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(54) Title: BISPECIFIC ANTIBODY COMPOSITIONS AND RELATED METHODS FOR IMPROVED PRETARGETED RADIOIMMUNOTHERAPIES

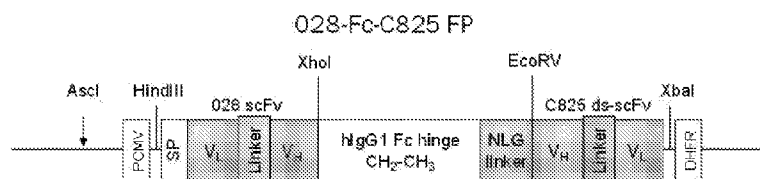


FIG. 1A

(57) Abstract: The present disclosure provides compositions and methods for improved pre-targeted radioimmunotherapeutics (PRIT) to treat various hematological disorders, such as B cell hyperproliferative disorders and solid tumors. The disclosed compositions include bispecific antibody compositions having a first domain that specifically bind to an antigen such as CD38, BCMA, Mucl, GPRC5D, or Slamf7, and a second domain that specifically binds to a radioactive ligand. Methods include administering the disclosed bispecific antibody reagent and separately administering the radioactive ligand. In some embodiments, a clearing agent is also administered. In some embodiments, the therapeutic methods comprise administering a combination of two or more bispecific antibody reagents. In some embodiments, an enhancing agent, such as ATRA, gamma secretase inhibitor, or dexamethasone, is also administered to enhance expression of the target antigen on the target cells.



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BISPECIFIC ANTIBODY COMPOSITIONS AND RELATED METHODS FOR IMPROVED PRETARGETED RADIOIMMUNOTHERAPIES

CROSS-REFERENCE TO RELATED APPLICATION

5 This application claims the benefit of U.S. Application No. 62/583,414, filed November 8, 2017, the disclosure of which is hereby expressly incorporated by reference in its entirety herein.

STATEMENT REGARDING SEQUENCE LISTING

10 The sequence listing associated with this application is provided in text format in lieu of a paper copy and is hereby incorporated by reference into the specification. The name of the text file containing the sequence listing is 67150_Sequence_Listing_final.txt. The text file is 237 KB; was created on November 8, 2018; and is being submitted via EFS-Web with the filing of the specification.

STATEMENT OF GOVERNMENT LICENSE RIGHTS

15 This invention was made with government support under CA205248 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

20 Effective agents and therapeutic strategies have been developed in hematological oncology over the past decade to improve cancer patