technology that recognizes and binds to the radiolabeled-DOTA hapten and a STEAP1 antigen target, wherein the complex is configured to localize to a tumor expressing the STEAP1 antigen target recognized by the bispecific antibody of the complex. The therapeutic effectiveness of such a complex may be determined by computing the area under the curve (AUC) tumor: AUC normal tissue ratio. In some embodiments, the complex has a AUC tumor: AUC normal tissue ratio of about 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 90:1, 95:1 or 100:1.

[00317] *Ex vivo armed T cells.* In one aspect, the present disclosure provides an *ex vivo* armed T cell that is coated or complexed with an effective amount of an anti-STEAP1 multi-specific antibody of the present technology, wherein the anti-STEAP1 multi-specific antibody includes a CD3 binding domain comprising a heavy chain immunoglobulin variable domain (V_H) of SEQ ID NO: 80 and a light chain immunoglobulin variable domain (V_L) of SEQ ID NO: 81, wherein the anti-STEAP1 multi-specific antibody is an immunoglobulin comprising two heavy chains and two light chains, wherein each of the light chains is fused to a single chain variable fragment (scFv). In some embodiments, at least one scFv of the anti-STEAP1 multi-specific antibody comprises the CD3 binding domain. Additionally or alternatively, in some embodiments, at least one scFv of the anti-STEAP1 multi-specific antibody comprises a DOTA binding domain. In certain embodiments, the DOTA binding domain comprises a V_H sequence and a V_L sequence comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 76 and SEQ ID NO: 77, and SEQ ID NO:

78, and SEQ ID NO: 79. Also disclosed herein are methods for treating a STEAP1-associated cancer in a subject in need thereof comprising administering to the subject an effective amount of the *ex vivo* armed T cell disclosed herein.

[00318] *Toxicity.* Optimally, an effective amount (*e.g.*, dose) of an anti-STEAP1 antibody described herein will provide therapeutic benefit without causing substantial toxicity to the subject. Toxicity of the anti-STEAP1 antibody described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, by determining the LD₅₀ (the dose lethal to 50% of the population) or the LD₁₀₀ (the dose lethal to 100% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index. The data obtained from these cell culture assays and animal studies can be used in formulating a dosage range that is not toxic for use in human. The dosage of the anti-STEAP1 antibody described herein lies within a range of circulating concentrations that include the effective dose with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the subject's condition. *See, e.g., Fingl et al., In: The Pharmacological Basis of Therapeutics, Ch. 1 (1975).*

[00319] *Formulations of Pharmaceutical Compositions.* According to the methods of the present technology, the anti-STEAP1 antibody can be incorporated into pharmaceutical compositions suitable for administration. The pharmaceutical compositions generally comprise recombinant or substantially purified antibody and a pharmaceutically-acceptable carrier in a form suitable for administration to a subject. Pharmaceutically-acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions for administering the antibody compositions (*See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA 18th ed., 1990*). The pharmaceutical compositions are generally formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

[00320] The terms "pharmaceutically-acceptable," "physiologically-tolerable," and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents,

are used interchangeably and represent that the materials are capable of administration to or upon a subject without the production of undesirable physiological effects to a degree that would prohibit administration of the composition. For example, “pharmaceutically-acceptable excipient” means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients can be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous.

“Pharmaceutically-acceptable salts and esters” means salts and esters that are pharmaceutically-acceptable and have the desired pharmacological properties. Such salts include salts that can be formed where acidic protons present in the composition are capable of reacting with inorganic or organic bases. Suitable inorganic salts include those formed with the alkali metals, *e.g.*, sodium and potassium, magnesium, calcium, and aluminum. Suitable organic salts include those formed with organic bases such as the amine bases, *e.g.*, ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like. Such salts also include acid addition salts formed with inorganic acids (*e.g.*, hydrochloric and hydrobromic acids) and organic acids (*e.g.*, acetic acid, citric acid, maleic acid, and the alkane- and arene-sulfonic acids such as methanesulfonic acid and benzenesulfonic acid). Pharmaceutically-acceptable esters include esters formed from carboxy, sulfonyloxy, and phosphonoxo groups present in the anti-STEAP1 antibody, *e.g.*, C₁₋₆ alkyl esters. When there are two acidic groups present, a pharmaceutically-acceptable salt or ester can be a mono-acid-mono-salt or ester or a di-salt or ester, and similarly where there are more than two acidic groups present, some or all of such groups can be salified or esterified. An anti-STEAP1 antibody named in this technology can be present in unsalified or unesterified form, or in salified and/or esterified form, and the naming of such anti-STEAP1 antibody is intended to include both the original (unsalified and unesterified) compound and its pharmaceutically-acceptable salts and esters. Also, certain embodiments of the present technology can be present in more than one stereoisomeric form, and the naming of such anti-STEAP1 antibody is intended to include all single stereoisomers and all mixtures (whether racemic or otherwise) of such stereoisomers. A person of ordinary skill in the art, would have no difficulty determining the appropriate timing, sequence and dosages of administration for particular drugs and compositions of the present technology.

[00321] Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and compounds for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or compound is incompatible with the anti-STEAP1 antibody, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00322] A pharmaceutical composition of the present technology is formulated to be compatible with its intended route of administration. The anti-STEAP1 antibody compositions of the present technology can be administered by parenteral, topical, intravenous, oral, subcutaneous, intraarterial, intradermal, transdermal, rectal, intracranial, intrathecal, intraperitoneal, intranasal; or intramuscular routes, or as inhalants. The anti-STEAP1 antibody can optionally be administered in combination with other agents that are at least partly effective in treating various STEAP1-associated cancers.

[00323] Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial compounds such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating compounds such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and compounds for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00324] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating

action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, *e.g.*, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, *e.g.*, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal compounds, *e.g.*, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be desirable to include isotonic compounds, *e.g.*, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition a compound which delays absorption, *e.g.*, aluminum monostearate and gelatin.

[00325] Sterile injectable solutions can be prepared by incorporating an anti-STEAP1 antibody of the present technology in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the anti-STEAP1 antibody into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The antibodies of the present technology can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained or pulsatile release of the active ingredient.

[00326] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the anti-STEAP1 antibody can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding compounds, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth

or gelatin; an excipient such as starch or lactose, a disintegrating compound such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening compound such as sucrose or saccharin; or a flavoring compound such as peppermint, methyl salicylate, or orange flavoring.

[00327] For administration by inhalation, the anti-STEAP1 antibody is delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[00328] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, *e.g.*, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the anti-STEAP1 antibody is formulated into ointments, salves, gels, or creams as generally known in the art.

[00329] The anti-STEAP1 antibody can also be prepared as pharmaceutical compositions in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00330] In one embodiment, the anti-STEAP1 antibody is prepared with carriers that will protect the anti-STEAP1 antibody against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically-acceptable carriers. These can be prepared according to methods known to those skilled in the art, *e.g.*, as described in U.S. Pat. No. 4,522,811.

C. Kits

[00331] The present technology provides kits for the detection and/or treatment of STEAP1-associated cancers, comprising at least one immunoglobulin-related composition of the present technology (*e.g.*, any antibody or antigen binding fragment described herein), or a functional variant (*e.g.*, substitutional variant) thereof. Optionally, the above described components of the kits of the present technology are packed in suitable containers and labeled for diagnosis and/or treatment of STEAP1-associated cancers. The above-mentioned components may be stored in unit or multi-dose containers, for example, sealed ampoules, vials, bottles, syringes, and test tubes, as an aqueous, preferably sterile, solution or as a lyophilized, preferably sterile, formulation for reconstitution. The kit may further comprise a second container which holds a diluent suitable for diluting the pharmaceutical composition towards a higher volume. Suitable diluents include, but are not limited to, the pharmaceutically acceptable excipient of the pharmaceutical composition and a saline solution. Furthermore, the kit may comprise instructions for diluting the pharmaceutical composition and/or instructions for administering the pharmaceutical composition, whether diluted or not. The containers may be formed from a variety of materials such as glass or plastic and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper which may be pierced by a hypodermic injection needle). The kit may further comprise more containers comprising a pharmaceutically acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, culture medium for one or more of the suitable hosts. The kits may optionally include instructions customarily included in commercial packages of therapeutic or diagnostic products, that contain information about, for example, the indications, usage, dosage, manufacture, administration, contraindications and/or warnings concerning the use of such therapeutic or diagnostic products.

[00332] The kits are useful for detecting the presence of an immunoreactive STEAP1 protein in a biological sample, *e.g.*, any body fluid including, but not limited to, *e.g.*, serum, plasma, lymph, cystic fluid, urine, stool, cerebrospinal fluid, ascitic fluid or blood and including biopsy samples of body tissue. For example, the kit can comprise: one or more humanized, chimeric, or bispecific anti-STEAP1 antibodies of the present technology (or

antigen binding fragments thereof) capable of binding a STEAP1 protein in a biological sample; means for determining the amount of the STEAP1 protein in the sample; and means for comparing the amount of the immunoreactive STEAP1 protein in the sample with a standard. One or more of the anti-STEAP1 antibodies may be labeled. The kit components, (*e.g.*, reagents) can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect the immunoreactive STEAP1 protein.

[00333] For antibody-based kits, the kit can comprise, *e.g.*, 1) a first antibody, *e.g.* a humanized, chimeric or bispecific STEAP1 antibody of the present technology (or an antigen binding fragment thereof), attached to a solid support, which binds to a STEAP1 protein; and, optionally; 2) a second, different antibody which binds to either the STEAP1 protein or to the first antibody, and is conjugated to a detectable label.

[00334] The kit can also comprise, *e.g.*, a buffering agent, a preservative or a protein-stabilizing agent. The kit can further comprise components necessary for detecting the detectable-label, *e.g.*, an enzyme or a substrate. The kit can also contain a control sample or a series of control samples, which can be assayed and compared to the test sample. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit. The kits of the present technology may contain a written product on or in the kit container. The written product describes how to use the reagents contained in the kit, *e.g.*, for detection of a STEAP1 protein *in vitro* or *in vivo*, or for treatment of STEAP1-associated cancers in a subject in need thereof. In certain embodiments, the use of the reagents can be according to the methods of the present technology.

EXAMPLES

[00335] The present technology is further illustrated by the following Examples, which should not be construed as limiting in any way. The following Examples demonstrate the preparation, characterization, and use of illustrative anti-STEAP1 antibodies of the present technology. The following Examples demonstrate the production of chimeric, humanized, and bispecific antibodies of the present technology, and characterization of their binding specificities and *in vitro* and *in vivo* biological activities.

Example 1: Structure of the Anti-STEAP1 Immunoglobulin-related Compositions of the Present Disclosure

[00336] A bivalent modular platform was chosen to build STEAP1-CD3 BsAb. As shown in **FIG. 1B**, the humanized anti-STEAP1 antibodies of the present disclosure were made by attaching a single chain Fv fragment (scFv) to the carboxyl end of a light chain of an anti-STEAP1 antibody, wherein the scFv binds to an antigen other than STEAP1. In some embodiments, the humanized anti-STEAP1 antibodies of the present disclosure were constructed by attaching the anti-CD3 humanized OKT3 (huOKT3) single chain Fv fragment (ScFv) to the carboxyl end of the X120 IgG1 light chain. The following considerations were taken into account while designing the humanized anti-STEAP1 antibodies of the present disclosure: (1) an optimal size (100-200 kd) to maximize tumor uptake, (2) bivalency towards the tumor target to maintain avidity, (3) a scaffold that is naturally assembled like any IgG (heavy chain and light chain) in CHO cells, purifiable by standard protein A affinity chromatography, (4) structural arrangement to render the anti-CD3 component functionally monovalent, hence reducing nonspecific activation of T cells, (5) a platform with proven tumor targeting efficiency in animal models. Anti-STEAP1 BsAb recruits T-cells via the CD3 receptor and can generate anti-tumor responses with an EC₅₀ in the picomolar range.

Example 2: Humanization of Mouse X120

[00337] The anti-STEAP1 antibody X120 was rehumanized to >85% humanness. The CDRs of the heavy and light chains of X120 were grafted onto human IgG1 frameworks based on their homology with human frameworks IGHV4-30-4*01- IGHJ6*01 for VH, IGKV4-1*01- IGKJ4*01 for VL, respectively. From six heavy chain and four light chain designs, 24 versions of huX120 were gene synthesized and expressed in CHO cells.

[00338] The V_H and V_L of anti-STEAP1 antibody clone X120 was rehumanized. **FIG. 10A** shows the amino acid sequences of the murine and humanized X120 heavy chain variable domains (V_H). The V_H domain of the murine X120 is set forth in SEQ ID NO: 1, which comprises V_H CDR1 (GYSITSD; SEQ ID NO: 2), V_H CDR2 (NSGS; SEQ ID NO: 3), and V_H CDR3 (ERNYDYDDYYAMDY; SEQ ID NO: 4) (**FIG. 10A**). SEQ ID NO: 5-11 are the humanized versions of V_H domain of the X120. Of these, the SEQ ID NO: 5, which has 81.8% humanness, was disclosed in US Patent No. 8,889,847. The sequences X120_VH-

1 (SEQ ID NO: 6), X120_VH-2 (SEQ ID NO: 7), X120_VH-3 (SEQ ID NO: 8), X120_VH-4 (SEQ ID NO: 9), X120_VH-5 (SEQ ID NO: 10), and X120_VH-6 (SEQ ID NO: 11) are six variants of the humanized X120 heavy chain variable domain disclosed herein, which feature >85% humanness (FIG. 10A).

[00339] FIG. 10B shows the amino acid sequences of the murine and humanized X120 light chain variable domains (VL). The VL domain of the murine X120 is set forth in SEQ ID NO: 12, which comprises VL CDR1 (KSSQSLLYRSNQKNYLA; SEQ ID NO: 13), VL CDR2 (WASTRES; SEQ ID NO: 14), and VL CDR3 (QQYYNYPRT; SEQ ID NO: 15) (FIG. 10B). SEQ ID NO: 16-20 are the humanized versions of VL domain of the X120. Of these, the SEQ ID NO: 16, which has 83.2% humanness, was disclosed in US Patent No. 8,889,847. The sequences X120_VL-1 (SEQ ID NO: 17), X120_VL-2 (SEQ ID NO: 18), X120_VL-3 (SEQ ID NO: 19), and X120_VL-4 (SEQ ID NO: 20) are four variants of the humanized X120 light chain variable domain disclosed herein, which feature >85% humanness (FIG. 10B).

[00340] From the six heavy chain and four light chain designs disclosed herein (See FIGs. 10A and 10B), twenty-four versions of humanized X120 were gene synthesized and expressed in CHO cells. FIGs. 11A and 11B show the amino acid sequences of the light chain (SEQ ID NO: 21) and heavy chain (SEQ ID NO: 22) of final humanized anti-STEAP1 amino acid sequence that combines the X120_VL-2 and X120_VH-2 humanized variable domains disclosed herein. The humanized antibodies were screened.

[00341] The humanized anti-STEAP1 BsAb antibodies of the present disclosure were made by attaching a single chain Fv fragment (scFv) to the carboxyl end of a light chain of an anti-STEAP1 antibody, wherein the scFv binds to an antigen other than STEAP1 (FIG. 1B). An anti-STEAP1-BsAb using the IgG-scFv format was synthesized. As shown in FIG. 11B, N297A mutation in a standard hIgG1 Fc region was introduced to remove glycosylation. A K322A mutation was also introduced. The light chain was constructed by extending a humanized X120 IgG1 light chain with a C-terminal (G4S)₃ linker followed by huOKT3 scFv.

[00342] FIGs. 12A and 12B show the nucleotide and amino acid sequences of the light chain (SEQ ID NOs: 23-24) and heavy chain (SEQ ID NOs: 25-26) of BiClone261 (BC261)

BsAb, respectively, which comprises X120_VL-2 and X120_VH-2 humanized variable domains disclosed herein, and an anti-CD3 scFv based on the hOKT3 antibody. Based on the six heavy chain and four light chain designs disclosed herein, twenty-four versions of anti-STEAP1-CD3 BsAb were prepared (**FIG. 4A**). A chimeric BsAb clone was prepared by combining the murine X120 V_H and V_L with anti-CD3 scFv (**FIG. 4A**). Likewise, by changing the specificity of the scFv fragment, numerous BsAbs were prepared. For example, **FIGs. 13A** and **13B** show the amino acid sequences of the light chain (SEQ ID NOs: 27 and 28) comprising the X120_VL-2 humanized anti-STEAP1 light chain with an anti-DOTA scFv based on mouse C825 or humanized C825 antibody. These light chains may be combined with heavy chains such as those disclosed in **FIGs. 11B** or **12B** to generate an anti-STEAP1-DOTA BsAb.

[00343] Also disclosed herein are the amino acid sequences of the humanized X120 x C825 (anti-DOTA) BsAbs of the single-chain bispecific tandem fragment variable (scBsTaFv) format (SEQ ID NOs: 29-40 and 61-64). **FIGs. 14A to 14P** show the amino acid sequences that feature a self-assembly disassembly (SADA) polypeptide containing a tetramerization domain from p53, p63, p73 (variants with or without histidine tag sequence). The scBsTaFvs of **FIGs. 14A to 14P** contain the X120_VL-2 and X120_VH-2 humanized variable domains disclosed herein. The scBsTaFvs may include any of the other humanized V_H or V_L domains disclosed herein.

Example 3: Purification and Biochemical Characterization of Anti-STEAP1 Immunoglobulin-related Compositions of the Present Disclosure

[00344] The DNA encoding both heavy chain and light chain was inserted into a mammalian expression vector, transfected into CHO-S cells, and stable clones of highest expression were selected. Supernatants were collected from shaker flasks and purified on protein A affinity chromatography.

[00345] To determine the biochemical purity of the BsAbs of the present disclosure, the purified BsAbs were resolved using size-exclusion chromatography-high-performance liquid chromatography (SEC-HPLC). The protein in the eluate was detected based on absorbance of UV light at 280 nm. An exemplary SEC-HPLC chromatogram is shown in **FIG. 1C**. The BsAb peaks were identified based on the retention time on SEC-HPLC. Biochemical purity

was assessed based on the area of the BsAb peak (85.7% for the 15.7 mins peak, and 11.1% for the 13.4 mins peak (dimerized peak)). The BsAb remained stable by SDS-PAGE and SEC-HPLC after multiple freeze and thaw cycles (data not shown).

Example 4: Comparative Binding of the Anti-STEAP1 Immunoglobulin-Related Compositions to Ewing's Sarcoma Cell Line TC32

[00346] To evaluate binding of the anti-STEAP1-BsAbs to STEAP1, flow cytometry was carried out following staining of Ewing's sarcoma cell lines with increasing concentrations of anti-STEAP1-BsAbs. As shown in **FIG. 2A**, the anti-STEAP1-BsAb BC261 bound specifically to STEAP1(+) Ewing's sarcoma cell line TC32. Control anti-human bispecific antibodies did not bind to TC32 cells (**FIG. 2A**). Binding of the anti-STEAP1-BsAb BC261 to an array of Ewing's sarcoma cell lines was tested using flow cytometry. As shown in **FIG. 2B**, all Ewing's sarcoma cell lines that were tested, except SKNMC, showed significant binding to BC261.

[00347] The six humanized V_H and four humanized V_L sequences of the murine X120 antibody disclosed herein were paired against each other, and twenty-four humanized BsAb versions were developed. As shown in **FIGs. 10A** and **10B**, the humanized BsAb sequences had identical CDR sequences. The sequences differed with respect to only some amino acids of the framework regions of V_H or V_L. To assess the affinity of the twenty-four humanized BsAbs to STEAP1, different doses of the antibodies were used to stain TC32 Ewing's sarcoma cells (STEAP1 positive). As shown in **FIG. 4A**, the BsAbs showed differing extent of binding to TC32 cells. The 4955 BsAb corresponds to a BsAb comprising the original X120 mouse antibody. Quantitation of the binding affinities of the twenty-four humanized BsAbs is presented in **FIGs. 20A-20B**.

[00348] After initial staining (**FIG. 4A**), ten different clones, including the chimeric BsAb clone, were chosen for further study. To assess binding of these ten clones to TC32 cells, following incubation of the BsAbs, the cells were subjected to ten washes with PBS. An aliquot of the binding reaction after each wash was stained with a fluorochrome labelled anti-human secondary antibody. The extent of binding of the anti-STEAP1 BsAbs to TC32 cells was measured using flow cytometry. As shown in **FIG. 4B**, there was a spectrum of affinities from low to high for these clones showing that the antibody affinity could be

changed by changing the sequence of antibody framework without altering the CDR sequences.

[00349] These results demonstrate that the antibodies or antigen binding fragments of the present technology can detect tumors that express STEAP1. Accordingly, the immunoglobulin-related compositions disclosed herein are useful for detecting a STEAP1-associated cancer in a subject in need thereof.

Example 5: The Anti-STEAP1 CD3-BsAb Redirected T-cells to Kill STEAP1 (+) Ewing's Sarcoma Cells

[00350] To evaluate whether anti-STEAP1-BsAb could redirect T cells to kill ES cells and prostate cancer cells, T cell cytotoxicity was tested in a standard 4-hour ⁵¹Cr release assays in various ES cell lines. When anti-STEAP1-BsAb was present, substantial killing was observed in STEAP1(+) TC32 (**FIG. 3A**), TC71-Luc (**FIG. 3B**), SK-ES-1 cells (**FIG. 3C**), A4573 (**FIG. 3D**), SKEAW (**FIG. 3E**), SKELP (**FIG. 3F**), SKERT (**FIG. 3G**), SKNMC (**FIG. 3H**), LNCaP-AR (**FIG. 3I**), CWR22(**FIG. 3J**), and VCaP (**FIG. 3K**). Without wishing to be bound by theory, it is believed that STEAP1 antigens may form microclusters at the cell surface, thus increasing the likelihood of clustering TCR and activating T cells. LNCaP-AR CWR22, and VCaP cells (**FIGs. 3I-K**) were tested in standard 4-hour ⁵¹Cr release assays. In the presence of STEAP1-BsAb BC261, substantial killing of ES tumor cell lines was observed with an EC₅₀ as low as 3.6 pM (for TC32 cells, 0.0009 µg/mL). A control bispecific antibody (an anti-GPA33 × CD3 BsAb BC123 that does not bind TC32 cells) did not kill the ES cell lines nor the prostate cancer cell line in these assays (**FIGs. 3A-3K**). When tested on prostate cancer cell line LNCaP-AR (**FIG. 3I**), BC261 mediated tumor killing at an EC₅₀ as low as 1.69 pM (0.000345 µg/mL).

[00351] These results demonstrate that the antibodies or antigen binding fragments of the present technology can detect tumors and inhibit the progression of tumor growth and/or metastasis. Accordingly, the immunoglobulin-related compositions disclosed herein are useful for treating a STEAP1-associated cancer in a subject in need thereof.

Example 6: The Killing of the STEAP1 (+) Ewing's Sarcoma Cells by T-cells Redirected by Anti-STEAP1 CD3-BsAb Correlates with BsAb Affinity

[00352] To evaluate the effect of antibody affinity on cytotoxicity potency, four humanized versions among the twenty-four humanized clones were selected based on their binding to STEAP1(+) positive cell lines (as determined by flow cytometry) and on their stabilities (as evaluated by HPLC) (**FIGs. 4A-4C**). T cell dependent cytotoxicity on STEAP1(+) TC32 cells in the presence of different doses of these four bispecific antibodies was tested in standard 4-hour ^{51}Cr release assays. As shown in **FIGs. 5A-5E**, the BsAbs with the higher affinity to STEAP1 exhibited higher levels of killing of TC32 (lower EC_{50}). BC261 (VL-2 + VH-2) was selected as the lead construct because of its high binding to STEAP1(+) cells (by flow cytometry), stability at 40°C over time (**FIG. 4C**), and the extent of its humanness of the $\text{V}_\text{L}/\text{V}_\text{H}$ sequences (which met the WHO criteria (>85%)).

[00353] These results demonstrate that the antibodies or antigen binding fragments of the present technology can detect tumors and inhibit the progression of tumor growth and/or metastasis. Accordingly, the immunoglobulin-related compositions disclosed herein are useful for treating a STEAP1-associated cancer in a subject in need thereof.

Example 7: In Vivo Therapy Studies Using the Anti- STEAP1 Immunoglobulin-related Compositions

[00354] For *in vivo* therapy studies, C.Cg-Rag2^{tm1Fwa}Il2rg^{tm1Sug}/JicTac, CIEA BRG male mice were used. To compare the efficacy of anti-STEAP1-BsAbs (BC259, BC260, BC261, BC262) against human Ewing's sarcoma xenograft TC32 in humanized mice, CIEA BRG male mice were injected subcutaneously with 3 million TC32 cells on day 0. Eight days later, the tumor volumes were measured (TM900, Peira) and the mice were distributed into 8 groups: 1. Activated T cell (ATC) only; 2. T cell plus 10 µg BC123 (an anti-GPA33 × CD3 BsAb that does not bind TC32 cells); 3. T cell plus BC259 (VH-1 + VL-1 BsAb variant, 10 µg/injection); 4. T cell plus BC260 (VH-2 + VL-1 BsAb variant, 10 µg/injection); 5. T cell plus BC261 (VH-2 + VL-2 BsAb variant, 10 µg/injection); 6. T cell plus BC262 (VH-5 + VL-1 BsAb variant, 10 µg/injection); 7. T cell plus 10µg BC120 (a HER2 × CD3 control BsAb that also does bind TC32 cells); and 8. Tumor only group.

[00355] Treatment was initiated on day 10, when the tumors (Ewing's sarcoma xenograft model) were fully established. For two weeks, mice received weakly injection of 20 million T cells mixed with BsAb. After the last dose of T cells, antibody treatment was continued for 2 more doses and then stopped. To support T cell survival *in vivo*, 1000IU IL2 was administered subcutaneously twice per week. The progression of TC32 Ewing's sarcoma cell line was monitored by measuring the tumor volumes (TM900, Peira). As shown in **FIG. 7A**, tumors in the tumors only group quickly grew into the range of 2000 mm³. The control BsAbs BC120 or BC123 did not suppress TC32 tumors. In contrast, each of BC259, BC260, BC261, and BC262 treated mice showed anti-tumor effects (**FIG. 7A**). Surprisingly, the low binding variant BC262 could suppress tumor growth, and only 1 mouse experienced recurrent tumor after the treatment was stopped. BC259, BC260, and BC261 treated mice showed prolonged survival and were healthy (**FIG. 7A**). In this model, BC261 showed slightly more efficient tumor suppression in terms of the rate of tumor volume shrinkage compared to BC259 or BC260.

[00356] These results demonstrate that the antibodies or antigen binding fragments of the present technology can detect tumors and inhibit the progression of tumor growth and/or metastasis. Accordingly, the immunoglobulin-related compositions disclosed herein are useful for treating a STEAP1-associated cancer in a subject in need thereof.

Example 8: Efficacy Titration of Anti-STEAP1-BsAb (BC261) Against Human Ewing's Sarcoma TC32 Xenograft

[00357] To further evaluate the efficacy of anti-STEAP1-BsAb (BC261) against human Ewing's sarcoma xenograft TC32 in humanized mice, a dose titration was performed. For *in vivo* therapy studies, C.Cg-Rag2^{tm1Fwa}Il2rg^{tm1Sug}/JicTac, CIEA BRG male mice were used. Mice were injected subcutaneously with 3 million TC32 cells on day 0. Seven days later, the tumor volumes were measured (TM900, Peira) and the mice were distributed into 5 groups: 1. Tumor only; 2. T cell plus 5 µg BC120 (an anti-HER2 × CD3 control BsAb that does bind TC32 cells); 3. T cell plus BC261 (50 µg/injection); 4. T cell plus BC261 (10 µg/injection); 5. T cell plus BC261 (2 µg/injection).

[00358] Treatment was initiated on day 8, when the tumors (Ewing's sarcoma xenograft model) were established. For two weeks, mice received weakly injection of 20 million T

cells mixed with BsAb. After the last dose of T cells, antibody treatment was continued for 2 more doses and then stopped. To support T cell survival *in vivo*, 1000IU IL2 was administered subcutaneously twice per week. The progression of TC32 Ewing's sarcoma cell line was monitored by measuring the tumor volumes (TM900, Peira). As shown in **FIG. 6A**, as low as 2 µg /injection dose of antibody (0.1 µg per million T cells per injection) could redirect T cells to reduce tumor burden and improve survival significantly ($p = 0.0047$ for tumor-only vs ATC/BC261 2 µg) while 5 µg dose of BC120 was only static /suppressive for tumor cells in this *in vivo* model since tumors quickly started growth within 2 weeks after the treatment was stopped (**FIGs. 6A-6B**). Although 2 µg/injection dose could provide anti-tumor effect, two mice in this treatment group had recurrent tumor after 80 days post-treatment (**FIG. 6C**). This might suggest that 10 µg/injection dose is an ideal dose for this xenograft model. In addition, there was no significant loss on the mice body weight of the treatment groups, which suggests that no significant toxicity was associated with the treatment.

[00359] These results demonstrate that the antibodies or antigen binding fragments of the present technology can detect tumors and inhibit the progression of tumor growth and/or metastasis. Accordingly, the immunoglobulin-related compositions disclosed herein are useful for treating a STEAP1-associated cancer in a subject in need thereof.

Example 9: Efficacy of Anti-STEAP1-BsAb (BC261) Against Large Tumors in Human Ewing's Sarcoma TC32 Xenograft Model

[00360] To test the efficacy of anti-STEAP1-BsAb (BC261) against large tumors, in human Ewing's sarcoma TC32 xenograft model, C.Cg-Rag2^{tm1Fwa}Il2rg^{tm1Sug}/JicTac, CIEA BRG male mice were injected subcutaneously with 3 million TC32 cells on day 0. Seven days later, the tumor volumes were measured (TM900, Peira) and the mice were distributed into three groups: 1. Group 8_Tumor only; 2. Group 1_ATC only; and Group 9_BC261 tumor Late treatment. Group 9 mice were not treated until 27 days after TC32 tumor was implanted. This group received 8 doses of ATC plus 10 µg BC261. As shown in **FIG.7B**, surprisingly, the tumors quickly shrank to the range of 500 mm³ range after 6 doses in 3 weeks, however 1 mouse did not survive due to graft versus host disease (GVHD) symptoms even though the tumor did shrink. Overall, 4 out of 5 mice in this group survived against

very aggressive tumor burden, and they all appeared to have GVHD symptoms after 8 doses of treatment but slowly recovered for the following 8 weeks.

[00361] These results demonstrate that the antibodies or antigen binding fragments of the present technology can detect tumors and inhibit the progression of tumor growth and/or metastasis. Accordingly, the immunoglobulin-related compositions disclosed herein are useful for treating a STEAP1-associated cancer in a subject in need thereof.

Example 10: Efficacy of Anti-STEAP1-BsAb (BC261) Against Human Ewing's Sarcoma Xenograft Models Based on TC71 or SKES1 Cell Lines

[00362] To further test the anti-tumor effect of BC261, Ewing's sarcoma xenograft models based on TC71 or SKES1 cell lines were used. CIEA BRG male mice were injected subcutaneously with either 5 million TC71 or SKES1 cells on day 0. Ten to eighteen days later, the tumor volumes were measured (TM900, Peira) and the mice were distributed into four groups each: 1. Activated T cell (ATC) only; 2. T cell plus 10 μ g BC123 (an anti-GPA33 $\times$ CD3 control BsAb); 3. T cell plus BC261 (10 μ g/injection); 4. BC261 only (10 μ g/injection).

[00363] Treatment was initiated when the tumor was fully established ($>200\text{mm}^3$). The data are shown in **FIGs. 8A-8B**. Since some TC71 tumors grew slowly compared to TC32 and SKES1, the treatment was not initiated until 21 days after the tumor implantation. As a result, only 3 out of 5 mice treated with BC261 survived compared to 100% anti-tumor effects for TC32 implantations. Two mice with escaping tumors were excluded from **FIG. 8A**. On the other hand, in case of SKES1, only 4 out of 5 mice treated with T cell plus BC261 were able to survive. One mouse, which died because of rapid tumor growth compared to the control groups was excluded from **FIG. 8B**. **FIGs. 8A-8B** demonstrate that BC261 with activated T cells exhibit an anti-tumor effect against STEAP1(+) cell lines in Ewing's sarcoma xenograft models based on TC71 or SKES1, compared to controls.

[00364] These results demonstrate that the antibodies or antigen binding fragments of the present technology can detect tumors and inhibit the progression of tumor growth and/or metastasis. Accordingly, the immunoglobulin-related compositions disclosed herein are useful for treating a STEAP1-associated cancer in a subject in need thereof.

Example 11: The Analysis of the STEAP1 Epitope for BC261

[00365] The epitope of the X120 antibody is unknown (see US Patent No. 7,494,646). In order to use protein engineering to improve the anti-tumor effect of BC261, defining the epitope is critical. The bispecific BC261 BsAb showed affinity for human but not mouse STEAP1 based on cell binding assays, and it had affinity for dog STEAP1 expressed on canine osteosarcoma cell lines as evidenced by FACS analysis (**FIG. 9C** and data not shown). Based on known sequence homology and structural information on STEAP1, these staining studies suggested that the binding epitope would most likely be present in the second extracellular domain (2nd ECD) of STEAP1, although the 3rd ECD could not be ruled based on STEAP1 sequence information (**FIG. 9A**).

[00366] To accurately determine the epitope of BC261 BsAb, the following four STEAP1 variants were constructed: human STEAP1 (STP1h), mouse STEAP1 (STP1m), mouse STEAP1 with human 2nd ECD (STP1mH2), and mouse STEAP1 with human 3rd ECD (STP1mH3). To express the STEAP1 variants on the cell surface, these variants were transfected into HEK293 cells using a lentiviral vector. GFP was a part of the transgene and used as a selection marker to FACS sort GFP (+) cells. As shown in **FIG. 9B**, the expression levels of all four STEAP1 variants on the cell surface was comparable as measured by the intensity of GFP fluorescence. Since GFP was a part of the transgene, GFP expression was an indirect measure of STEAP1 expression.

[00367] The variants were stained using the BC261 BsAb and binding was detected using flow cytometry. As shown in **FIG. 9C**, BC261 bound with only HEK 293 cells bearing the STP1mH2 variant with comparable mean fluorescence intensity as STP1h. These data demonstrate that BC261 recognizes an epitope located within the 2nd ECD domain of STEAP1.

[00368] Besides STEAP1, the 2nd ECD sequence of STEAP1 is found on the extracellular domain of STEAP1B, another related gene which is encoded on human chromosome 7, the opposite arm of STEAP1. STEAP1B has two isoforms, STEAP1B1 and STEAP1B2, both sharing the exact sequence as STEAP1. Therefore, the STEAP1B isoforms are expected to react with BC261. Since STEAP1B is expressed in human cancers, these isomers provide additional targets for BC261 and BC261 derived therapeutics.

[00369] FIG. 16 shows the staining of canine osteosarcoma cell lines by anti-STEAP1 BsAb BC261. The canine cell lines, D-17 and DSN, exhibited significant binding of BC261, and DSDH and DAN were also positive for anti-STEAP1 BsAb staining. The FACS analysis results demonstrate canine osteosarcoma can be treated by anti-STEAP1 BsAbs of the present disclosure. FIGs. 17A-17D show the antibody dependent T cell mediated cytotoxicity (ADTC) of anti-STEAP1-BsAb BC261 on STEAP1(+) canine osteosarcoma cell lines, specifically on D-17 (FIG. 17A), DSN (FIG. 17B), DSDh (FIG. 17C), and DAN cells (FIG. 17D). Substantial killing in four canine osteosarcoma cell lines was detected, which was consistent with the observation that STEAP1-BsAb BC261 binds to canine STEAP1 as determined by FACS analysis (FIG. 16) and sequence alignment (FIG. 9). These results demonstrate that STEAP1-BsAbs are useful for treating osteosarcoma in canine subjects. FIG. 18 demonstrates that BC261 showed picomolar range EC50 against Ewing sarcoma, prostate cancer and dog osteosarcoma cell lines.

[00370] These results demonstrate that the antibodies or antigen binding fragments of the present technology can detect tumors and inhibit the progression of tumor growth and/or metastasis. Accordingly, the immunoglobulin-related compositions disclosed herein are useful for detecting and/or treating a STEAP1-associated cancer in a subject in need thereof.

Example 12: BC261 Showed Exceptional Anti-tumor Potency in Ablating Prostate Patient Derived Prostate Xenografts (PDX) in NSG mice

[00371] The BC261 antibody was next tested against prostate cancer PDXs that were xenografted in NSG mice. Prostate cancer PDX (TM00298) was obtained from the *Jackson Laboratory* and passaged subcutaneously in NSG mice. On day 21 after tumor implantation, tumor size was measured using an electronic caliper (TM900, Peira), and mice were randomly assigned into 3 groups: Group 1: Human T cells expanded *in vitro* using anti-CD3/CD28 beads, 20 million cells per mouse iv q week; Group 2: iv human T cells plus 10 µg iv BC123 (control BsAb, GPA33 × CD3 that does not bind TC32 cells, twice a week); Group 3: iv human T cells plus iv BC261 (H2L2 BsAb variant, 10 µg/mouse, twice a week). Treatment began on day 28 when the tumor was fully established (>200mm³). The PDX tumors continued to grow to >500-1000 mm³ in the following week before responding to BC261/T-cells treatment. After 3 weeks of treatment, animals treated with BC261 + T cells

showed robust anti-tumor effects when compared to the control group – a potency rarely seen with BsAb. See FIGs. 15A-15B.

[00372] FIG. 15C shows the quantification of tumor volumes from DKO (BALB/cA-*Rag2^{tm1Fwa}/Il2rg^{tm1Sug}* (BRG)) mice harboring Prostate cancer patient derived xenografts (PDX: TM00298 from JAX lab) treated with BC261 or BC123 (anti-GPA33 × CD3 negative control) BsAbs and T cells. The BRG model shows a decrease in GVHD phenotype, which permits a more robust assessment of survival. As shown in FIG. 15C, BRG mice treated with BC261 + T cells showed prolonged survival curve compared with the control group.

[00373] These results demonstrate that the antibodies or antigen binding fragments of the present technology can detect tumors and inhibit the progression of tumor growth and/or metastasis. Accordingly, the immunoglobulin-related compositions disclosed herein are useful for detecting and/or treating a STEAP1-associated cancer in a subject in need thereof.

Example 13: Use of Anti-STEAP1 BsAb in PRIT

[00374] *IgG-based STEAP1-C825 BsAbs.* STEAP1(+) leukemic cells will be injected subcutaneously, intraperitoneally, intravenously, or via other routes into animals. After tumor establishment (depending on the type of tumor and the route of injection), treatment will be initiated. Treatment is composed of one or more cycles. Each cycle will comprise administration of the test BsAb (250 µg intravenously), followed by injection of a clearing agent (DOTA dextran or DOTA dendrimer; dose is 5-15% of the BsAb dose, see Cheal SM *et al.*, *Mol Cancer Ther* 13:1803-12, 2014) after 24 to 48 hours. After 4 hours, DOTA-¹⁷⁷Lu (up to 1.5mCi) or DOTA-²²⁵Ac (1µCi) will be injected intravenously. Generally, DOTA-²²⁵Ac is more potent than DOTA-¹⁷⁷Lu and may require fewer cycles for tumor eradication.

[00375] *Tetramerized BsAbs.* STEAP1(+) leukemic cells will be injected subcutaneously, intraperitoneally, intravenously, or via other routes into animals and after tumor establishment (depending on the type of tumor and the route of injection), treatment will be initiated. Treatment is composed of one or more cycles. Each cycle consists of administration of the BsAb (250 µg intravenously) followed by intravenous injection of DOTA-¹⁷⁷Lu (up to 1.5mCi) or DOTA-²²⁵Ac (1µCi) after 24-48 hours. Generally, DOTA-²²⁵Ac is more potent than DOTA-¹⁷⁷Lu and may require fewer cycles for tumor eradication.

[00376] These results will demonstrate that the antibodies or antigen binding fragments of the present technology can detect tumors and inhibit the progression of tumor growth and/or metastasis using PRIT. Accordingly, the immunoglobulin-related compositions disclosed herein are useful for detecting and treating a STEAP1-associated cancer in a subject in need thereof.

Example 14: Comparison of IgG[L]-scFv Anti-STEAP1× CD3 Bispecific Antibody with Other BsAb Formats

[00377] Five other STEAP1× CD3 bispecific antibody platforms (See **FIGs. 19A-19D**) will be compared directly with the IgG[L]-scFv format to test T cell-mediated tumor killing activities *in vitro* and *in vivo*.

[00378] It is anticipated that the STEAP1× CD3 IgG[L]-scFv format will show consistent anti-tumor effects *in vivo* when given intravenously to humanized mice. Additionally, it is expected that when arming T cells *ex vivo* with these 6 different antibody platforms, the IgG[L]-scFv format will produce the most potent anti-tumor effect *in vivo* compared to the other formats.

[00379] These results demonstrate that the antibodies or antigen binding fragments of the present technology can detect tumors and inhibit the progression of tumor growth and/or metastasis. Accordingly, the immunoglobulin-related compositions disclosed herein are useful for detecting and/or treating a STEAP1-associated cancer in a subject in need thereof.

EQUIVALENTS

[00380] The present technology is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the present technology. Many modifications and variations of this present technology can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within the scope of the present technology, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the present technology. It is to be understood that this present technology is not limited to particular methods, reagents, compounds compositions or biological systems,

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

[00381] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[00382] As will be understood by one skilled in the art, for any and all purposes, particularly in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, *etc.* As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, *etc.* As will also be understood by one skilled in the art all language such as “up to,” “at least,” “greater than,” “less than,” and the like, include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 cells refers to groups having 1, 2, or 3 cells. Similarly, a group having 1-5 cells refers to groups having 1, 2, 3, 4, or 5 cells, and so forth.

[00383] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

WHAT IS CLAIMED IS:

1. An antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein:

(a) the V_H comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11; and/or

(b) the V_L comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

2. The antibody or antigen binding fragment of claim 1, further comprising a Fc domain of an isotype selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, IgD, and IgE.

3. The antibody of claim 2, comprising an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A and K322A.

4. The antibody of claim 2, comprising an IgG4 constant region comprising a S228P mutation.

5. The antigen binding fragment of claim 1, wherein the antigen binding fragment is selected from the group consisting of Fab, F(ab')₂, Fab', scFv, and Fv.

6. The antibody or antigen binding fragment of any one of claims 1-5, wherein the antibody or antigen binding fragment binds to a STEAP1 polypeptide comprising amino acids 185 to 216 of any of SEQ ID NOs: 41, 42, or 60.

7. The antibody of any one of claims 1-4 or 6, wherein the antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody, or a bispecific antibody.

8. An antibody comprising a heavy chain (HC) amino acid sequence comprising SEQ ID NO: 22, SEQ ID NO: 26, or a variant thereof having one or more conservative amino acid substitutions, and/or a light chain (LC) amino acid sequence comprising SEQ ID NO: 21, SEQ ID NO: 24, SEQ ID NO: 27, SEQ ID NO: 28, or a variant thereof having one or more conservative amino acid substitutions.

9. The antibody of any one of claim 8, comprising a HC amino acid sequence and a LC amino acid sequence selected from the group consisting of:

SEQ ID NO: 22 and SEQ ID NO: 21;
SEQ ID NO: 22 and SEQ ID NO: 24;
SEQ ID NO: 22 and SEQ ID NO: 27;
SEQ ID NO: 22 and SEQ ID NO: 28;
SEQ ID NO: 26 and SEQ ID NO: 21;
SEQ ID NO: 26 and SEQ ID NO: 24;
SEQ ID NO: 26 and SEQ ID NO: 27; and
SEQ ID NO: 26 and SEQ ID NO: 28, respectively.

10. An antibody comprising (a) a light chain immunoglobulin variable domain sequence that is at least 95% identical to the light chain immunoglobulin variable domain sequence of any one of SEQ ID NOs: 17, 18, 19, or 20; and/or

(b) a heavy chain immunoglobulin variable domain sequence that is at least 95% identical to the heavy chain immunoglobulin variable domain sequence of any one of SEQ ID NOs: 6, 7, 8, 9, 10, or 11.

11. An antibody comprising:

(a) a LC sequence that is at least 95% identical to the LC sequence present in any one of SEQ ID NO: 21, SEQ ID NO: 24, SEQ ID NO: 27, or SEQ ID NO: 28; and/or

(b) a HC sequence that is at least 95% identical to the HC sequence present in SEQ ID NO: 22 or SEQ ID NO: 26.

12. The antibody of any one of claims 8-11, wherein the antibody is a chimeric antibody, a humanized antibody, or a bispecific antibody.

13. The antibody of any one of claims 8-12, wherein the antibody binds to a STEAP1 polypeptide comprising amino acids 185 to 216 of any of SEQ ID NOs: 41, 42, or 60.

14. The antibody of any one of claims 8-13, wherein the antibody comprises an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A and K322A.

15. The antibody of any one of claims 8-13, wherein the antibody comprises an IgG4 constant region comprising a S228P mutation.

16. A bispecific antibody or antigen binding fragment comprising an amino acid sequence that is at least 95% identical to an amino acid sequence selected from any one of SEQ ID NOs: 29-40 or 61-64.

17. The antibody or antigen binding fragment of claim 16, wherein the antibody or antigen binding fragment comprises an amino acid sequence selected from any one of SEQ ID NOs: 29-40 or 61-64.

18. A recombinant nucleic acid sequence encoding the antibody or antigen binding fragment of any one of claims 1-17.

19. A recombinant nucleic acid sequence selected from the group consisting of: SEQ ID NOs: 23, and 25.

20. A host cell or vector comprising the recombinant nucleic acid sequence of claim 18 or claim 19.

21. A composition comprising the antibody or antigen binding fragment of any one of claims 1-7 and a pharmaceutically-acceptable carrier, wherein the antibody or antigen binding fragment is optionally conjugated to an agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof.

22. A composition comprising the antibody or antigen binding fragment of any one of claims 8-17 and a pharmaceutically-acceptable carrier, wherein the antibody is optionally conjugated to an agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof.

23. The antibody of any one of claims 1-4, 6 or 7, wherein the antibody lacks α -1,6-fucose modifications.

24. The antibody of any one of claims 8-15, wherein the antibody lacks α -1,6-fucose modifications.

25. The bispecific antibody of claim 7 or 12, wherein the bispecific antibody binds to T cells, B-cells, myeloid cells, plasma cells, or mast-cells.

26. The bispecific antibody or antigen binding fragment of claim 7, 12, 16, or 17, wherein the bispecific antibody or antigen binding fragment binds to CD3, CD4, CD8, CD20, CD19, CD21, CD23, CD46, CD80, HLA-DR, CD74, CD22, CD14, CD15, CD16, CD123, TCR gamma/delta, NKp46, KIR, or a small molecule DOTA hapten.

27. A method for treating a STEAP1-associated cancer in a subject in need thereof, comprising administering to the subject an effective amount of an antibody comprising a HC amino acid sequence and a LC amino acid sequence selected from the group consisting of:

SEQ ID NO: 22 and SEQ ID NO: 21;

SEQ ID NO: 22 and SEQ ID NO: 24;

SEQ ID NO: 22 and SEQ ID NO: 27;

SEQ ID NO: 22 and SEQ ID NO: 28;

SEQ ID NO: 26 and SEQ ID NO: 21;

SEQ ID NO: 26 and SEQ ID NO: 24;

SEQ ID NO: 26 and SEQ ID NO: 27; and

SEQ ID NO: 26 and SEQ ID NO: 28, respectively.

wherein the antibody specifically binds to STEAP1.

28. A method for treating a STEAP1-associated cancer in a subject in need thereof, comprising administering to the subject an effective amount of a bispecific antibody or antigen binding fragment comprising an amino acid sequence selected from any one of SEQ ID NOs: 29-40 or 61-64.

29. The method of any one of claims 27 or 28, wherein the STEAP1-associated cancer is Ewing's sarcoma, prostate cancer, osteosarcoma, bladder cancer, breast cancer, ovary cancer, colon cancer, lung cancer, or kidney cancer.

30. The method of any one of claims 27-29, wherein the antibody or antigen binding fragment is administered to the subject separately, sequentially or simultaneously with an additional therapeutic agent.

31. The method of claim 30, wherein the additional therapeutic agent is one or more of alkylating agents, platinum agents, taxanes, vinca agents, anti-estrogen drugs, aromatase inhibitors, ovarian suppression agents, VEGF/VEGFR inhibitors, EGF/EGFR inhibitors, PARP inhibitors, cytostatic alkaloids, cytotoxic antibiotics, antimetabolites, endocrine/hormonal agents, bisphosphonate therapy agents.

32. A method for detecting a tumor in a subject *in vivo* comprising

(a) administering to the subject an effective amount of the antibody or antigen binding fragment of any one of claims 1-17, wherein the antibody or antigen binding fragment is configured to localize to a tumor expressing STEAP1 and is labeled with a radioisotope; and

(b) detecting the presence of a tumor in the subject by detecting radioactive levels emitted by the antibody or antigen binding fragment that are higher than a reference value.

33. The method of claim 32, wherein the subject is diagnosed with or is suspected of having cancer.

34. The method of claim 32 or 33, wherein the radioactive levels emitted by the antibody or antigen binding fragment are detected using positron emission tomography or single photon emission computed tomography.

35. The method of any one of claims 32-34, further comprising administering to the subject an effective amount of an immunoconjugate comprising the antibody or antigen binding fragment of any one of claims 1-17 conjugated to a radionuclide.

36. The method of claim 35, wherein the radionuclide is an alpha particle-emitting isotope, a beta particle-emitting isotope, an Auger-emitter, or any combination thereof.

37. The method of claim 36, wherein the beta particle-emitting isotope is selected from the group consisting of ^{86}Y , ^{90}Y , ^{89}Sr , ^{165}Dy , ^{186}Re , ^{188}Re , ^{177}Lu , and ^{67}Cu .

38. A kit comprising the antibody or antigen binding fragment of any one of claims 1-17 and instructions for use.

39. The kit of claim 38, wherein the antibody or antigen binding fragment of any one of claims 1-17 is coupled to at least one detectable label selected from the group consisting of a radioactive label, a fluorescent label, and a chromogenic label.

40. The kit of claim 38 or 39, further comprising a secondary antibody that specifically binds to the antibody of any one of claims 1-17.

41. The bispecific antibody or antigen binding fragment of claim 7, 12, 16, or 17, wherein the bispecific antibody binds to a radiolabeled DOTA hapten and a STEAP1 antigen.

42. A method for selecting a subject for pretargeted radioimmunotherapy comprising

(a) administering to the subject an effective amount of a complex comprising a radiolabeled DOTA hapten and the bispecific antibody or antigen binding fragment of claim 41, wherein the complex is configured to localize to a STEAP1 expressing tumor;

(b) detecting radioactive levels emitted by the complex; and

(c) selecting the subject for pretargeted radioimmunotherapy when the radioactive levels emitted by the complex are higher than a reference value.

43. A method for increasing tumor sensitivity to radiation therapy in a subject diagnosed with a STEAP1-associated cancer comprising administering to the subject an effective amount of a complex comprising a radiolabeled DOTA hapten and the bispecific antibody or antigen binding fragment of claim 41, wherein the complex is configured to localize to a STEAP1 expressing tumor.

44. A method for treating cancer in a subject in need thereof comprising administering to the subject an effective amount of a complex comprising a radiolabeled DOTA hapten and the bispecific antibody or antigen binding fragment of claim 41, wherein the complex is configured to localize to a STEAP1 expressing tumor.

45. A method for increasing tumor sensitivity to radiation therapy in a subject diagnosed with a STEAP1-associated cancer comprising

(a) administering an effective amount of the bispecific antibody or antigen binding fragment of claim 41, wherein the bispecific antibody or antigen binding fragment is configured to localize to a STEAP1 expressing tumor; and

(b) administering an effective amount of a radiolabeled-DOTA hapten to the subject, wherein the radiolabeled-DOTA hapten is configured to bind to the bispecific antibody or antigen binding fragment.

46. A method for treating cancer in a subject in need thereof comprising

(a) administering an effective amount of the bispecific antibody or antigen binding fragment of claim 41, wherein the bispecific antibody or antigen binding fragment is configured to localize to a STEAP1 expressing tumor; and

(b) administering an effective amount of a radiolabeled-DOTA hapten to the subject, wherein the radiolabeled-DOTA hapten is configured to bind to the bispecific antibody or antigen binding fragment.

47. The method of claim 45 or 46, further comprising administering an effective amount of a clearing agent to the subject prior to administration of the radiolabeled-DOTA hapten.

48. The method of any one of claims 42-47, wherein the subject is human.

49. The method of any one of claims 42-44, wherein the complex is administered intravenously, intramuscularly, intraarterially, intrathecally, intracapsularly, intraorbitally, intradermally, intraperitoneally, transtracheally, subcutaneously, intracerebroventricularly, orally, intratumorally, or intranasally.

50. The method of any one of claims 42-49, wherein the radiolabeled-DOTA hapten comprises an alpha particle-emitting isotope, a beta particle-emitting isotope, or an Auger-emitter.

51. The method of any one of claims 42-50, wherein the radiolabeled-DOTA hapten comprises ^{213}Bi , ^{211}At , ^{225}Ac , ^{152}Dy , ^{212}Bi , ^{223}Ra , ^{219}Rn , ^{215}Po , ^{211}Bi , ^{221}Fr , ^{217}At ,

²⁵⁵Fm, ⁸⁶Y, ⁹⁰Y, ⁸⁹Sr, ¹⁶⁵Dy, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁷⁷Lu, ⁶⁷Cu, ¹¹¹In, ⁶⁷Ga, ⁵¹Cr, ⁵⁸Co, ^{99m}Tc, ^{103m}Rh, ^{195m}Pt, ¹¹⁹Sb, ¹⁶¹Ho, ^{189m}Os, ¹⁹²Ir, ²⁰¹Tl, ²⁰³Pb, ⁶⁸Ga, ²²⁷Th, or ⁶⁴Cu.

52. A bispecific antigen binding fragment comprising a first polypeptide chain, wherein:

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

- i. a heavy chain variable domain of a first immunoglobulin that is capable of specifically binding to a first epitope;
- ii. a flexible peptide linker comprising the amino acid sequence (GGGGS)₆;
- iii. a light chain variable domain of the first immunoglobulin;
- iv. a flexible peptide linker comprising the amino acid sequence (GGGGS)₄;
- v. a heavy chain variable domain of a second immunoglobulin that is capable of specifically binding to a second epitope;
- vi. a flexible peptide linker comprising the amino acid sequence (GGGGS)₆;
- vii. a light chain variable domain of the second immunoglobulin;
- viii. a flexible peptide linker sequence comprising the amino acid sequence TPLGDTTHT; and
- ix. a self-assembly disassembly (SADA) polypeptide;

wherein the heavy chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and/or the light chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20.

53. A bispecific antigen binding fragment comprising a first polypeptide chain, wherein:

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

- i. a light chain variable domain of a first immunoglobulin that is capable of specifically binding to a first epitope;
- ii. a flexible peptide linker comprising the amino acid sequence (GGGGS)₆;
- iii. a heavy chain variable domain of the first immunoglobulin;
- iv. a flexible peptide linker comprising the amino acid sequence (GGGGS)₄;

- v.a heavy chain variable domain of a second immunoglobulin that is capable of specifically binding to a second epitope;
- vi.a flexible peptide linker comprising the amino acid sequence (GGGS)₆;
- vii.a light chain variable domain of the second immunoglobulin;
- viii.a flexible peptide linker sequence comprising the amino acid sequence TPLGDTTHT; and
- ix.a self-assembly disassembly (SADA) polypeptide;

wherein the heavy chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and/or the light chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20.

54. The antigen binding fragment of claim 52 or 53, wherein the SADA polypeptide comprises a tetramerization, pentamerization, or hexamerization domain.

55. The antigen binding fragment of claim 54, wherein the SADA polypeptide comprises a tetramerization domain of any one of p53, p63, p73, hnRNPC, SNA-23, Stefin B, KCNQ4, or CBFA2T1.

56. The antigen binding fragment of any one of claims 52-55, wherein the antigen binding fragment comprises an amino acid sequence selected from SEQ ID NOs: 29-40 or 61-64.

57. A bispecific antibody comprising a first polypeptide chain, a second polypeptide chain, a third polypeptide chain and a fourth polypeptide chain, wherein the first and second polypeptide chains are covalently bonded to one another, the second and third polypeptide chains are covalently bonded to one another, and the third and fourth polypeptide chain are covalently bonded to one another, and wherein:

- a. each of the first polypeptide chain and the fourth polypeptide chain comprises in the N-terminal to C-terminal direction:

- i. a light chain variable domain of a first immunoglobulin that is capable of specifically binding to a first epitope;
 - ii. a light chain constant domain of the first immunoglobulin;
 - iii. a flexible peptide linker comprising the amino acid sequence (GGGGS)₃; and
 - iv. a light chain variable domain of a second immunoglobulin that is linked to a complementary heavy chain variable domain of the second immunoglobulin, or a heavy chain variable domain of a second immunoglobulin that is linked to a complementary light chain variable domain of the second immunoglobulin, wherein the light chain and heavy chain variable domains of the second immunoglobulin are capable of specifically binding to a second epitope, and are linked together via a flexible peptide linker comprising the amino acid sequence (GGGGS)₆ to form a single-chain variable fragment; and
- b. each of the second polypeptide chain and the third polypeptide chain comprises in the N-terminal to C-terminal direction:
 - i. a heavy chain variable domain of the first immunoglobulin that is capable of specifically binding to the first epitope; and
 - ii. a heavy chain constant domain of the first immunoglobulin; and

wherein the heavy chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and/or the light chain variable domain of the first immunoglobulin is selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20.

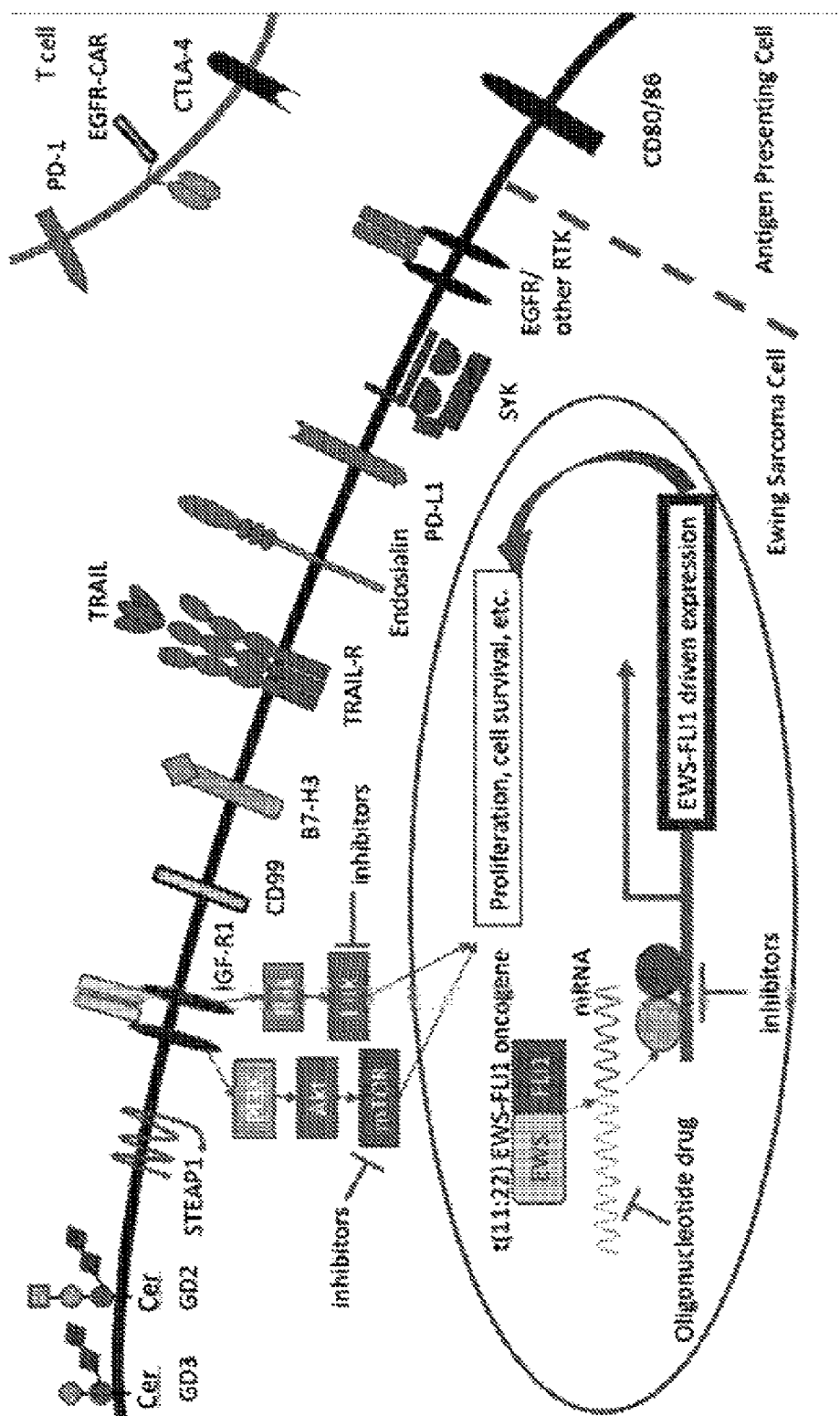
58. The bispecific antibody of claim 57, wherein the second immunoglobulin binds to CD3, CD4, CD8, CD20, CD19, CD21, CD23, CD46, CD80, HLA-DR, CD74, CD22, CD14, CD15, CD16, CD123, TCR gamma/delta, NKp46, KIR, or a small molecule DOTA hapten.

59. The bispecific antibody or antigen binding fragment of claim 7, 12, or 57-58 wherein the bispecific antibody binds to CD3 and a STEAP1 antigen.

60. An *ex vivo* armed T cell that is coated or complexed with an effective amount of the bispecific antibody of claim 59, wherein the bispecific antibody includes a CD3 binding domain comprising a heavy chain immunoglobulin variable domain (V_H) of SEQ ID NO: 80 and a light chain immunoglobulin variable domain (V_L) of SEQ ID NO: 81, wherein the bispecific antibody is an immunoglobulin comprising two heavy chains and two light chains, wherein each of the light chains is fused to a single chain variable fragment (scFv).

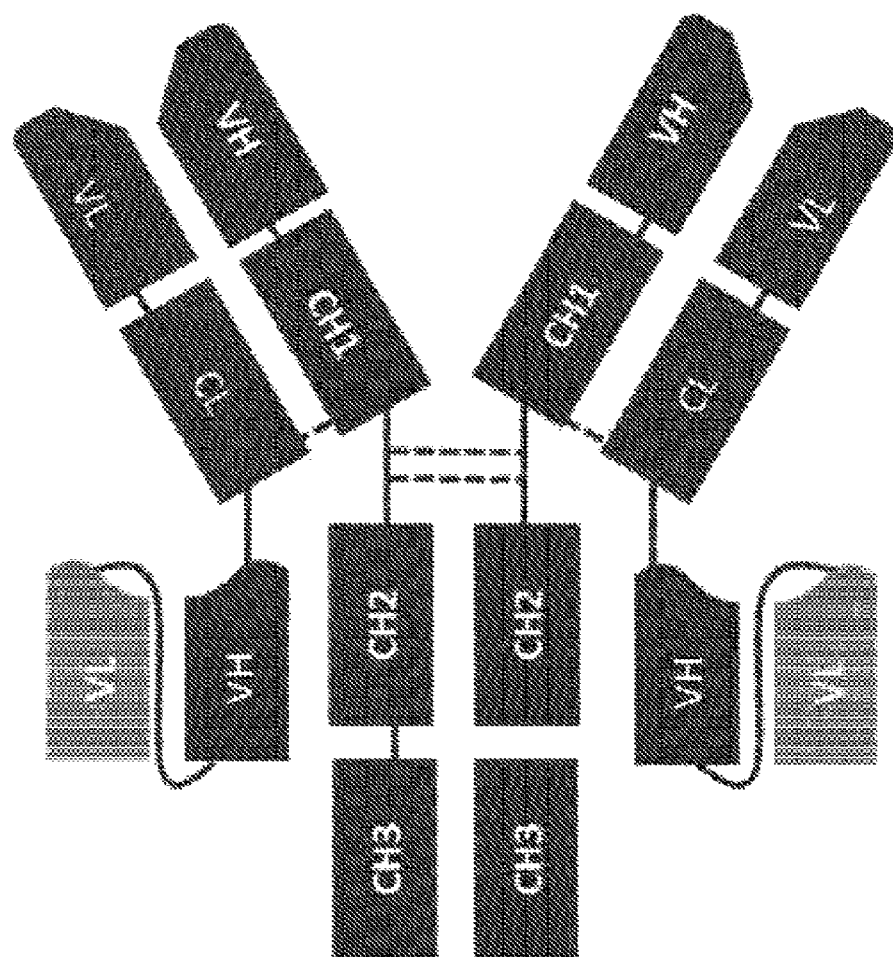
61. The *ex vivo* armed T cell of claim 60, wherein at least one scFv of the bispecific antibody comprises the CD3 binding domain.

62. A method for treating a STEAP1-associated cancer in a subject in need thereof comprising administering to the subject an effective amount of the *ex vivo* armed T cell of claim 60 or 61.

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FIG. 1A

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FIG. 1B

Modular IgG-scFv



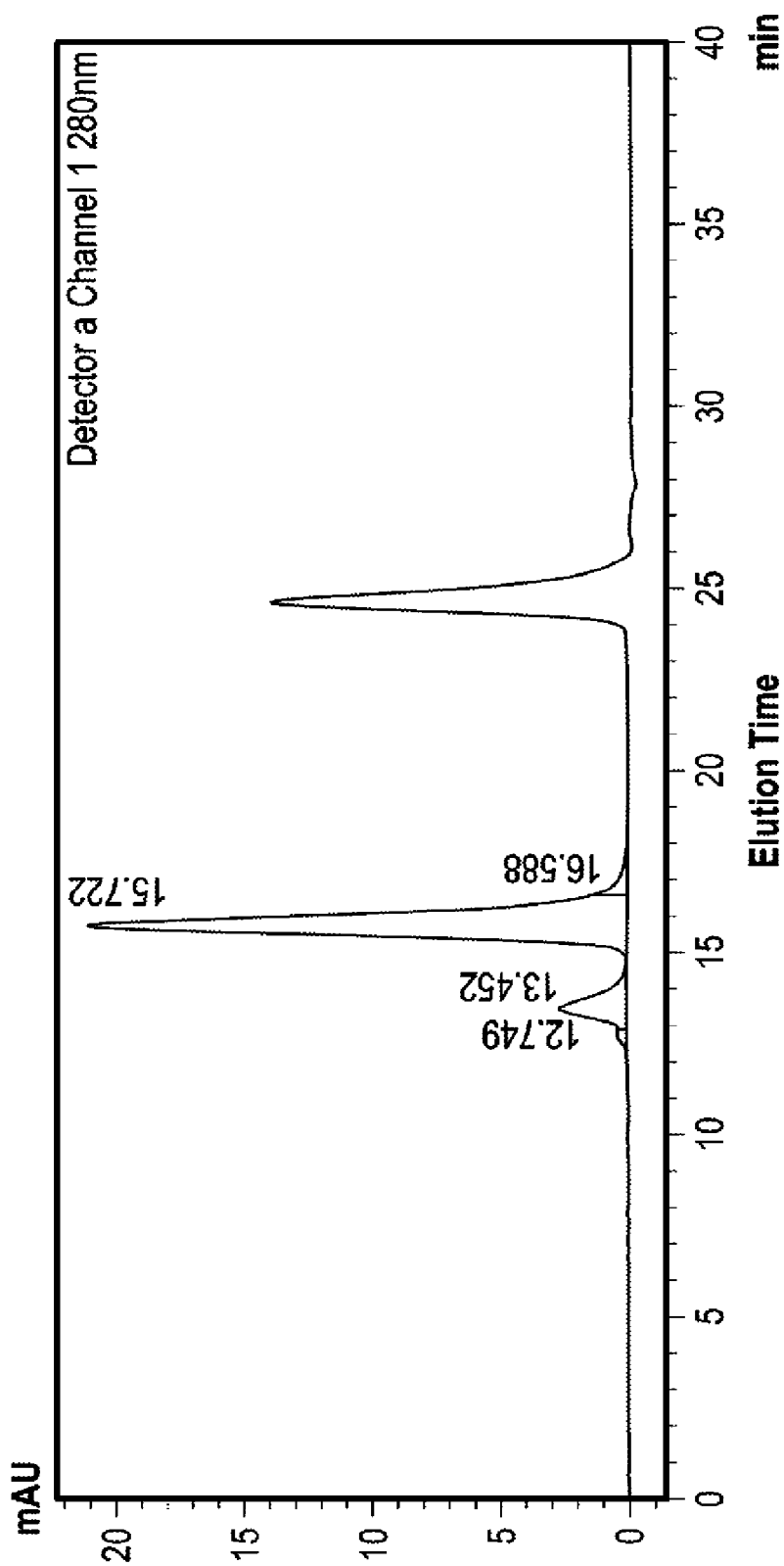
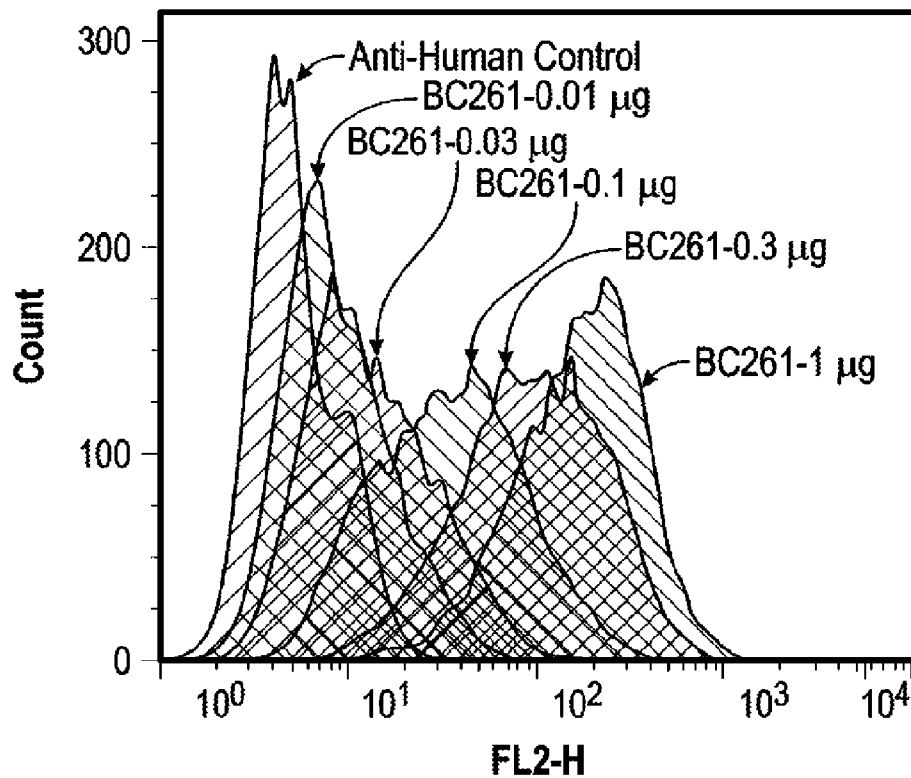


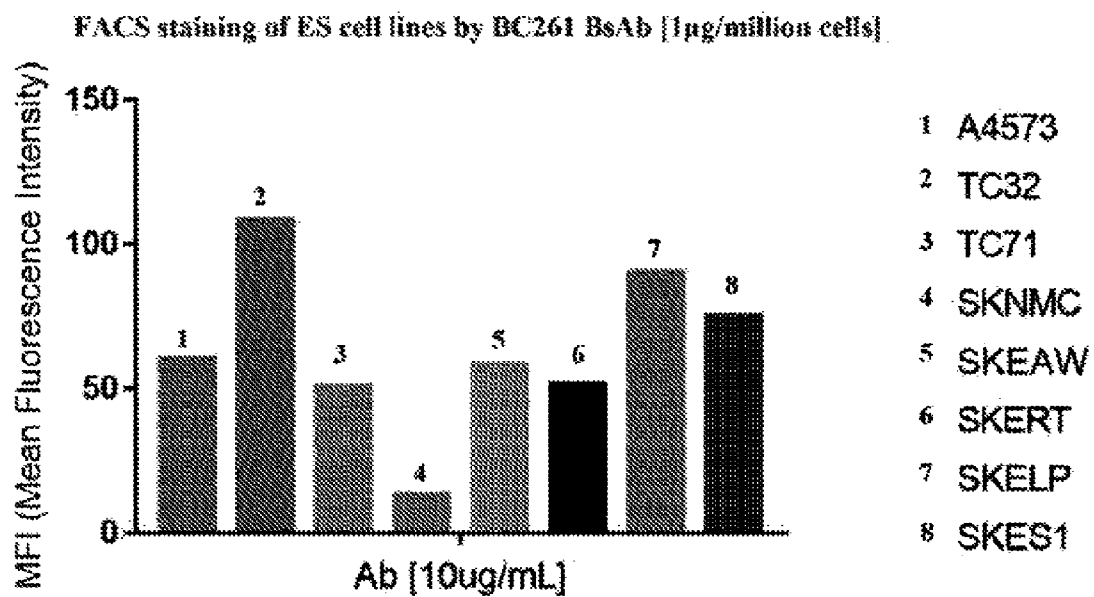
FIG. 1C



SAMPLE	Count	Geometrix Mean: FL2-H
Anti-human ctrl	9239	5.22
BC261-1 μ g	9496	160
BC261-0.3 μ g	9494	92.7
BC261-0.1 μ g	9430	33.9
BC261-0.03 μ g	9287	12.9
BC261-0.01 μ g	9397	8.73

FIG. 2A

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FIG. 2B



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FIG. 3A

ADTC of ES cell line TC32

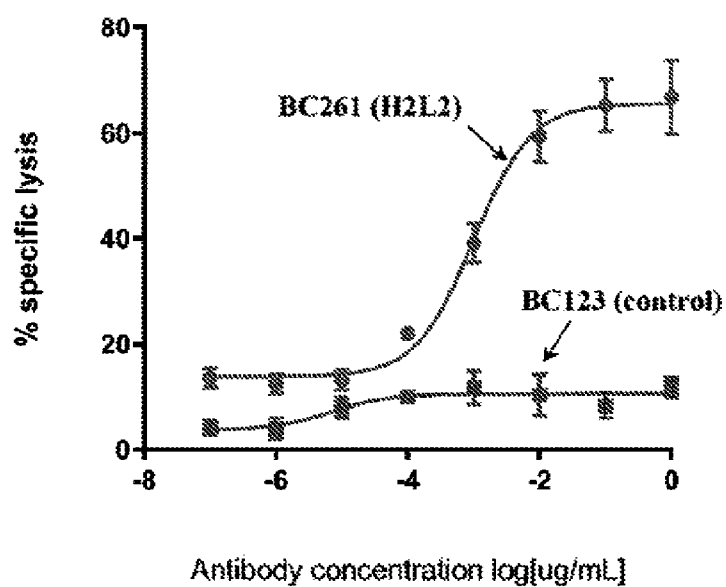
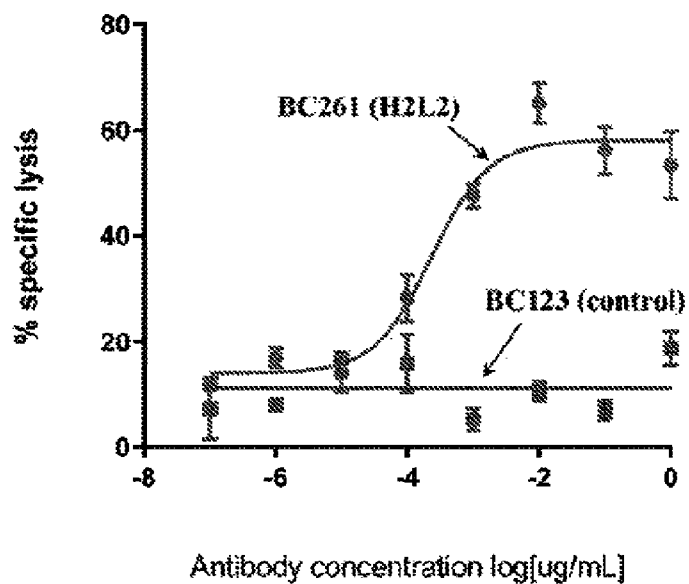


FIG. 3B

ADTC of ES cell line TC71



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FIG. 3C

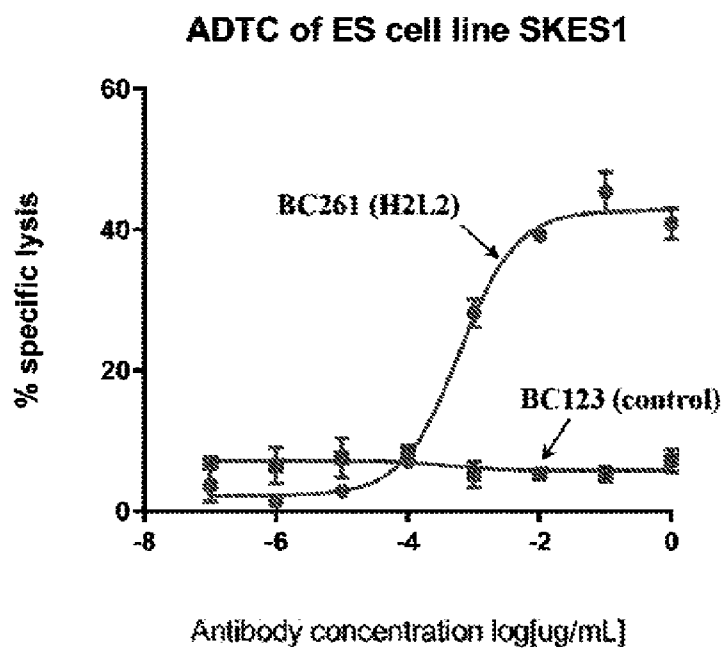


FIG. 3D

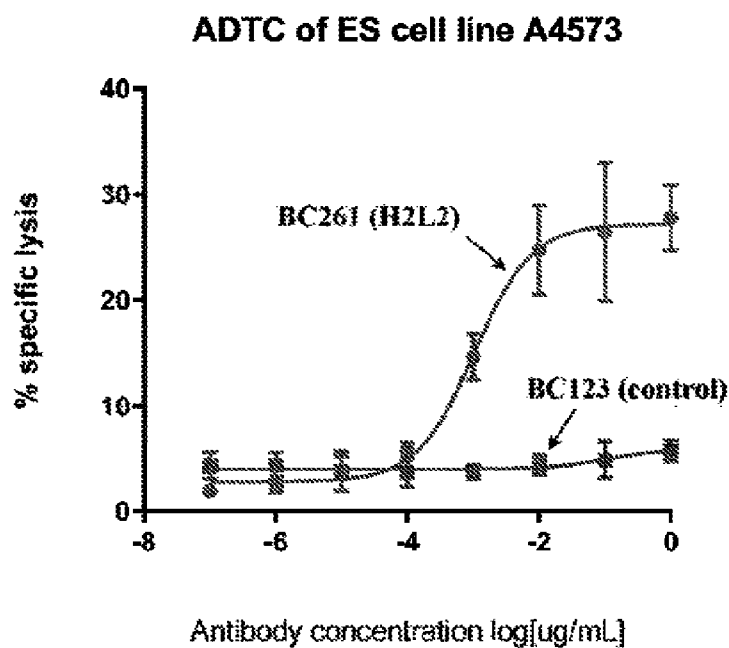


FIG. 3E

ADTC of ES cell line SKEAW

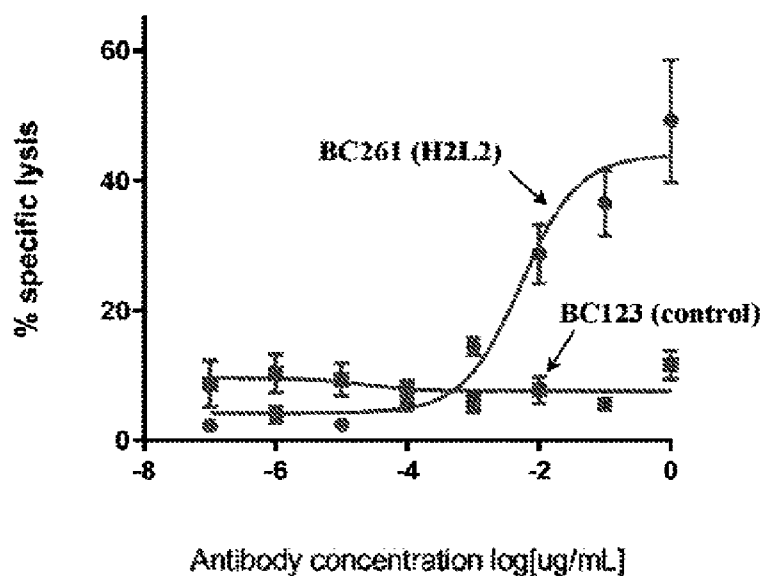
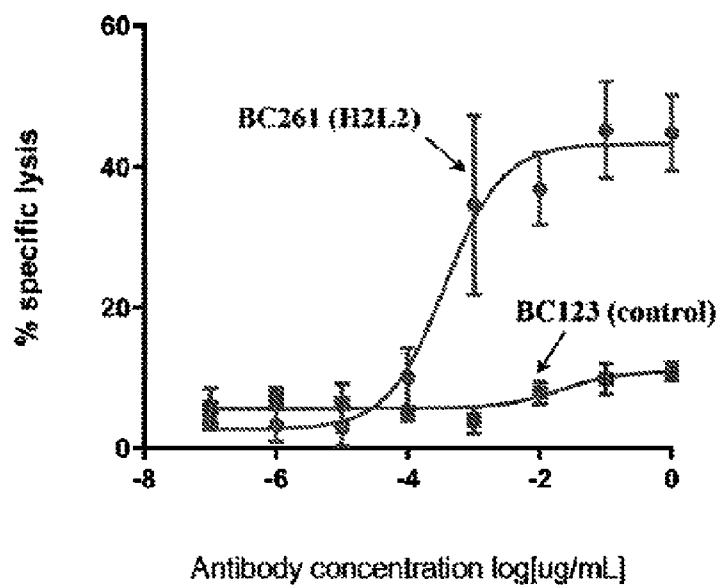


FIG. 3F

ADTC of ES cell line SKELP



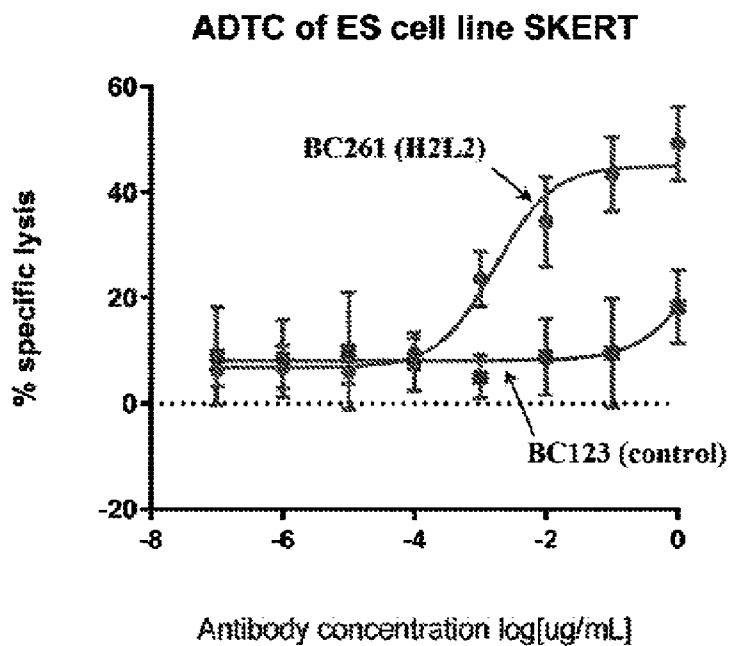
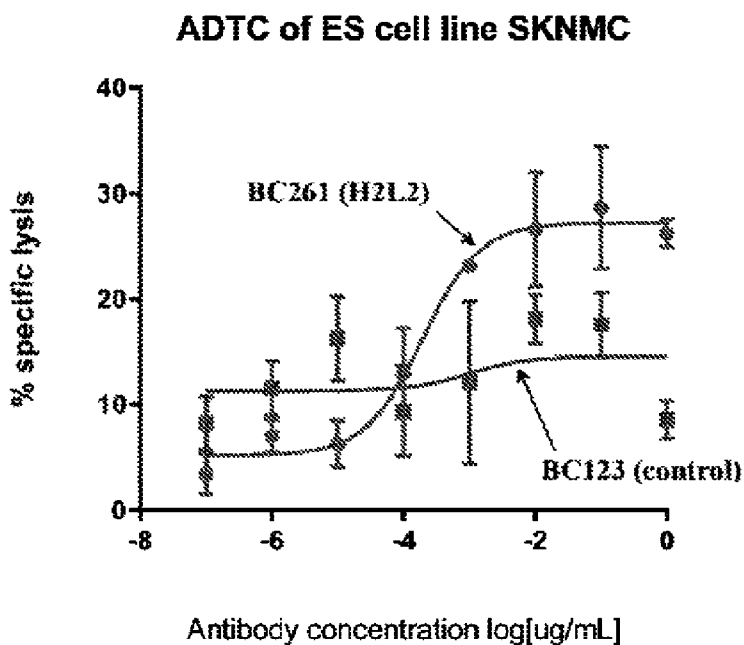
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FIG. 3G

FIG. 3H



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FIG. 3I

ADTC of Prostate cancer cell line LNCaP-AR

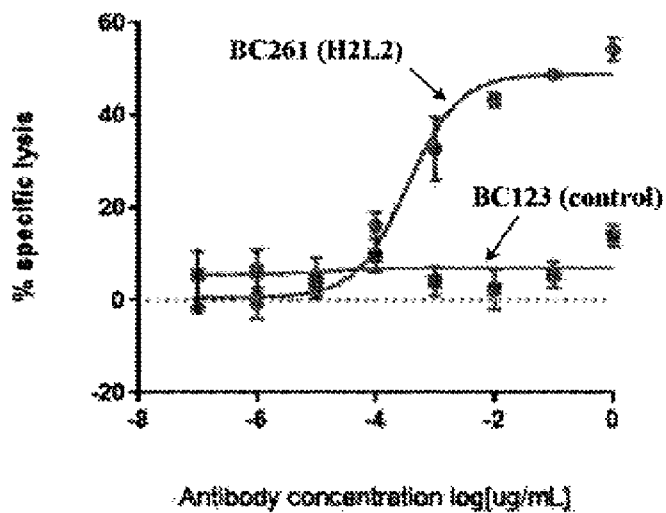
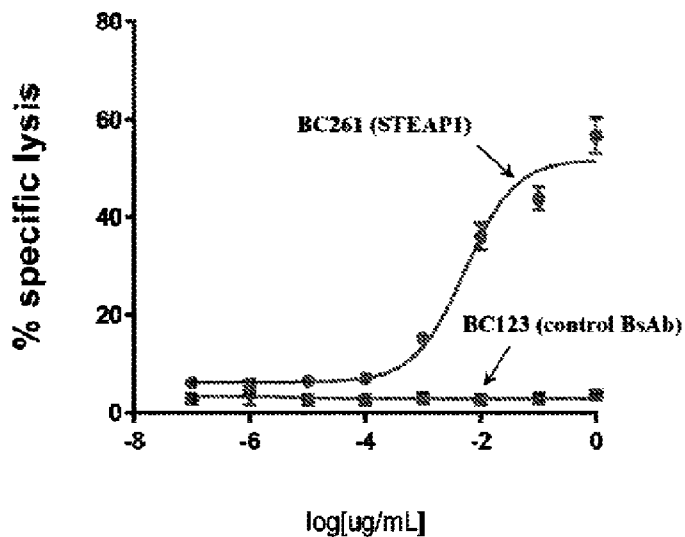


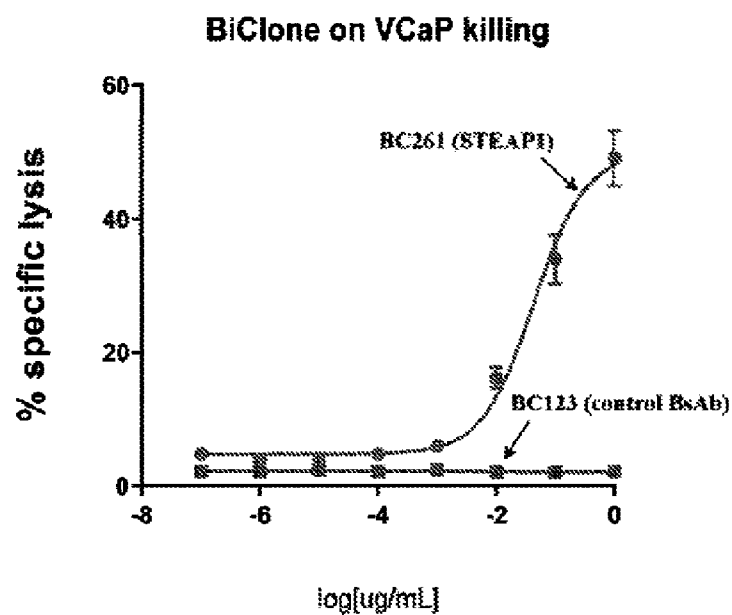
FIG. 3J

BiClone on CWR22 killing



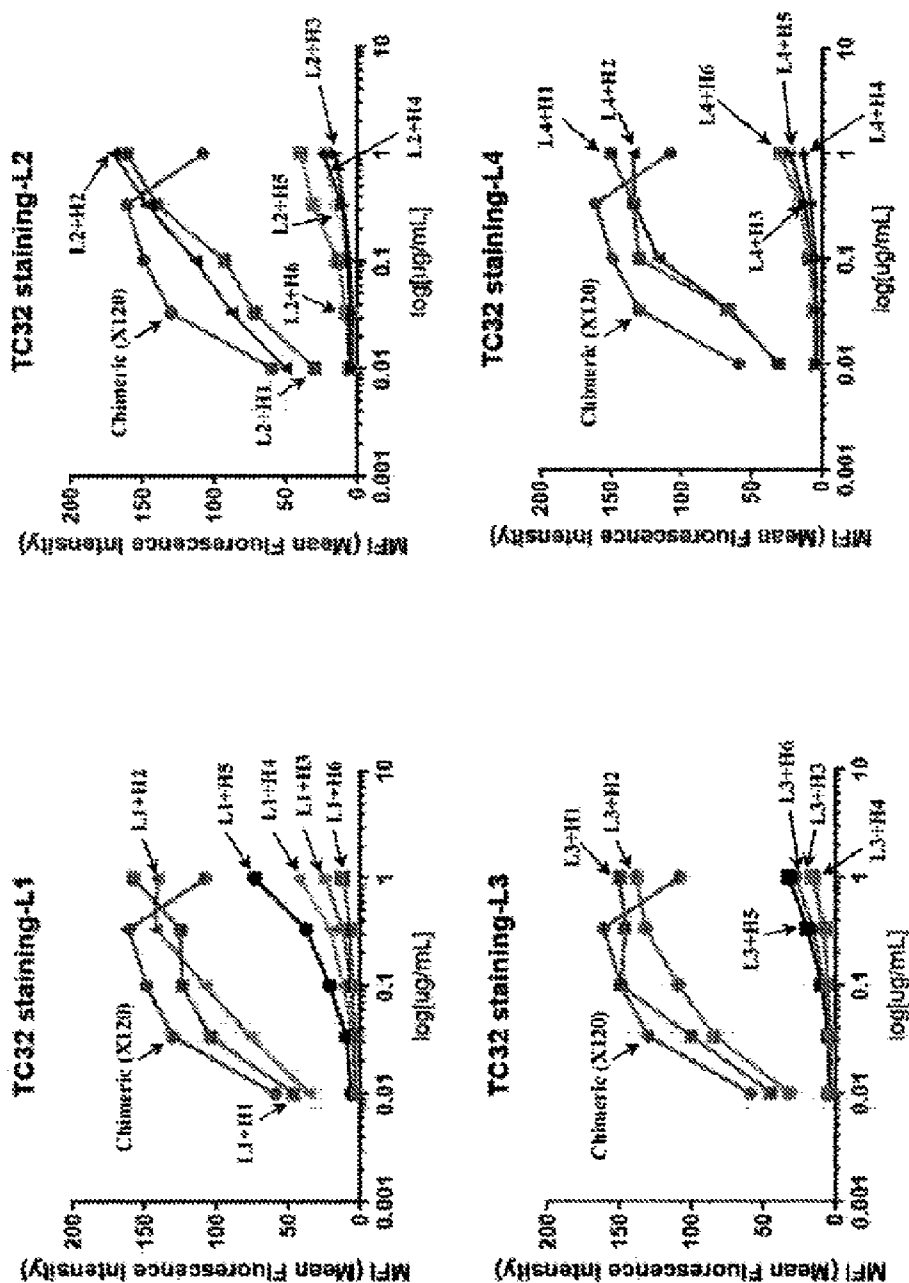
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FIG. 3K



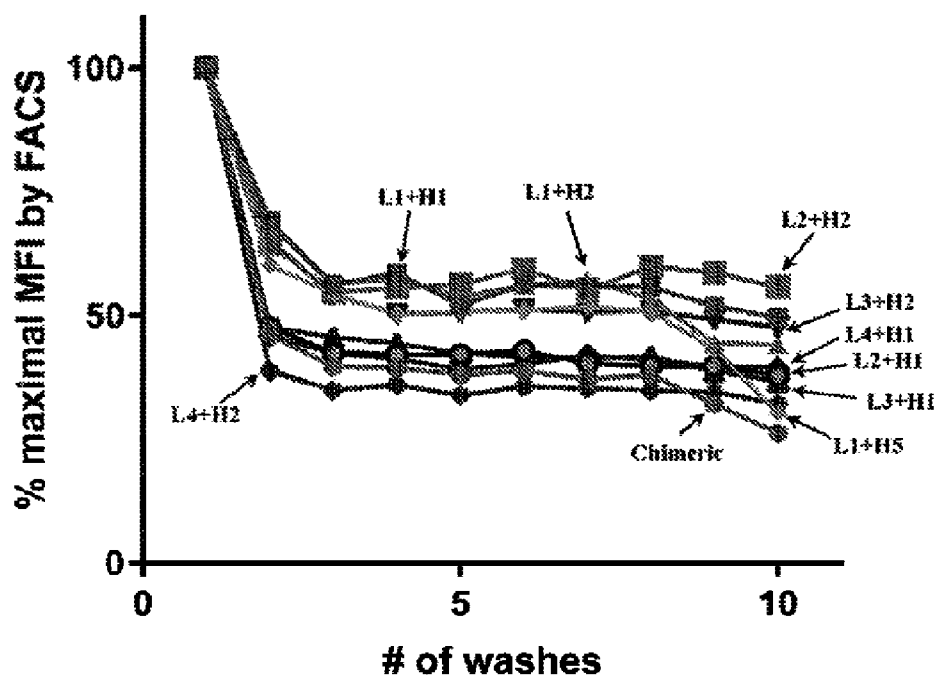
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FIG. 4A



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FIG. 4B

Binding avidity by washing

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FIG. 4C

Protein Name	OD280nm	Conc. (mg/ml)	time 0	F/T	40C D7	40C D14	40C D21	40C D28
chimeric (X120)	2.58	1.58	99.2	98.0	90.4	86.0	80.5	76.1
VL1+VH1	3.34	2.03	99.4	98.9	91.9	87.4	81.5	77.3
VL1+VH2	3	1.82	99.5	93.2	90.9	88.4	82.4	78.2
VL1+VH3	3.25	1.96	99.5	96.3	92.0	88.6	83.5	79.7
VL1+VH4	3.14	1.89	99.4	95.8	91.1	88.6	84.3	80.5
VL1+VH5	2.75	1.66	99.5	91.0	91.2	88.5	83.7	79.5
VL1+VH6	3.21	1.93	99.4	98.5	92.4	88.9	83.5	79.6
VL2+VH1	3.49	2.12	99.5	97.9	92.8	88.7	83.8	80.2
VL2+VH2	3.67	2.23	99.4	98.0	91.8	88.8	83.7	79.6
VL2+VH3	3.34	2.01	99.4	98.1	91.8	89.3	83.7	79.7
VL2+VH4	3.27	1.97	99.4	94.1	90.7	88.7	83.3	79.3
VL2+VH5	3.24	1.95	99.5	97.3	90.6	88.0	81.9	77.4
VL2+VH6	3.3	1.99	99.3	94.6	90.3	87.2	80.4	76.1
VL3+VH1	3.48	2.12	99.4	97.2	91.6	88.4	82.1	78.0
VL3+VH2	2.34	1.42	99.2	97.6	80.5	68.5	57.4	50.6
VL3+VH3	3.37	2.03	99.3	99.0	84.1	71.3	59.7	52.3
VL3+VH4	2.93	1.77	99.3	98.2	84.3	72.1	61.2	53.9
VL3+VH5	2.7	1.63	99.3	96.5	84.8	73.4	67.9	65.2
VL3+VH6	3.1	1.87	99.2	97.3	85.1	72.7	61.8	54.5
VL4+VH1	3.26	1.98	99.4	98.6	86.1	73.7	62.0	54.1
VL4+VH2	3.14	1.91	99.4	99.2	85.8	73.1	61.7	53.1
VL4+VH3	3.77	2.27	99.3	98.2	84.3	71.5	60.3	52.6
VL4+VH4	3.23	1.95	99.4	98.7	83.9	71.2	61.4	55.1
VL4+VH5	3.37	2.03	99.3	97.9	82.2	69.7	58.1	50.5
VL4+VH6	2.27	1.37	99.3	97.4	84.3	70.7	57.8	48.8

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FIG. 5A

ADTC on TC32

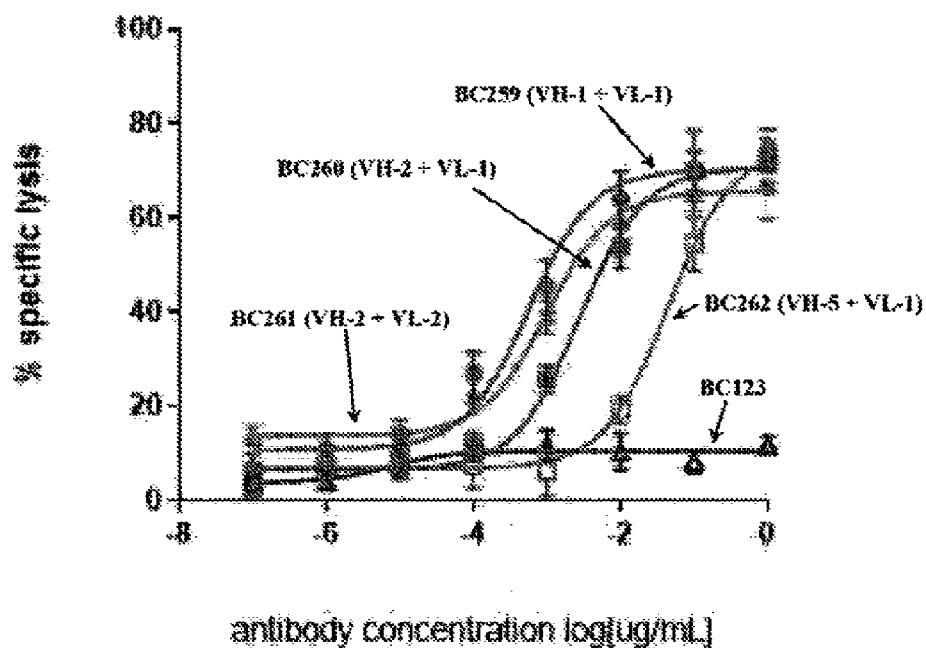
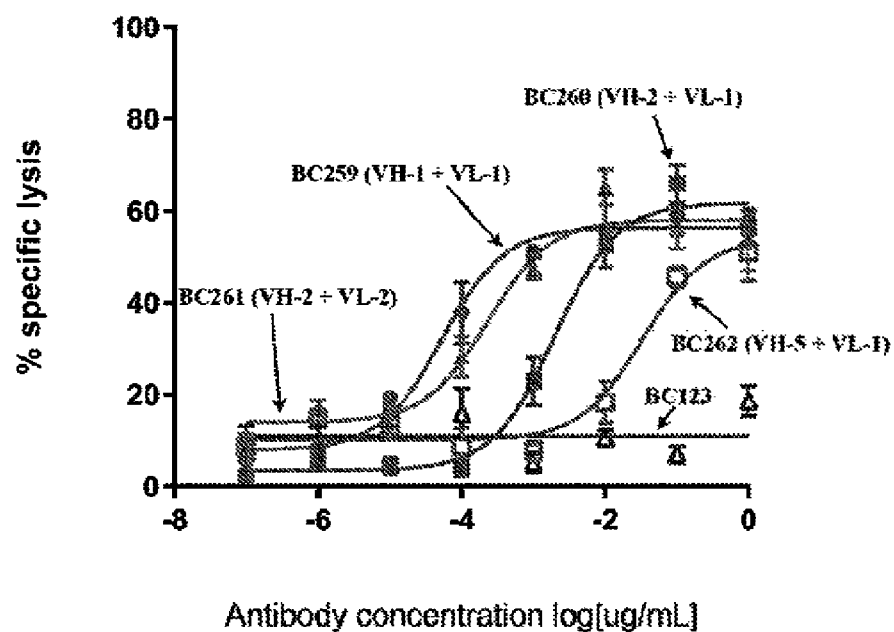


FIG. 5B

ADTC on TC71



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ADTC on SKES1

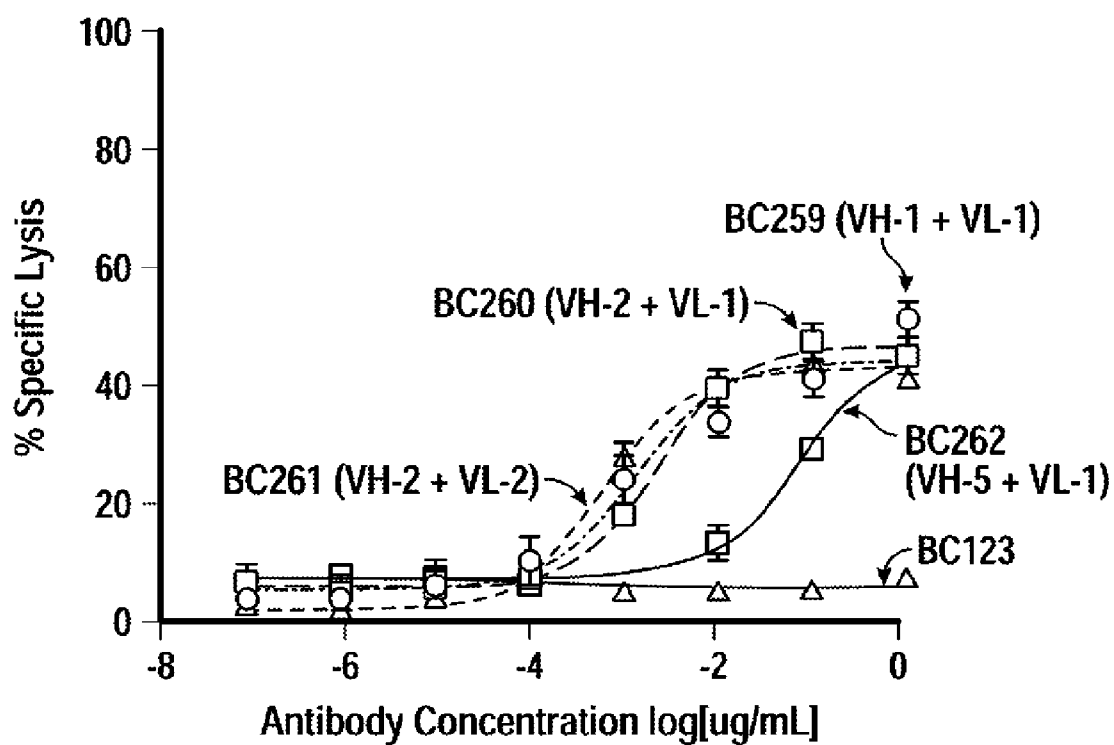


FIG. 5C

EC50 in pM			
BsAb/Cell Lines	TC32	TC71	SKES1
BC259	2.8	0.3	6.3
BC260	13.5	9.6	11.4
BC261	4.9	1.1	3.0
BC262	214.3	161.3	342.2
Control BsAb	N/A	N/A	N/A

FIG. 5D

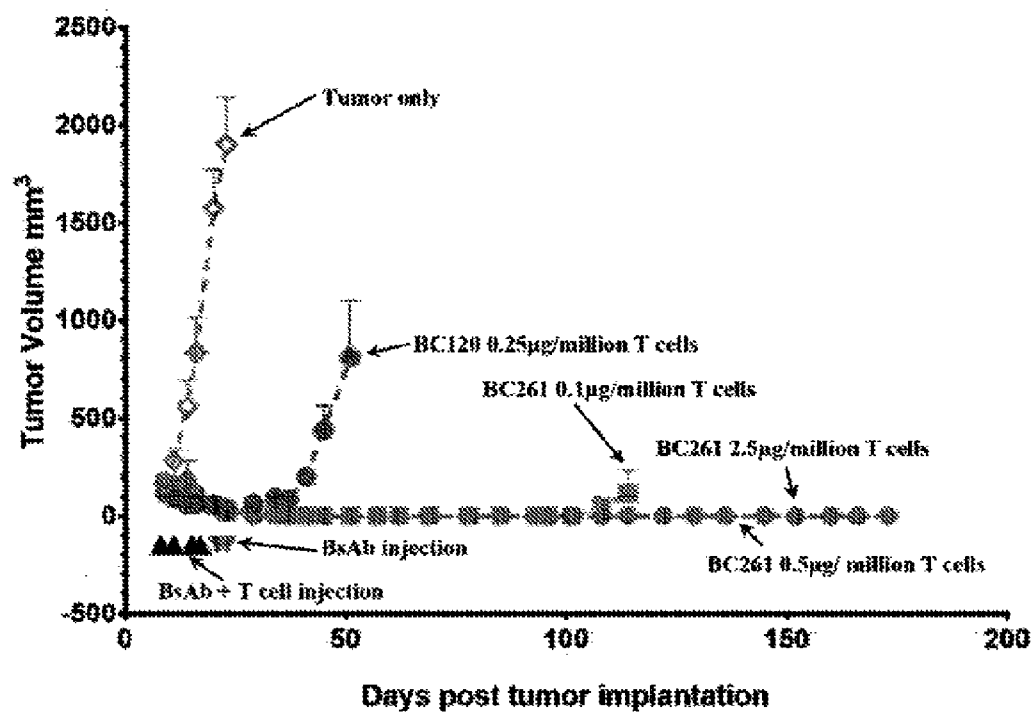
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FIG. 5E

300ml expression	concentration	HPLC	F/T	40C D7	40C D14	40C D21	40C D28
BC259	1.76	88.215	86.259	85.158	79.533	72.326	63.365
BC260	1.23	87.936	86.116	84.803	79.736	75.596	66.191
BC261	1.18	85.696	83.366	82.375	77.125	74.504	70.037
BC262	1.34	88.366	86.444	84.895	81.119	77	64.492

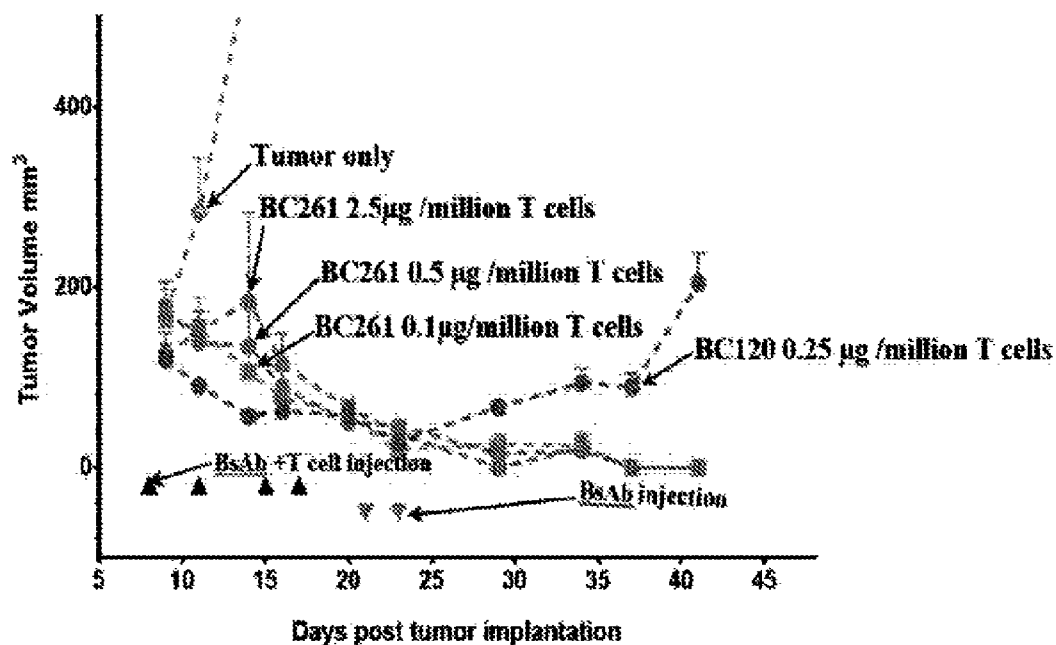
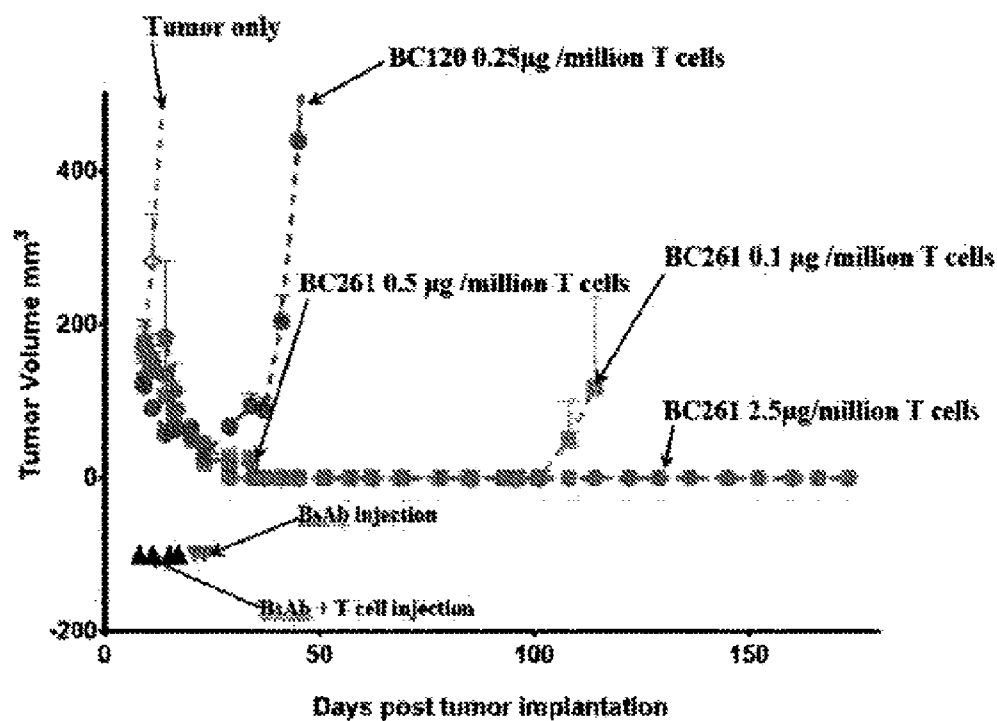
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FIG. 6A



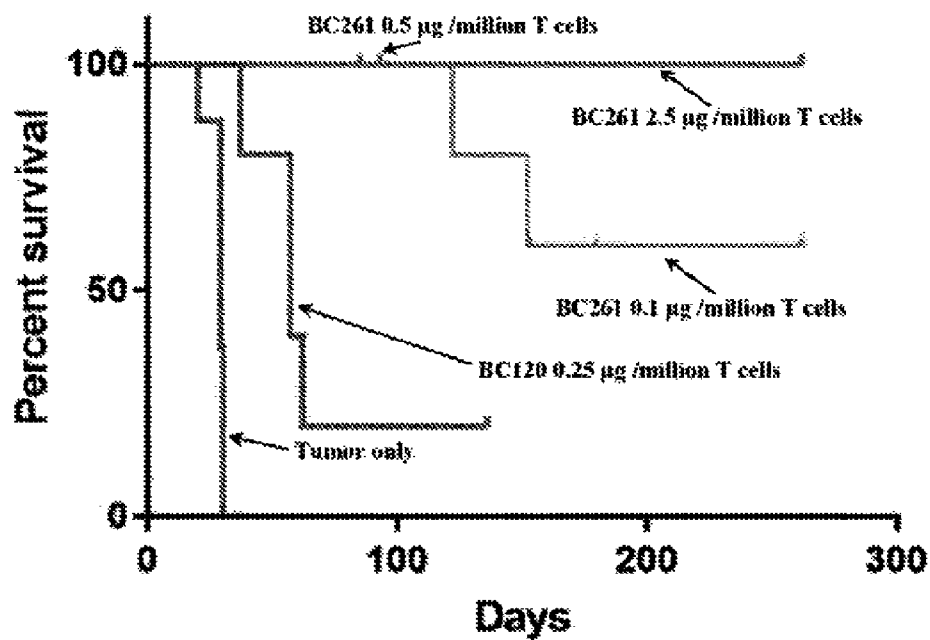
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FIG. 6B



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FIG. 6C

Exp 301 survival curve

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FIG. 7A

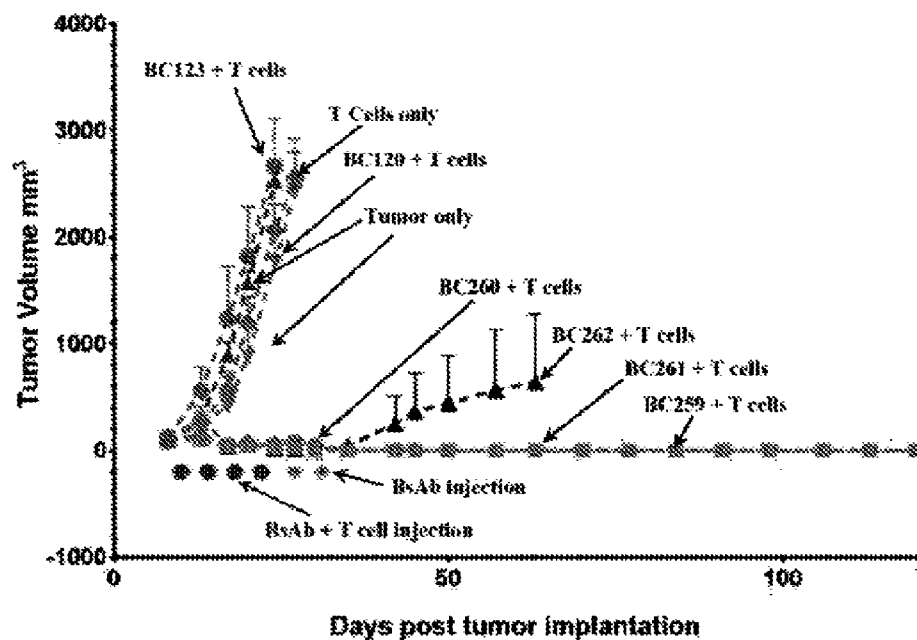
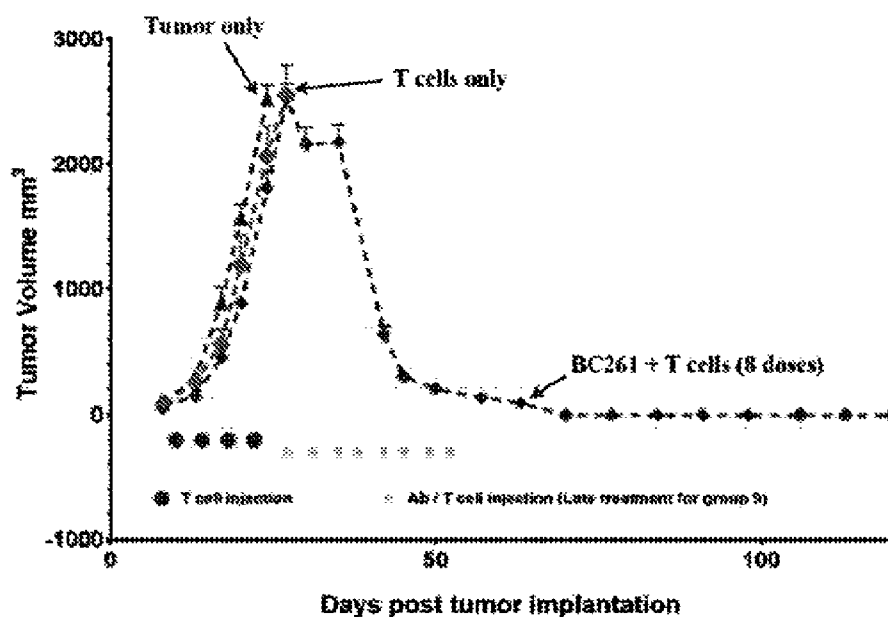


FIG. 7B



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FIG. 8A

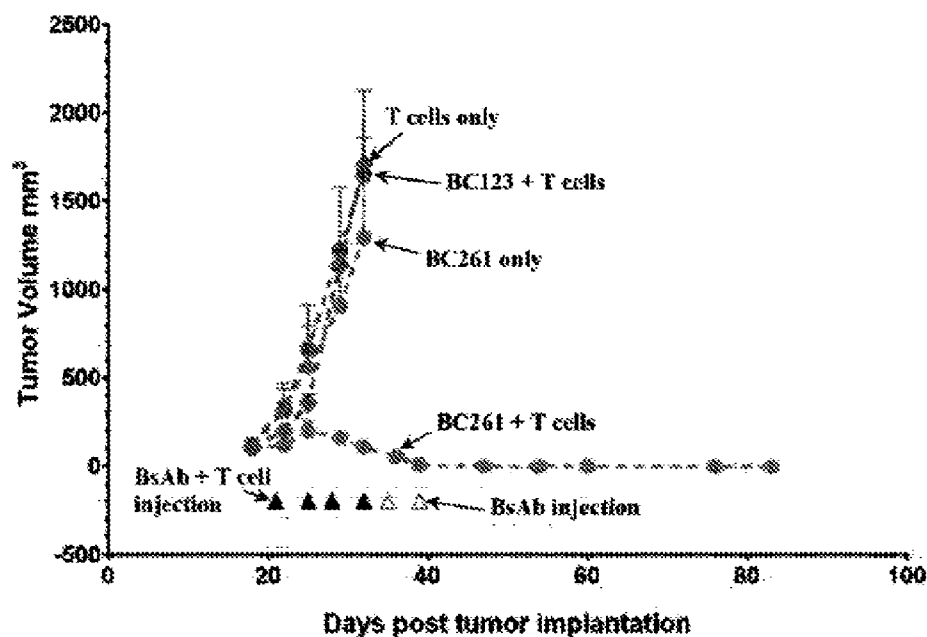
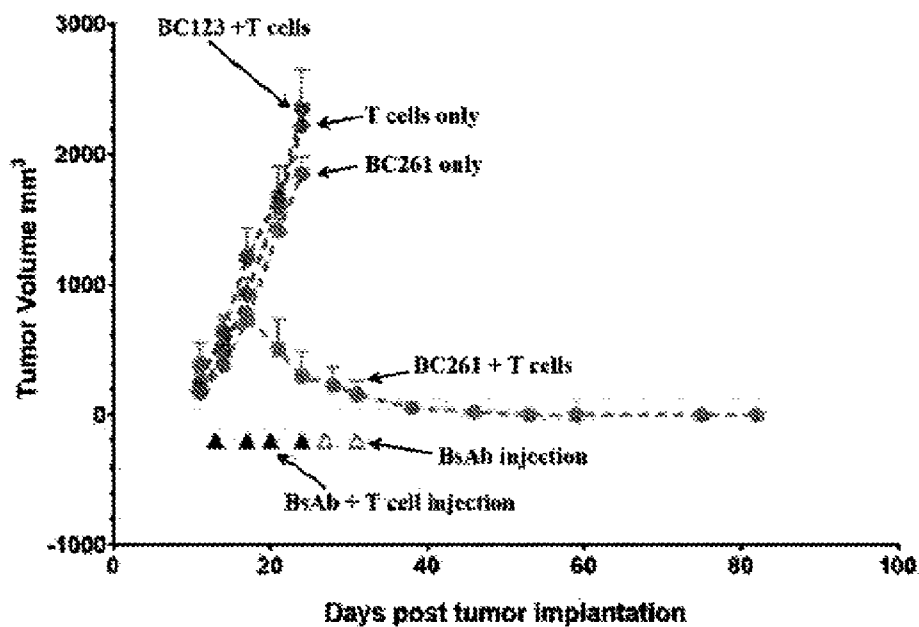


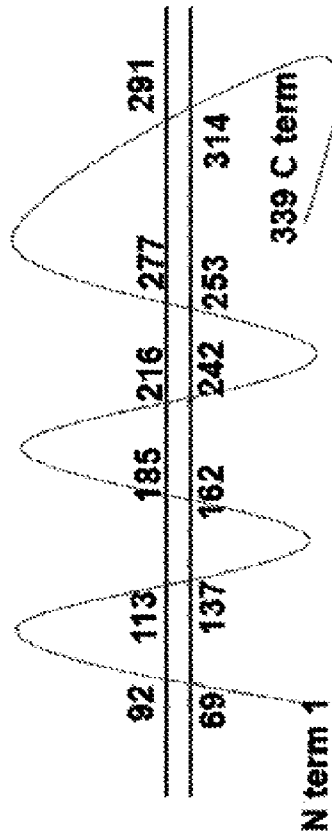
FIG. 8B



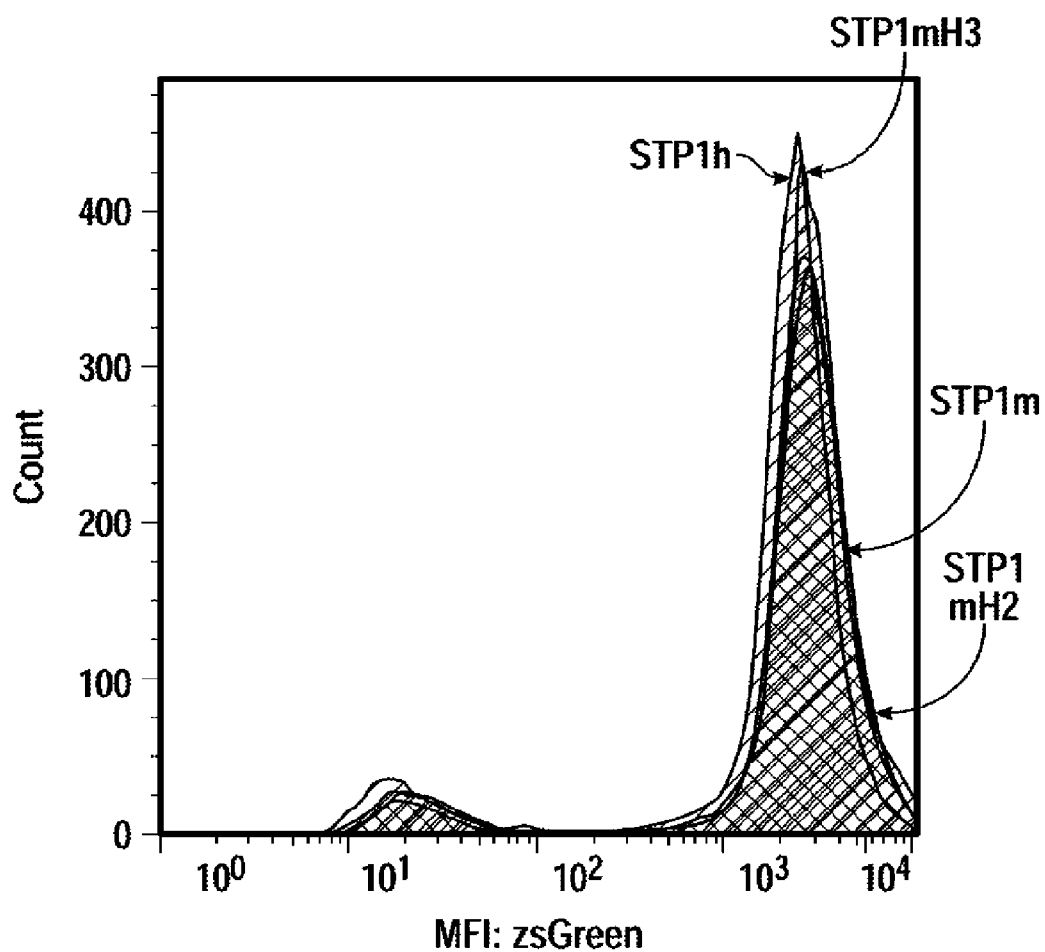
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FIG. 9A

Epitope mapping of STEAP1 Ab

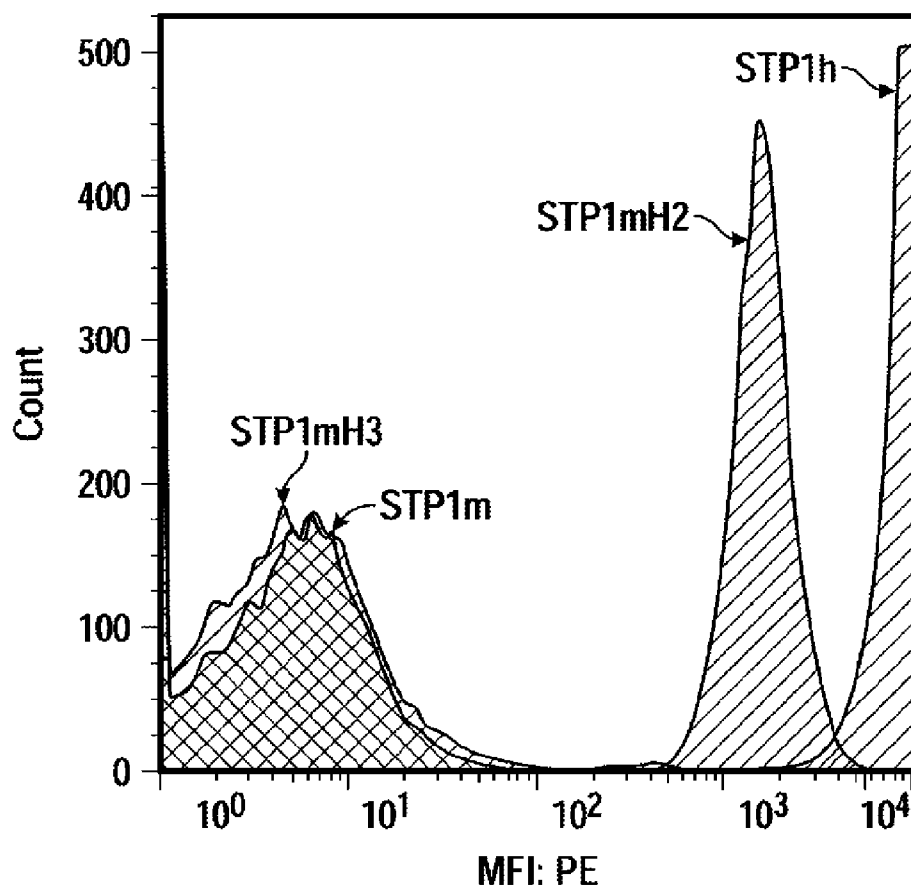


STEAP 1st	ECD	ECD difference
Protein position	90 100 110	90 100 110
humanSTEAP1	SLTFLYTLREVIHPLATSHQOYFKIPIL	LIREVIHPLATSHQOYFKIPILVIN
mouseSTEAP1	SLTFLYTLREVIHPLATSHQOYFKIPIL	...I.Y..V..RE.....
canineSTEAP1	SLTFLYTLREVIHPLATSHQOYFKIPIL	...I...EV.....
STEAP 2nd	ECD	
Protein position	190 200 210	190 200 210
humanSTEAP1	RRSYRYKLLNWAYQQVQQNKEDAWIHDVWMEI	RRSYRYKLLNWAYQQVQQNKELAWIHDVWMEI
mouseSTEAP1	RRSYRYKLLNWAYQQVQQNKEDAWIHDVWMEI	K.....V.....
canineSTEAP1	RRSYRYKLLNWAYQQVQQNKEDAWIHDVWMEI	
STEAP 3rd	ECD	
Protein position	280 290	280 290
humanSTEAP1	AWNKWDIKQFVWYTPPTETMIAVFLP	RWIDIKQFVWYTPPTETM
mouseSTEAP1	AWNKWDIKQFVWYTPPTETMIAVFLP	..V.VS.....M.....
canineSTEAP1	AWNKWDIKQFVWYTPPTETMIAVFLP	..V.....



ID		MFI: zsGreen
	STP1h	1334
	STP1m	1862
	STP1mH2	1793
	STP1mH3	1842

FIG. 9B



ID		MFI: PE
	STP1h	7423
	STP1m	5.00
	STP1mH2	1415
	STP1mH3	5.04

FIG. 9C

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FIG. 10A

mX120_VH (murine, humanness 74.5%) (SEQ ID NO: 1)

DVQVQESGPGGLVKPSQSLSLTCTVTGYSITSDYAWNWIWIRQFPGNKLEWMGYISNSGSTS
 YNPSLKSRLTISRDTSKNQFFLQLISVTTEDTATYYCARERNYDYDDYYYAMDYWGQ
 GTTLTVSA

Genentech humanized VH sequence (humanness 81.8%) (SEQ ID NO: 5)

EVQLVESGGGLVQPGGSLRLSCAVSGYSITSDYAWNWIWVRQAPGKGLEWVGYISNSGST
 SYNPSLKSRLTISRDTSKNNTLYLQMNSLRAEDTAVYYCARERNYDYDDYYYAMDYWG
 QGTLTVSS

VH-1 (humanness 86.9%) (SEQ ID NO: 6)

QVQVQESGPGGLVKPSQTLSTCTVTGYSITSDYAWNWIWIRQPPGKGLEWMGYISNSGSTS
 YNPSLKSRLTISRDTSKNQFSLKLSSVTAADTAVYYCARERNYDYDDYYYAMDYWGQ
 GTTLTVSA

VH-2 (humanness 86.7%) (SEQ ID NO: 7)

DVQVQESGPGGLVKPSQTLSTCTVTGYSITSDYAWNWIWIRQPPGKGLEWMGYISNSGSTS
 YNPSLKSRLTISRDTSKNQFSLKLSSVTAADTAVYYCARERNYDYDDYYYAMDYWGQ
 GTTLTVSA

VH-3 (humanness 86.9%) (SEQ ID NO: 8)

QVQVQESGPGGLVKPSQTLSTCTVTGYSITSDYAWNWIWIRQPPGKGLEWMGYISNSGST
 YNPSLKSRLTISRDTSKNQFSLKLSSVTAADTATYYCARERNYDYDDYYYAMDYWGQ
 GTTLTVSA

VH-4 (humanness 86.9%) (SEQ ID NO: 9)

QVQVQESGPGGLVKPSQTLSTCTVTGYSITSDYAWNWIWIRQPPGKGLEWMGYISNSGST
 YNPSLKSRLTISRDTSKNQFSLKLSSVTAADTAVYYCARERNYDYDDYYYAMDYWG
 QGTLTVSA

VH-5 (humanness 86.7%) (SEQ ID NO: 10)

DVQVQESGPGGLVKPSQTLSTCTVTGYSITSDYAWNWIWIRQPPGKGLEWMGYISNSGST
 YNPSLKSRLTISRDTSKNQFSLKLSSVTAADTATYYCARERNYDYDDYYYAMDYWGQ
 GTTLTVSA

VH-6 (humanness 86.7%) (SEQ ID NO: 11)

DVQVQESGPGGLVKPSQTLSTCTVTGYSITSDYAWNWIWIRQPPGKGLEWMGYISNSGST
 YNPSLKSRLTISRDTSKNQFSLKLSSVTAADTAVYYCARERNYDYDDYYYAMDYWG
 QGTLTVSA

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FIG. 10B

mX120_VL (murine, humanness 83.2%) (SEQ ID NO: 12)

DIVMSQSPSSLAVSVGEKVTMSCKSSOSLLYRSNOKNYLAWYQQKPGQSPKLLIYWAS
TRESGVDPDRFTGSGSGTDFTLTISSVKAEDLAVYYCCOYYNYPRTFGGGTKVEIK

Genentech humanized VL (humanness 81.2) (SEQ ID NO: 16)

DIQMTQSPSSLSASVGDRVTITCKSSOSLLYRSNOKNYLAWYQQKPGKAPKLLIYWAS
TRESGVPSRFSQSGSGTDFTLTISLQPEDFATYYCCOYYNYPRTFGGGTKVEIK

VL-1 (humanness 91.1%) (SEQ ID NO: 17)

DIVMTQSPDSLAVSLGERVTMNCKSSOSLLYRSNOKNYLAWYQQKPGQSPKLLIYWA
STRESGVDPDRFSGSGSGTDFTLTISSVQAEDVAVYYCCOYYNYPRTFGGGTKVEIK

VL-2 (humanness 91.1%) (SEQ ID NO: 18)

DIVMTQSPDSLAVSVGERVTMNCKSSOSLLYRSNOKNYLAWYQQKPGQPPKLLIYWA
STRESGVDPDRFSGSGSGTDFTLTISSVQAEDVAVYYCCOYYNYPRTFGGGTKVEIK

VL-3 (humanness 91.1%) (SEQ ID NO: 19)

DIVMTQSPDSLAVSVGERATMNCKSSOSLLYRSNOKNYLAWYQQKPGQSPKLLIYWA
STRESGVDPDRFSGSGSGTDFTLTISSVQAEDVAVYYCCOYYNYPRTFGGGTKVEIK

VL-4 (humanness 91.1%) (SEQ ID NO: 20)

DIVMTQSPDSLAVSVGERVTMNCKSSOSLLYRSNOKNYLAWYQQKPGQSPKLLIYWA
STRESGVDPDRFSGSGSGTDFTLTISLQAEDVAVYYCCOYYNYPRTFGGGTKVEIK

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FIG. 11A

Light chain sequence (LC) (SEQ ID NO: 21)

DIVMTQSPDSLAVSVGERVTMNCKSSQSLLYRSNQKNYLAWYQQKPGQPPKLLIY
WASTRESGVPDFRSGSGSGTDFTLTISVQAEDVAVYYCQQYYNYPRTFGGGGTKVE
IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFFYPREAKVQWKVDNALQSGNSQESVT
EQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

FIG. 11B

Heavy chain sequence (HC) (Two mutations N297A and K322A) (SEQ ID NOs: 22)

DVQVQESGPGLVKPSQTLSTCTVTGYSITSDYAWNWIQPPGKGLEWMGYISNS
GSTSYNPSLKSRTISRDTSKNQFSLKLSSVTAADTAVYYCARERNYDYYDDYYAM
YWGQGTTTLTVSAASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD
SDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

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FIG. 12A

BiClone261 (BC261) Light Chain: Nucleotide Sequence (SEQ ID NO: 23)

ATGGGCTGGICCTGCATCATCCTGTTTCTGGTGGCTACCGCCACCGGGCTCCATAGC
 GACATCGTGATGACCCAGAGCCCTGATAGCCTGGCCGTGAGCGTGGGGGAAAGAGT
 GACAATGAACTGTAAGAGCAGCCAGTCCCTGCTGTACCGGTCTAACCAGAAGAATT
 ACCTGGCCTGGTATCAGCAGAAGCCAGGCCAGCCCCCTAAGCTGCTGATCTATTGGG
 CCTCTACCAGGGAGAGCGGAGTGCCAGACAGATTCTCTGGCAGCGGCTCCGGCACA
 GACTTCACCTGACAATCAGCTCCGTGCAGGCAGAGGACGTGGCCGTGTACTATTGT
 CAGCAGTATTACAACCTACCCACAGAACTTTTGGAGGCGGCACTAAGGTGGAAATCAA
 GCGGACCGTGGCCGCTCCCTCCGTGTTTCTCTTCCACCTTCCGACGAGCAGCTGAA
 GTCCGGCACCCGCTTCTGTCTGTGCCTGCTGAACAACCTTCTACCCCGCGAGGGCCAA
 GGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGCAACTCCCAGGAATCCGTGA
 CCGAGCAGGACTCCAAGGACAGCACCTACTCCCTGTCTCCACCTGACCTGTCCA
 AGGCCGACTACGAGAAGCACAAAGGTGTACGCCCTGCGAAGTGACCCACCAGGGCCTG
 TCTAGCCCCGTGACCAAGTCTTTCAACCGGGGCGAGTGCCTAGTGGCGGGCGGAGG
 ATCTGGCGGAGGTGGAAGTGGGGGAGGCGGATCTCAGGTGCAGCTGGTGCAGAGTG
 GTGGCGGAGTGGTGCAGCCTGGCAGATCCCTGAGACTGTCTTGCAAGGCCAGCGGC
 TACACCTTCACCCGGTACACCATGCCTGGGTGCGACAGGCCCTGGCAAGTGCCTG
 GAATGGATCGGCTACATCAACCCCTCCCGGGGCTACACCAACTACAACCAGAAGTT
 CAAGGACCGGTTTACCATCAGCCGGGACAACCTCCAAGAACACCGCCTTTCTGCAGA
 TGGACTCCCTGCGGCCTGAGGATACCGGCGTGTACTTTTGCGCCCGGTACTACGACG
 ACCACTACAGCCTGGACTACTGGGGCCAGGGAACCCCTGTGACAGTGTCTAGCGGA
 GGGGGAGGTTTCAAGTGGCGGTGGATCAGGGGGCGGAGGAAGTGGCGGGGGAGGTA
 GTGGTGGTGGTGGAAAGCGGAGGTGGCGGCTCCGATATCCAGATGACCCAGTCCCCC
 TCCAGCCTGTCTGCCTCTGTGGGAGACAGAGTGACAATTACATGCTCCGCCAGCTCC
 AGCGTGTCTACATGAATTGGTATCAGCAGACCCCTGGCAAGGCTCCCAAGCGGTG
 GATCTACGACACCTCCAAGCTGGCCTCCGGCGTGCCTCCAGATTTTCTGGCAGCGG
 CTCCGGCACAGACTATACCTTTACAATCAGCTCCCTGCAGCCCGAAGATATCGCCAC
 CTACTACTGCCAGCAGTGGTCTCCAACCCCTTCACCTTCGGCTGCGGCACAAAGCT
 GCAGATCACCAGATAG

BC261 Amino Acid Sequence: signal peptide- VL-2 - C_L-linker-hOKT3 V_H-linker-hOKT3 V_L
 (SEQ ID NO: 24)

MGWSCILFLVATATGVHSDIVMTQSPDSLAVSVGERVTMNCCKSSQSLLYRSNQKNY
 LAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISVQAEDVAVYYC
 QQYYNYPRTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKV
 QWKVDNALQSGNSQESVTEQDSKSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSP
 VTKSFNRGECTSGGGGSGGGGSGGGGSGVQLVQSGGGVVPGRSLRLSCKASGYTFT
 RYTMHWVRQAPGKCLEWIGYINPSRGYTNYNQKFKDRFTISRDN SKNTAFLQMD S
 LRPEDTGVYFCARYYDDHYSLDYWGQGTPTVSSGGGGSGGGGSGGGGSGGGGSGG
 GSGGGGSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTPGKAPKRWIYD
 TSKLASGVPSRFSGSGSGTDYFTTISLQPEDIAFYCCQQWSSNPFTFGCGTKLQITR

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FIG. 12B

BiClone261 (BC261) Heavy Chain Nucleotide Sequence: (SEQ ID NO: 25)

ATGGGCTGGTCTGCATCATCTGTTTCTGGTGGCTACCGCCACCGGCGTCCACTCC
 GATGTGCAGGTGCAGGAAAGCGGCCAGGACTGGTGAAGCCCTCCCAGACTCTGTC
 TCTGACTTGTACCGTGACCGGCTACAGCATCACCTCCGACTATGCCTGGAAGTGGAT
 CAGACAGCCACCTGGCAAGGGCCTGGAGTGGATGGGCTACATCTCTAACAGCGGCT
 CCACATCTTATAATCCCTCTCTGAAGAGCAGGATCACCATCTCCCGCGATACATCTA
 AGAACCAGTTCAGCCTGAAGCTGAGCTCCGTGACCGCAGCAGACACAGCCGTGTAC
 TATTGCGCCCGGGAGAGAAATTACGATTATGATGACTACTATTATGCTATGGATTAC
 TGGGGACAGGGGACTACTCTGACCGTCTCCGCCCGCTCCACCAAGGGCCCATCGGTC
 TTCCCCCTGGCACCTCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGC
 CTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAAGTCAAGGCGCCCTG
 ACCAGCGCGGTGCACACCTTCCCGGCCGTCTACAGTCCTCAGGACTCTACTCCCTC
 AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA
 CGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTT
 GTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCG
 TCAGTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCT
 GAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAA
 CTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGC
 AGTACGCCAGCACGTACCGTGTGGTCAGCGTCTCACCCTCCTGCACCAGGACTGGC
 TGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATC
 GAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCT
 GCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTGAGCTGACCTGCCTGGTCA
 AAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG
 AACAACTACAAGACCACGCCTCCCGTGTGACTCCGACGGCTCCTTCTTCTCTAC
 AGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
 CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCC
 GGGTAAATGA

BC261 Heavy Chain Amino Acid Sequence [signal peptide- VH-2 -C_H1-3] (SEQ ID NO: 26)

MGWSCIILFLVATATGVHSDVQVQESGPGLVKPSQTLSTCTVTGYISITSDYAWNWI
 RQPPGKGLEWMGYISNSGSTSYPNPSLKSRTISRDTSKNQFSLKLSSVTAADTAVYY
 CARERNYDYDDYYAMDYWGQGTTLTVSAASTKGPSVFPLAPSSKSTSGGTAAALGC
 LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH
 KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVTV
 DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK
 CAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
 WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
 QKSLSLSPGK

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FIG. 13A

anti-STEAP1 x C825 (huX120-VL-CL-(G4S)3-mouse C825-VH-(G4S)6-VL) (SEQ ID NO: 27)
 MGWSCIHLFLVATATGVHSDIVMTQSPDSLAVSVGERVTMNCCKSSQSLLYRSNQKNY
 LAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISVQAEDVAVYYC
 QQYYNYPRITFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
 QWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP
 VTKSFNRGECTSGGGGSGGGSGGGGSHVKLQESGPGLVQPSQSLSLTCTVSGFSLTD
YGVHWVRQSPGKGLEWLGVIWSGGGTAYNTALISRLNIYRDNSKNQVFLEMNSLQ
AEDTAMYYCARRGSYPYNYFDAWGCGTTVTVSSGGGGSGGGSGGGSGGGSGGGSGG
GGSGGGGSQAVVIQESALTPPGETVTLTCGSSTGAVTASNANWVQEKPDHCFTG
LIGGHNNRPPGVPARFSGSLIGDKAALTIAGTQTEDEAIYFCALWYSDHWVIGGGT
 RLTVLG

FIG. 13B

anti-STEAP1 x huC825 (huX120-VL-CL-(G4S)3-huC825-VH-(G4S)6-VL) (SEQ ID NO: 28)
 MGWSCIHLFLVATATGVHSDIVMTQSPDSLAVSVGERVTMNCCKSSQSLLYRSNQKNY
 LAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISVQAEDVAVYYC
 QQYYNYPRITFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
 QWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP
 VTKSFNRGECTSGGGSGGGSGGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLT
DYGVHWVRQAPGKGLEWLGVIWSGGGTAYNTALISRFTISRDN SKNTLYLQMNSL
RAEDTAVYYCARRGSYPYNYFDAWGCGTLVTVSSGGGGSGGGSGGGSGGGSGGGSG
GGSGGGGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTASNANWVQQKPGQCP
RGLIGGHNNRPPGVPARFSGSLLGGKAALTLLGAQPEDEAEYYCALWYSDHWVIG
 GGTKLTVLG

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FIG. 14A

huX120 VL2-(G4S)6-VH2-(G4S)4-huC825 VH-(G4S)6-VL-linker-P53 (SEQ ID NO: 29)
MGWSCILFLVATATGVHSDIVMTQSPDSLAVSVGERVTMNCCKSSQSLLYRSNQKNY
LAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYC
QQYYNYPRITFGGGTKVEIKGGGGSGGGSGGGSGGGSGGGSGGGSGGGSDVQVQES
GPGLVKPSQTLSTCTVTGYSITSDYAWNWIQQPGKGLEWMGYISNSGSTSYPNS
LKSRTISRDTSKNQFSLKLSSVTAADTAVYYCARERNYDYDDYYYAMDYWGQGT
TLTVSAGGGSGGGSGGGSGGGSGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLT
DYGVHWVRQAPGKGLEWLGVIWSGGGTAYNTALISRFTISRDN SKNTLYLQMNSL
RAEDTAVYYCARRGSYPYNYFDAWGCGTLVTVSSGGGGSGGGSGGGSGGGSGG
GGSGGGSGQAVVTQEPSTVSPGGTVTLTCGSSTGAVTASNANWVQQKPGQCP
RGLIGGHNNRPPGVPARFSGSLLGGAALTLGAQPEDEAEYYCALWYSDHWVIG
GGTKLTVLGTPLGDTTHTSGKPLDGEYFTLOIRGRERFEMFRELNEALELKDAOAGKE
PGGSGGAPHHHHHH

FIG. 14B

huX120 VL2-(G4S)6-VH2-(G4S)4-huC825 VH-(G4S)6-VL-linker-P53 (SEQ ID NO: 30)
MGWSCILFLVATATGVHSDIVMTQSPDSLAVSVGERVTMNCCKSSQSLLYRSNQKNY
LAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYC
QQYYNYPRITFGGGTKVEIKGGGGSGGGSGGGSGGGSGGGSGGGSGGGSDVQVQES
GPGLVKPSQTLSTCTVTGYSITSDYAWNWIQQPGKGLEWMGYISNSGSTSYPNS
LKSRTISRDTSKNQFSLKLSSVTAADTAVYYCARERNYDYDDYYYAMDYWGQGT
TLTVSAGGGSGGGSGGGSGGGSGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLT
DYGVHWVRQAPGKGLEWLGVIWSGGGTAYNTALISRFTISRDN SKNTLYLQMNSL
RAEDTAVYYCARRGSYPYNYFDAWGCGTLVTVSSGGGGSGGGSGGGSGGGSGG
GGSGGGSGQAVVTQEPSTVSPGGTVTLTCGSSTGAVTASNANWVQQKPGQCP
RGLIGGHNNRPPGVPARFSGSLLGGAALTLGAQPEDEAEYYCALWYSDHWVIG
GGTKLTVLGTPLGDTTHTSGKPLDGEYFTLOIRGRERFEMFRELNEALELKDAOAGKE
PGGSGGAP

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FIG. 14C

huX120 VL2-(G4S)6-VH2-(G4S)4-huC825 VH-(G4S)6-VL-linker-P63-H6 (SEQ ID NO: 31)
MGWSCILFLVATATGVHSDIVMTQSPDSLAVSVGERVTMNCKSSQSLLYRSNQKNY
LAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYC
QQYYNYPRTFGGGTKVEIKGGGGSGGGGSGGGGSGGGGSGGGGSDVQVQES
GPGLVKPSQTLSTCTVTGYSITSDYAWNWIQQPGKGLEWMGYISNSGSTSYPNS
LKSRTISRDTSKNQFSLKLSSVTAADTAVYYCARERNYDYDDYYYAMDYWGQGT
TLTVSAGGGGSGGGGSGGGGSGGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLT
DYGVHWVVRQAPGKGLEWLGVIWSGGGTAYNTALISRFTISRDN SKNTLYLQMNSL
RAEDTAVYYCARRGSYPYNYFDAWGCGTLVTVSSGGGGSGGGGSGGGGSGGGGSG
GGGSGGGGSQAVVTQEPSTVSPGGTVTLTCGSSTGAVTASNANWVQQKPGQCP
RGLIGGHNNRPPGVPARFSGSLLGGAALTLGAQPEDEAEYYCALWYSDHWVIG
GGTKLTVLGTPLGDTTHTSGRSPDDELLYLPVRGRETYEMLLKIKESLELMOYLPQHTI
ETYROOOOOOHOHLLOKOGGSGGAPHHHHH

FIG. 14D

VL2-(G4S)6-VH2-(G4S)4-huC825 VH-(G4S)6-VL-linker-P63 (SEQ ID NO: 32)
MGWSCILFLVATATGVHSDIVMTQSPDSLAVSVGERVTMNCKSSQSLLYRSNQKNY
LAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYC
QQYYNYPRTFGGGTKVEIKGGGGSGGGGSGGGGSGGGGSGGGGSDVQVQES
GPGLVKPSQTLSTCTVTGYSITSDYAWNWIQQPGKGLEWMGYISNSGSTSYPNS
LKSRTISRDTSKNQFSLKLSSVTAADTAVYYCARERNYDYDDYYYAMDYWGQGT
TLTVSAGGGGSGGGGSGGGGSGGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLT
DYGVHWVVRQAPGKGLEWLGVIWSGGGTAYNTALISRFTISRDN SKNTLYLQMNSL
RAEDTAVYYCARRGSYPYNYFDAWGCGTLVTVSSGGGGSGGGGSGGGGSGGGGSG
GGGSGGGGSQAVVTQEPSTVSPGGTVTLTCGSSTGAVTASNANWVQQKPGQCP
RGLIGGHNNRPPGVPARFSGSLLGGAALTLGAQPEDEAEYYCALWYSDHWVIG
GGTKLTVLGTPLGDTTHTSGRSPDDELLYLPVRGRETYEMLLKIKESLELMOYLPQHTI
ETYROOOOOOHOHLLOKOGGSGGAP

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FIG. 14E

VL2-(G4S)6-VH2-(G4S)4-huC825 VH-(G4S)6-VL-linker-P73-H6 (SEQ ID NO: 33)
MGWSCILFLVATATGVHSDIVMTQSPDSLAVSVGERVTMNCCKSSQSLLYRSNQKNY
LAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYC
QQYYNYPRTFGGGTKVEIKGGGGSGGGGSGGGGSGGGGSGGGGSDVQVQES
GPGLVKPSQTLSTCTVTGYSITSDYAWNWIQQPGKGLEWMGYISNSGSTSYPNS
LKSRTISRDTSKNQFSLKLSSVTAADTAVYYCARERNYDYDDYYYAMDYWGQGT
TLTVSAGGGGSGGGGSGGGGSGGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLT
DYGVHWVVRQAPGKGLEWLGVIWSGGGTAYNTALISRFTISRDN SKNTLYLQMNSL
RAEDTAVYYCARRGSYPYNYFDAWGCGTLVTVSSGGGGSGGGGSGGGGSGGGGSG
GGGSGGGGSQAVVTQEPSTVSPGGTIVTLTCSSTGAVTASNYANWVQQKPGQCP
RGLIGGHNNRPPGVPARFSGSLLGGAALTLGAQPEDEAEYYCALWYSDHWVIG
GGTKLTVLGTPLGDTTHTSGRHGDEDTYYLOVRGRENFEILMKLKESLELMELVPOPL
VDSYROOOOLLORPGGSGGAPHHHHHH

FIG. 14F

VL2-(G4S)6-VH2-(G4S)4-huC825 VH-(G4S)6-VL-linker-P73 (SEQ ID NO: 34)
MGWSCILFLVATATGVHSDIVMTQSPDSLAVSVGERVTMNCCKSSQSLLYRSNQKNY
LAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYC
QQYYNYPRTFGGGTKVEIKGGGGSGGGGSGGGGSGGGGSGGGGSDVQVQES
GPGLVKPSQTLSTCTVTGYSITSDYAWNWIQQPGKGLEWMGYISNSGSTSYPNS
LKSRTISRDTSKNQFSLKLSSVTAADTAVYYCARERNYDYDDYYYAMDYWGQGT
TLTVSAGGGGSGGGGSGGGGSGGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLT
DYGVHWVVRQAPGKGLEWLGVIWSGGGTAYNTALISRFTISRDN SKNTLYLQMNSL
RAEDTAVYYCARRGSYPYNYFDAWGCGTLVTVSSGGGGSGGGGSGGGGSGGGGSG
GGGSGGGGSQAVVTQEPSTVSPGGTIVTLTCSSTGAVTASNYANWVQQKPGQCP
RGLIGGHNNRPPGVPARFSGSLLGGAALTLGAQPEDEAEYYCALWYSDHWVIG
GGTKLTVLGTPLGDTTHTSGRHGDEDTYYLOVRGRENFEILMKLKESLELMELVPOPL
VDSYROOOOLLORPGGSGGAP

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FIG. 14G

huX120 VH2-(G4S)6-VL2-(G4S)4-huC825 VH-(G4S)6-VL-linker-P53-H6 (SEQ ID NO: 35)
MGWSCILFLVATATGVHSDVQVQESGPGLVKPSQTLSTCTVTGYSITSDYAWNWI
RQPPGKGLEWMGYISNSGSTSYNPSLKSRTISRDTSKNQFSLKLSSVTAADTAVYY
CARERNYDYDDYYYAMDYWGQGTTLTVSAGGGGSGGGGSGGGGSGGGGSGGGGSG
GGGSDIVMTQSPDSLAVSVGERVTMNCSSQSLLYRSNQKNYLAWYQQKPGQPPK
LLIYWASTRESGVPDRFSGSGSGTDFTLTISVQAEDVAVYYCQQYYNYPRTFGGG
TKVEIKGGGGSGGGGSGGGGSGGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLT
DYGVHWVRQAPGKGLEWLGVIWSGGGTAYNTALISRFTISRDN SKNTLYLQMNSL
RAEDTAVYYCARRGSYPYNYFDAWGCGTLVTVSSGGGGSGGGGSGGGGSGGGGSG
GGGSGGGGSQAVVTQEPSTVSPGGTVTLTCSSTGAVTASNANWVQQKPGQCP
RGLIGGHNNRPPGVPARFSGSLLGGAALTLGAQPEDEAEYYCALWYSDHWVIG
GGTKLTVLGTPLGDTTHTSGKPLDGEYFTLOIRGRERFEMFRELNEALELKDAOAGKE
PGGSGGAPHHHHHH

FIG. 14H

huX120 VH2-(G4S)6-VL2-(G4S)4-huC825 VH-(G4S)6-VL-linker-P53 (SEQ ID NO: 36)
MGWSCILFLVATATGVHSDVQVQESGPGLVKPSQTLSTCTVTGYSITSDYAWNWI
RQPPGKGLEWMGYISNSGSTSYNPSLKSRTISRDTSKNQFSLKLSSVTAADTAVYY
CARERNYDYDDYYYAMDYWGQGTTLTVSAGGGGSGGGGSGGGGSGGGGSGGGGSG
GGGSDIVMTQSPDSLAVSVGERVTMNCSSQSLLYRSNQKNYLAWYQQKPGQPPK
LLIYWASTRESGVPDRFSGSGSGTDFTLTISVQAEDVAVYYCQQYYNYPRTFGGG
TKVEIKGGGGSGGGGSGGGGSGGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLT
DYGVHWVRQAPGKGLEWLGVIWSGGGTAYNTALISRFTISRDN SKNTLYLQMNSL
RAEDTAVYYCARRGSYPYNYFDAWGCGTLVTVSSGGGGSGGGGSGGGGSGGGGSG
GGGSGGGGSQAVVTQEPSTVSPGGTVTLTCSSTGAVTASNANWVQQKPGQCP
RGLIGGHNNRPPGVPARFSGSLLGGAALTLGAQPEDEAEYYCALWYSDHWVIG
GGTKLTVLGTPLGDTTHTSGKPLDGEYFTLOIRGRERFEMFRELNEALELKDAOAGKE
PGGSGGAP

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FIG. 14I

huX120 VH2-(G4S)6-VL2-(G4S)4-huC825 VH-(G4S)6-VL-linker-P63-H6 (SEQ ID NO: 37)
MGWSCILFLVATATGVHSDVQVQESGPGLVKPSQTLSTCTVTGYSITSDYAWNWI
RQPPGKGLEWMGYISNSGSTSYNPSLKSRTISRDTSKNQFSLKLSSVTAADTAVYY
CARERNYDYDDYYYAMDYWGQGTTLTVSAGGGGSGGGGSGGGGSGGGGSGGGGSG
GGGSDIVMTQSPDSLAVSVGERVTMNCSSQSLLYRSNQKNYLAWYQQKPGQPPK
LLIYWASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQYYNYPRTFGGG
TKVEIKGGGGSGGGGSGGGGSGGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLT
DYGVHWVRQAPGKGLEWLGVIWSGGGTAYNTALISRFTISRDN SKNTLYLQMNSL
RAEDTAVYYCARRGSYPYNYFDAWGCGTLVTVSSGGGGSGGGGSGGGGSGGGGSG
GGGSGGGGSQAVVTQEPSTVSPGGTVTLTCSSTGAVTASNANWVQQKPGQCP
RGLIGGHNNRPPGVPARFSGSLLGGAALTLGAQPEDEAEYCALWYSDHWVIG
GGTKLTVLGTPLGDTTHTSGRSPDDELLYLPVRGRETYEMLLKIKESLELMOYLPQHTI
ETYROOOOOOHOHLLOKOGGSGGAPHHHHHH

FIG. 14J

huX120 VH2-(G4S)6-VL2-(G4S)4-huC825 VH-(G4S)6-VL-linker-P63 (SEQ ID NO: 38)
MGWSCILFLVATATGVHSDVQVQESGPGLVKPSQTLSTCTVTGYSITSDYAWNWI
RQPPGKGLEWMGYISNSGSTSYNPSLKSRTISRDTSKNQFSLKLSSVTAADTAVYY
CARERNYDYDDYYYAMDYWGQGTTLTVSAGGGGSGGGGSGGGGSGGGGSGGGGSG
GGGSDIVMTQSPDSLAVSVGERVTMNCSSQSLLYRSNQKNYLAWYQQKPGQPPK
LLIYWASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQYYNYPRTFGGG
TKVEIKGGGGSGGGGSGGGGSGGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLT
DYGVHWVRQAPGKGLEWLGVIWSGGGTAYNTALISRFTISRDN SKNTLYLQMNSL
RAEDTAVYYCARRGSYPYNYFDAWGCGTLVTVSSGGGGSGGGGSGGGGSGGGGSG
GGGSGGGGSQAVVTQEPSTVSPGGTVTLTCSSTGAVTASNANWVQQKPGQCP
RGLIGGHNNRPPGVPARFSGSLLGGAALTLGAQPEDEAEYCALWYSDHWVIG
GGTKLTVLGTPLGDTTHTSGRSPDDELLYLPVRGRETYEMLLKIKESLELMOYLPQHTI
ETYROOOOOOHOHLLOKOGGSGGAP

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FIG. 14K

huX120 VH2-(G4S)6-VL2-(G4S)4-huC825 VH-(G4S)6-VL-linker-P73-H6 (SEQ ID NO: 39)
MGWSCILFLVATATGVHSDVQVQESGPGLVKPSQTLSTCTVTGYSITSDYAWNWI
RQPPGKGLEWMGYISNSGSTSYNPSLKSRTISRDTSKNQFSLKLSSVTAADTAVYY
CARERNYDYDDYYYAMDYWGQGTTLTVSAGGGGSGGGGSGGGGSGGGGSGGGGSG
GGGSDIVMTQSPDSLAVSVGERVTMNCSSQSLLYRSNQKNYLAWYQQKPGQPPK
LLIYWASTRESGVPDRFSGSGSGTDFTLTISVQAEDVAVYYCQQYYNYPRTFGGG
TKVEIKGGGGSGGGGSGGGGSGGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLT
DYGVHWVRQAPGKGLEWLGVIWSGGGTAYNTALISRFTISRDN SKNTLYLQMNSL
RAEDTAVYYCARRGSYPYNYFDAWGCGTLVTVSSGGGGSGGGGSGGGGSGGGGSG
GGGSGGGGSQAVVTQEPSTVSPGGTVTLTCSSTGAVTASNANWVQQKPGQCP
RGLIGGHNNRPPGVPARFSGSLLGGAALTLGAQPEDEAEYYCALWYSDHWVIG
GGTKLTVLGTPLGDTTHTSGRHGDEDTYYLOVRGRENFEILMKLKESLELMELVPOPL
VDSYROOOOLLORPGSGGAPHHHHHH

FIG. 14L

huX120 VH2-(G4S)6-VL2-(G4S)4-huC825 VH-(G4S)6-VL-linker-P73 (SEQ ID NO: 40)
MGWSCILFLVATATGVHSDVQVQESGPGLVKPSQTLSTCTVTGYSITSDYAWNWI
RQPPGKGLEWMGYISNSGSTSYNPSLKSRTISRDTSKNQFSLKLSSVTAADTAVYY
CARERNYDYDDYYYAMDYWGQGTTLTVSAGGGGSGGGGSGGGGSGGGGSGGGGSG
GGGSDIVMTQSPDSLAVSVGERVTMNCSSQSLLYRSNQKNYLAWYQQKPGQPPK
LLIYWASTRESGVPDRFSGSGSGTDFTLTISVQAEDVAVYYCQQYYNYPRTFGGG
TKVEIKGGGGSGGGGSGGGGSGGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLT
DYGVHWVRQAPGKGLEWLGVIWSGGGTAYNTALISRFTISRDN SKNTLYLQMNSL
RAEDTAVYYCARRGSYPYNYFDAWGCGTLVTVSSGGGGSGGGGSGGGGSGGGGSG
GGGSGGGGSQAVVTQEPSTVSPGGTVTLTCSSTGAVTASNANWVQQKPGQCP
RGLIGGHNNRPPGVPARFSGSLLGGAALTLGAQPEDEAEYYCALWYSDHWVIG
GGTKLTVLGTPLGDTTHTSGRHGDEDTYYLOVRGRENFEILMKLKESLELMELVPOPL
VDSYROOOOLLORPGSGGAP

FIG. 14M

DIVMTQSPDSLAVSVGERVTMNCCKSSQSLLYRSNQKNYLAWYQQKPGQPPLLIY
WASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQYVNYPRTFGCGTKVE
IKRGGGGSGGGGSGGGGSGGGGSGGGGSDVQVQESGPGLVKPSQTLSTCTV
TGYSITSDYAWNWIROPPEGKCLEWMGYISNSGSTSYNPSLKSRTISRDTSKNQFSL
KLSSVTAADTAVYYCARERNYDYDDYYAMDYWGQGTTLTVSAGGGGSGGGGSG
GGGSGGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLTDYGVHWVRQAPGKGLE
WLGVIWSGGGTAYNTALISRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARRGSP
YNYFDAWGCGLTVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSAVVVTQEPS
LTVSPGGTVTLTCGSSTGAVTASNANWVQQKPGQCPRLIGGHNNRPPGVPARF
SGSLLGGAALTLGAQPEDEAEYYCALWYSDHWVIGGGTKLTVLGTPLGDTTHI
SGKPLDGEYFTLOIRGRERFEMFRELNEALELKDAOAGKEPGSGGAPHHHHHH

huX120 VL2-(G4S)6-VH2-(G4S)4 with ds [G101C:G43C] huC825 VH-(G4S)6-VL-linker-P53-6His (SEQ ID NO: 62)

DIVMTQSPDSLAVSVGERVTMNCSSQSLLYRSNQKNYLAWYQKPKGPQPKLLIY
WASTRESGVPDRFSGSGSGTDFTLTISIVQAEDVAVYYCQYYNYPRTFGGCTKVE
IKRGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSDVQVQESGPGLVKPSQTLSTCTV
TGYSITSDYAWNWIROPPEGKCLEWMGYISNSGSTSYNPSLKSRTISRDTSKNQFSL
KLSSVTAADTAVYYCARERNYDYDDYYAMDYWGQGTTLTVSAGGGGSGGGGSG
GGGSGGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLT DYGVHWVRQAPGKGLE
WLGVWSGGGTAYNTALISRTISRDN SKNTLYLQMNSLRAEDTAVYYCARRGSP
YNYFDAWGCGTLTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGQAVVTQEPS
LTVSPGGTVTLTCGSSTGAVTASNANWYQKPKGCPRGLIGGHNNRPPGVPARF
SGSLLGGAALTLGAQPEDEAEYYCALWYSDHWVIGGGTKLTVLGTPLGDTTHT
SGKPLDGEYFTLOIRGRERFEMFRELNEALELKDAOAGKEPGSGGAPHHHHHH

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FIG. 14O

huX120 VL2-(G4S)6-VH2-(G4S)4 with VL2[D1R] and with ds [G101C:G43C] | huC825 VH-(G4S)6-VL-linker-P53-6His (SEQ ID NO: 63)

DIVMTQSPDSLAVSVGERVTMNCKSSQSLLYRSNQKNYLAWYQOKPGQPPLLIYW
 ASTRESGVPDRFSGSGSGTDFTLTISVQAEDVAVYYCQQYYNYPRTFGGCTKVEIK
 RGGGSGGGGSGGGGSGGGGSGGGGSGGGGSRVQVQESGPGLVKPSQTLSTCTVTG
 YSITSDYAWNWIROPKCLEWMGYISNSGSTSYNPSLKSRTISRDTSKNQFSLKLS
 SVTAADTAVYYCARERNYDYDDYYAMDYWGQGTTLTVSAGGGGSGGGGSGGGGS
 GGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLTLDYGVHWVRQAPGKGLEWLV
 IWSGGGTAYNTALISRTISRDN SKNTLYLQMNSLRAEDTAVYYCARRGSYPNYFD
 AWGCGTLTVSSGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPG
 GTVTLTLCGSSTGAVTASNANWVQKPGQCPRGLIGGHNNRPPGVPARFSGSLLGG
 KAALTLLGAQPEDEAEYYCALWYSDHWVIGGGTKLTVLGTPLGDTTHTSGKPLDGE
 YFTLOIRGRERFEMFRELNEALELKDAOAGKEPGGSGGAPHHHHHH

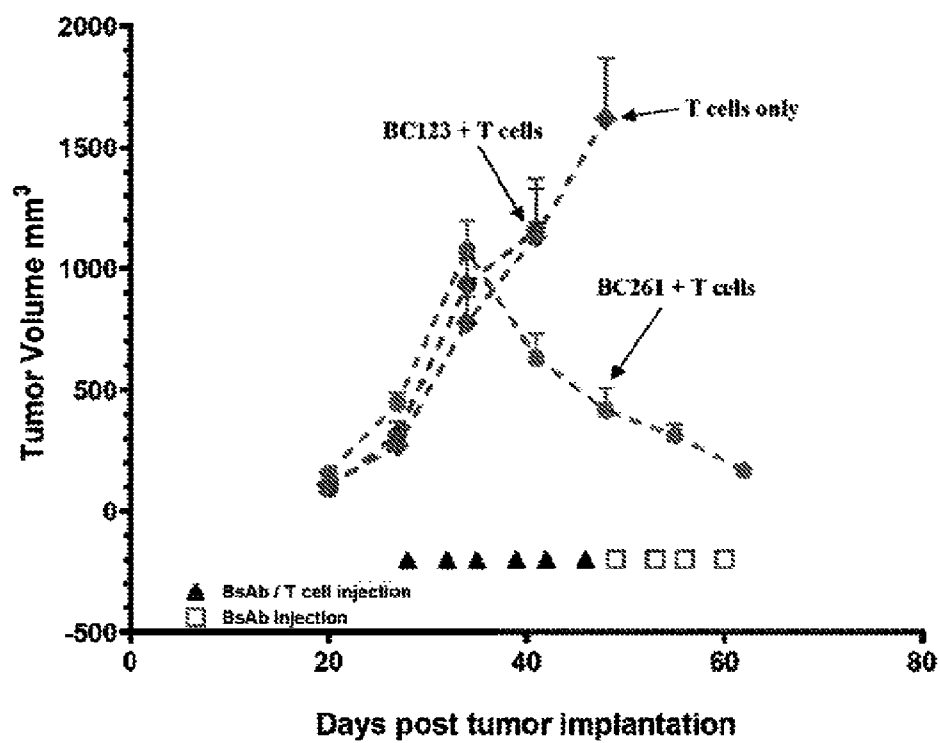
FIG. 14P

huX120 VL2-(G4S)6-VH2-(G4S)4 with ds [G101C:G43C] huC825 VH-(G4S)6-VL-linker-P53 [N36R]-6His (SEQ ID NO: 64)

DIVMTQSPDSLAVSVGERVTMNCKSSQSLLYRSNQKNYLAWYQOKPGQPPLLIYW
 ASTRESGVPDRFSGSGSGTDFTLTISVQAEDVAVYYCQQYYNYPRTFGCGTKVEIK
 RGGGSGGGGSGGGGSGGGGSGGGGSGGGGSDVQVQESGPGLVKPSQTLSTCTVTG
 YSITSDYAWNWIROPKCLEWMGYISNSGSTSYNPSLKSRTISRDTSKNQFSLKLS
 SVTAADTAVYYCARERNYDYDDYYAMDYWGQGTTLTVSAGGGGSGGGGSGGGGS
 GGGGSHVQLVESGGGLVQPGGSLRLSCAASGFSLTLDYGVHWVRQAPGKGLEWLV
 IWSGGGTAYNTALISRTISRDN SKNTLYLQMNSLRAEDTAVYYCARRGSYPNYFD
 AWGCGTLTVSSGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPG
 GTVTLTLCGSSTGAVTASNANWVQKPGQCPRGLIGGHNNRPPGVPARFSGSLLGG
 KAALTLLGAQPEDEAEYYCALWYSDHWVIGGGTKLTVLGTPLGDTTHTSGKPLDGE
 YFTLOIRGRERFEMFRELREALELKDAOAGKEPGGSGGAPHHHHHH

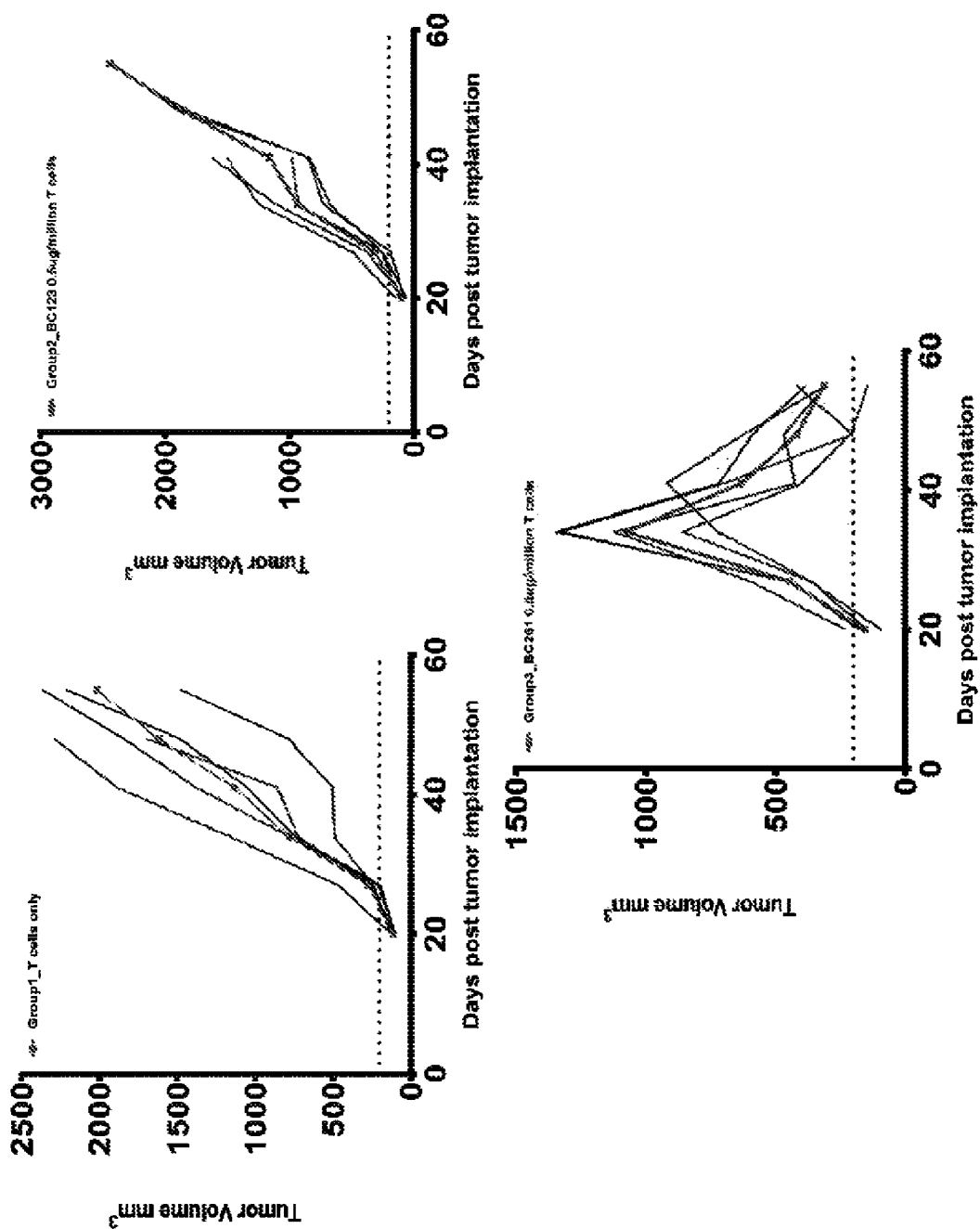
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FIG. 15A



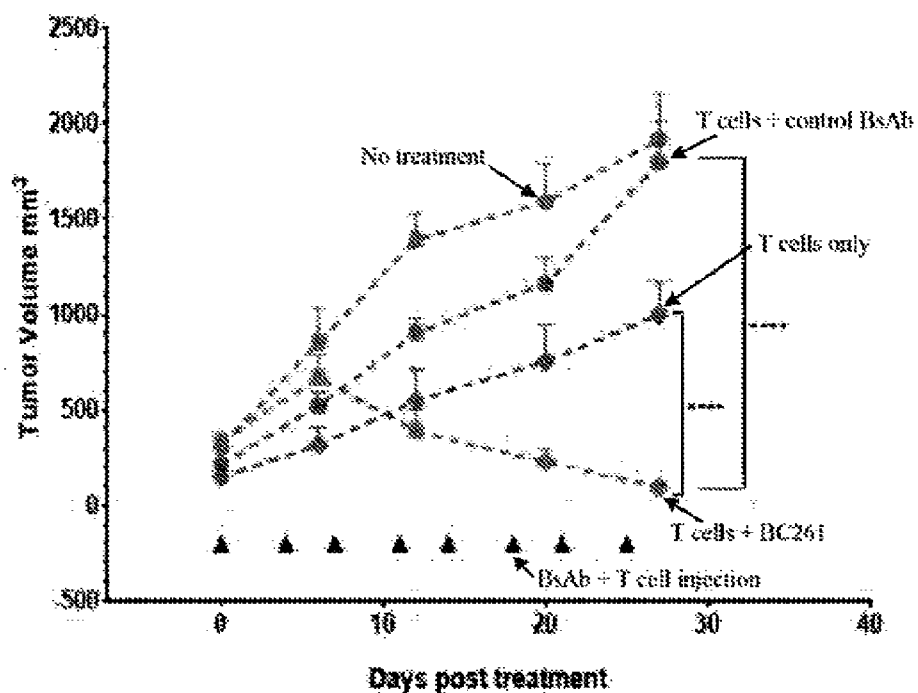
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FIG. 15B

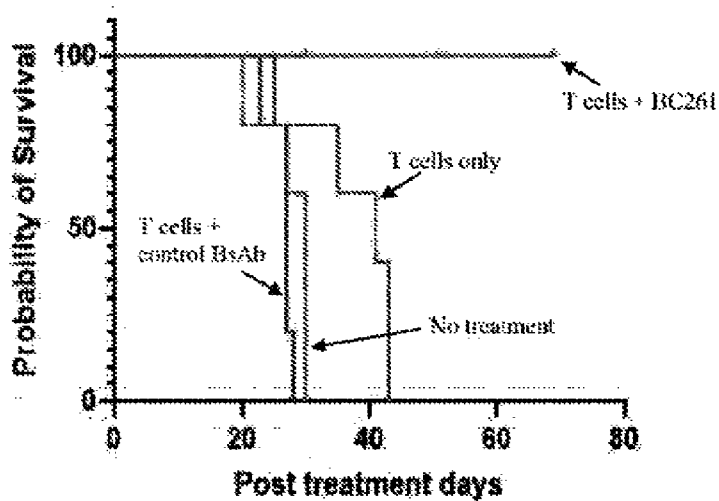


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FIG. 15C

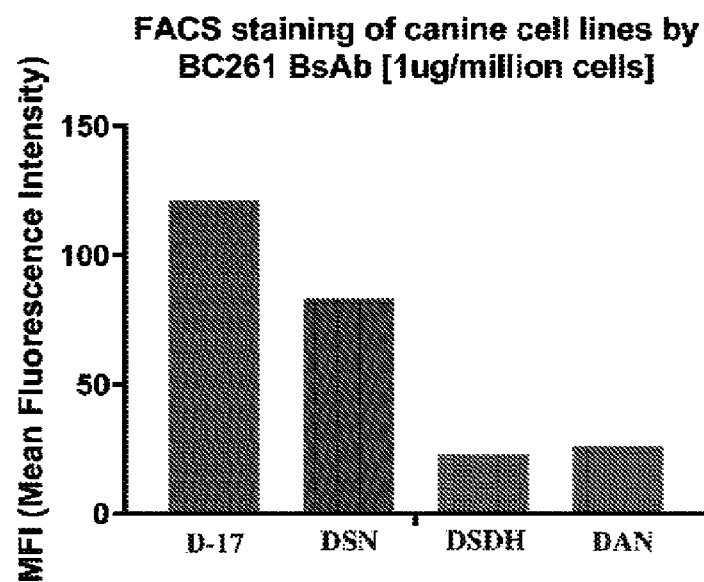


Survival proportions: TM00298 PDX



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FIG. 16



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FIG. 17A

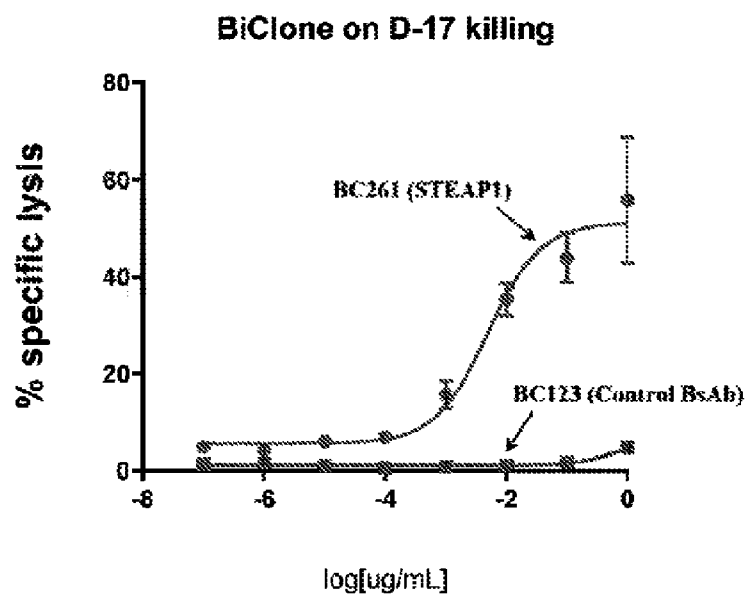
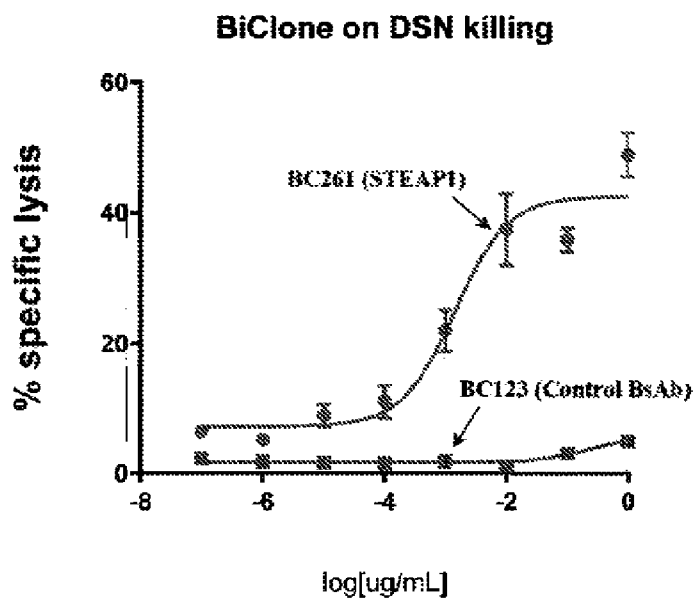


FIG. 17B



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FIG. 17C

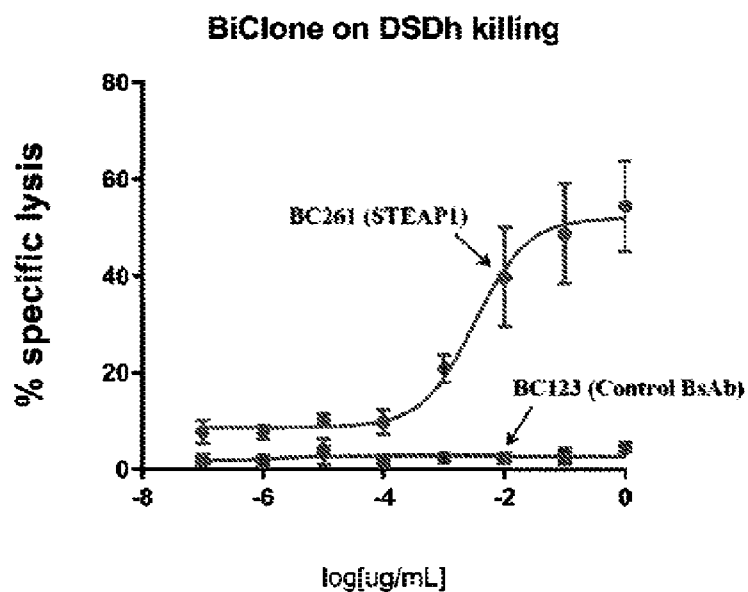
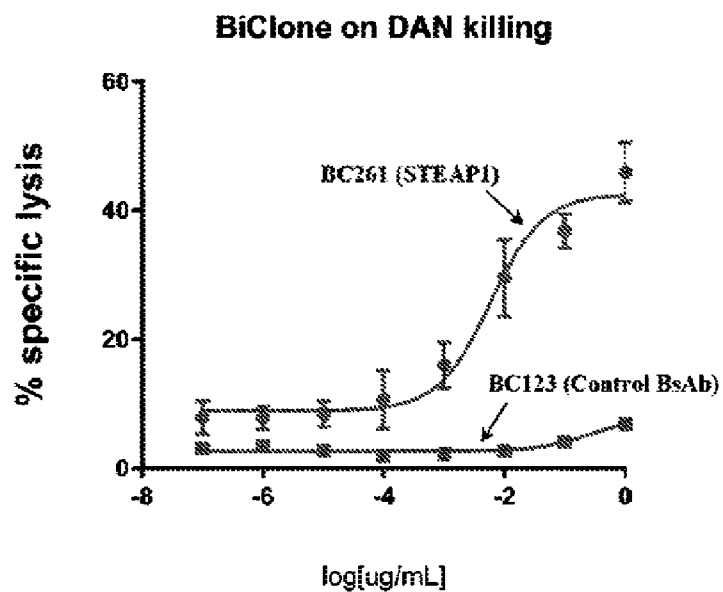


FIG. 17D



EC50 in pM	
Cell Lines/BsAb	BC261
TC32	4.9
TC71	1.1
SKES1	3.0
A4573	5.0
SKEAW	25.8
SKELP	1.8
SKERT	8.2
SKNMC	10.9
LNCaP-AR	1.7
CWR22	26.7
VCaP	214.8
D-17	24.8
DSN	7.2
DAN	29.5
DSDh	16.2

FIG. 18

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FIG. 19A

BC327: STEAP1 × CD3_BiTE with DS [G100C:G43C] (SEQ ID NO: 65)

DIVMTQSPDSLAVSVGERVTMNCKSSQSLLYRSNQKNYLAWYQQKPGQPPKLLIY
 WASTRESGVPDFRFSGSGSGTDFTLTISSVQAEDVAVYYCQQYYNYPRTFGCGTKVE
 IKRGGGGSGGGGSGGGGSGGGGSDVQVQESGPGLVKPSQTLSTCTVTGYSITSDYA
 WNWIRQPPGKCLEWMGYISNSGSTSYNPSLKSRLTISRDTSKNQFSLKLSSVTAADT
 AVYYCARERNYDYDDYYAMDYWGQGTTLTVSAGGGGSGGGGSGGGGSGVQLVQ
 SGGGVVQPGRLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYN
 QKFKDRFTISRDNKNTAFLQMDSLRLPEDTGVYFCARYYDDHYSLDYWGQGPVT
 VSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWY
 QQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFITISLQPEDATYYCQQWSS
 NPFTFGQGTKLQITR

BC328: STEAP1 × CD3_BiTE without DS (SEQ ID NO: 66)

DIVMTQSPDSLAVSVGERVTMNCKSSQSLLYRSNQKNYLAWYQQKPGQPPKLLIY
 WASTRESGVPDFRFSGSGSGTDFTLTISSVQAEDVAVYYCQQYYNYPRTFGGGTKVE
 IKRGGGGSGGGGSGGGGSGGGGSDVQVQESGPGLVKPSQTLSTCTVTGYSITSDYA
 WNWIRQPPGKGLEWMGYISNSGSTSYNPSLKSRLTISRDTSKNQFSLKLSSVTAADT
 AVYYCARERNYDYDDYYAMDYWGQGTTLTVSAGGGGSGGGGSGGGGSGVQLVQ
 SGGGVVQPGRLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYN
 QKFKDRFTISRDNKNTAFLQMDSLRLPEDTGVYFCARYYDDHYSLDYWGQGPVT
 VSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWY
 QQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFITISLQPEDATYYCQQWSS
 NPFTFGQGTKLQITR

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FIG. 19B

BC329: STEAP1 × CD3_Dimeric BiTE (SEQ ID NO: 67)

DIVMTQSPDSLAVSVGERVTMNCKSSQSLLYRSNQKNYLAWYQQKPGQPPKLLIY
 WASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQYYNYPRTFGCGTKVE
 IKRGGGGSGGGGSGGGGSGGGGSDVQVQESGPGLVKPSQTLSTCTVTGYSITSDYA
 WNWIRQPPGKCLEWMGYISNSGSTSYNPSLKSRLTISRDTSKNQFSLKLSSVTAADT
 AVYYCARERNYDYDDYYAMDYWGQGTTLTVSAGGGGSGGGGSGGGGSGVQLVQ
 SGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYN
 QKFKDRFTISRDNKNTAFLQMDSLRLPEDTGVYFCARYYDDHYSLDYWGQGTPTV
 VSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWY
 QQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYFTFISLQPEDATYYCQQWSS
 NPFTFGQGTKLQITRTPLGDTTHTSGMVSKLSQLQTLLAALLESGLSKEALIQALGEGSG
 GAPHHHHHH

BC330: STEAP1 × CD3_BiTE-Fc (SEQ ID NO: 68)

DIVMTQSPDSLAVSVGERVTMNCKSSQSLLYRSNQKNYLAWYQQKPGQPPKLLIY
 WASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQYYNYPRTFGCGTKVE
 IKRGGGGSGGGGSGGGGSGGGGSDVQVQESGPGLVKPSQTLSTCTVTGYSITSDYA
 WNWIRQPPGKCLEWMGYISNSGSTSYNPSLKSRLTISRDTSKNQFSLKLSSVTAADT
 AVYYCARERNYDYDDYYAMDYWGQGTTLTVSAGGGGSGVQLVQSGGGVVQPG
 RSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKFKDRFTI
 SRDNKNTAFLQMDSLRLPEDTGVYFCARYYDDHYSLDYWGQGTPTVTVSSGGGGSG
 GGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTPGKAP
 KRWIYDTSKLASGVPSRFSGSGSGTDYFTFISLQPEDATYYCQQWSSNPFTFGQ
 TKLQITRGGGGSGGGGSGGGGSGGGGSGPDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
 LMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV
 LHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
 LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFC
 VMHEALHNHYTQKSLSLSPGK

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FIG. 19C

BC330: STEAP1 × CD3_BiTE-Fc –long linker (SEQ ID NO: 69)

DIVMTQSPDSLAVSVGERVTMNCKSSQSLLYRSNQKNYLAWYQQKPGQPPKLLIY
 WASTRESGVPDFRSGSGSGTDFTLTISSVQAEDVAVYYCQQYYNYPRTFGCGTKVE
 IKRGGGGSGGGSGGGSGGGSGGGSDVQVQESGPGLVKPSQTLSTCTVTGYSITSDYA
 WNWIRQPPGKCLEWMGYISNSGSTSYNPSLKSRTISRDTSKNQPSLKLSSVTAADT
 AVYYCAREERNYDYYDYAMDYWGQGTTLTVSAGGGSGGGSGGGSGVQLVQ
 SGGGVVQPGRLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYN
 QKFKDRFTISRDNKNTAFLQMDSLRLPEDTGVYFCARYYDDHYSLDYWGQGPVT
 VSSGGGGSGGGSGGGSGGGSGGGSDIQMTQSPSSLSASVGDRVITITCSASSSVSYMNWY
 QQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYFTTISLQPEDATYYCQQWSS
 NPFTFGQGTKLQITRGGGGSGGGSGGGSGGGSGGGSGPDKTHTCPPCPAPELLGGPSVF
 LFPPKPKDTLMISRTPVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAST
 YRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRW
 QQGNVFSCSVMHREALHNHYTQKSLSLSPGK

STEAP1 IgG-(H)-Scfv (BC365)

Heavy chain (SEQ ID NO: 70)

DVQVQESGPGLVKPSQTLSTCTVTGYSITSDYAWNWIWIRQPPGKCLEWMGYISNSG
 STSYNPSLKSRTISRDTSKNQPSLKLSSVTAADTAVYYCAREERNYDYYDYAMDY
 WGQGTTLTVSAASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH
 TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPVTCVVVDVSHEDPEVKFNWYVDGVE
 VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKG
 QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD
 DGSFFLYSKLTVDKSRWQQGNVFSCSVMHREALHNHYTQKSLSLSPGTSGGGGSGGGGS
 GGGGSQVQLVQSGGGVVQPGRLRLSCKASGYTFTRYTMHWVRQAPGKCLEWIG
 YINPSRGYTNYNQKFKDRFTISRDNKNTAFLQMDSLRLPEDTGVYFCARYYDDHYS
 LDYWGQGPVTVSSGGGGSGGGSGGGSGGGSGGGSGGGSDIQMTQSPSSLSA
 SVGDRVITITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTD
 YFTTISLQPEDATYYCQQWSSNPFTFGCGTKLQITR

Light chain (SEQ ID NO: 71)

DIVMTQSPDSLAVSVGERVTMNCKSSQSLLYRSNQKNYLAWYQQKPGQPPKLLIY
 WASTRESGVPDFRSGSGSGTDFTLTISSVQAEDVAVYYCQQYYNYPRTFGCGTKVE
 IKRTVAAPSVFIFPPSDEQLKSGTASVCLNNFYPREAKVQWKVDNALQSGNSQESVT
 EQDSKDSSTLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC

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FIG. 19D

STEAP1 x CD3 Heterodimer: HD148

STEAP1 (N297A, K322A, K409R)

HC (SEQ ID NO: 72)

DVQVQESGPGLVKPSQTLSTCTVTGYSITSDYAWNWIROPPGKGLEWMGYISNS
 GSTSYNPSLKSRTISRDTSKNQFSLKLSSVTAADTAVYYCARERNYDYDDYYAM
 YWGQGTTTLTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
 TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDKT
 HTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
 EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAK
 GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 SDGSFFLYSRLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

LC (SEQ ID NO: 73)

DIVMTQSPDSLAVSVGERVTMNCKSSQSLLYRSNQKNYLAWYQQKPGQPPKLLIY
 WASTRESGVPDRFSGSGSGTDFTLTISVVQAEDVAVYYCQQYYNYPRTFGGGTKVE
 IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV
 TQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC

OKT3 (N297A, K322A, F405L)

HC (SEQ ID NO: 74)

QVQLVQSGGGVVPGRSLRLSCKASGYTFTRYIMHWVRQAPGKGLEWIGYINPS
 RGYTNYNQKFKDRFTISRDNSTNTAFLOMDSLRLPEDTGVYFCARYYDDHYSLDYW
 GQGTPVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
 VHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDKTHTC
 PPCAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
 NAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQP
 REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
 SFLLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

LC (SEQ ID NO: 75)

DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASG
 VPSRFSGSGSGTDYFTFISLQPEDATYYCQQWSSNPFTFGQGTKLQITRTVAAPSV
 FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYS
 LSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC

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FIG. 20A

	chimeric	L1+H1	L1+H2	L1+H3	L1+H4	L1+H5	L1+H6	L2+H1	L2+H2	L2+H3	L2+H4	L2+H5
One site -- Specific binding												
Best-fit values												
B _{max}	146.5	149.7	150.6	37.24	71.25	115.4	11.63	166.6	166.7	17.34	37.64	31.96
K _d	0.7385	1.478	2.55	39.13	47.33	39.65	7.178	4.246	2.417	13.59	42.83	23.93
Std. Error												
B _{max}	20.06	10.17	4.668	8.865	13.32	17.77	2.701	10.55	8.999	3.847	13.53	4.97
K _d	0.6033	0.485	0.3361	19.33	17.25	12.83	5.663	1.027	0.5623	8.7	31.03	9.081

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FIG. 20B

	L2+H6	L3+H1	L3+H2	L3+H3	L3+H4	L3+H5	L3+H6	L4+H1	L4+H2	L4+H3	L4+H4	L4+H5	L4+H6
One site -- Specific binding													
Best-fit values													
B _{max}	49.55	160.9	143.7	22.86	16.78	46.81	39.48	158.1	146	31.17	13.86	28.79	41.61
K _d	15.04	1.574	2.171	32.23	13.68	27.65	29.96	2.826	2.651	28.27	10.42	17.68	29.78
Std. Error													
B _{max}	4.924	9.488	5.258	8.740	3.067	5.878	7.509	9.677	6.335	6.72	3.556	5.1	9.38
K _d	4.193	0.4414	0.3531	27.35	7.206	8.289	12.93	0.7209	0.4851	14.1	8.286	6.384	15.24

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FIG. 21

huC825 sequences

VH (SEQ ID NO: 76)

HVQLVESGGGLVQPGGSLRLSCAASGFSLT DYGVHWVRQAPGKGLEWLGVIWSGGGTA
YNTALISRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARRGSYPYNYFDAWGCGTLTV
SS

VL (SEQ ID NO: 77)

QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTASN YANWVQOKPGQCPRGLIGGHNNRPPG
VPA RFSGSLLGGKAALTLLGAQPEDEAEYYCALWYSDHWVIGGGTKLTVLG

mC825 sequences

VH (SEQ ID NO: 78)

HVKLQESQGLVQPSQSLTCTVSGFSLT DYGVHWVRQSPGKGLEWLGVIWSGGGTA
YNTALISRLNIYRDNSKNQVFLEMNSLQAEDTAMYYCARRGSYPYNYFDAWGCGTTVT
VSS

VL (SEQ ID NO: 79)

QAVVIQESALTTPPGETVTLTCGSSTGAVTASN YANWVQEKPDHCFTGLIGGHNNRPPG
VPA RFSGSLIGDKAALTIA GTQTEDEAIYFCALWYSDHWVIGGGTRLTVLG

OKT3

VH (SEQ ID NO: 80)

QVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTMH WVRQAPGKGLEWIGYINPS
RGYTNYNQKFKDRFTISRDN SKNTAFLQMDSL RPEDTGVYFCARYYDDHYS LDYW
GQGTPVTVSS

VL (SEQ ID NO: 81)

DIQMTQSPSSLSASVGDRTITCSASSSVSYM NWYQQTPGKAPKRWIYDTSKLASG
VPSRFSGSGSGTDYTFITISLQPEDIA TYQCQQWSSNPFTFGQGTKLQITR

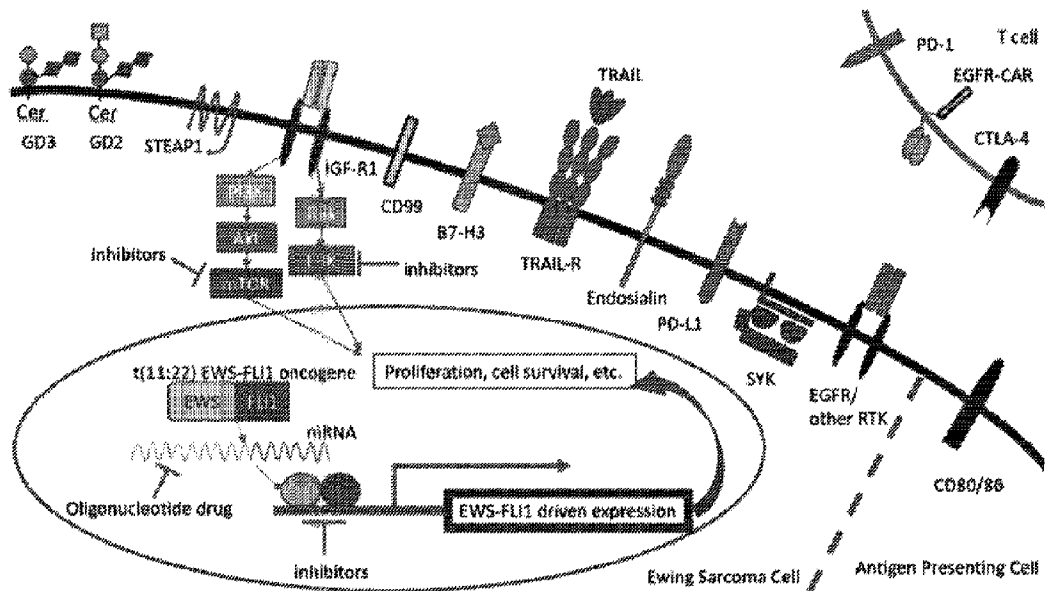


FIG. 1A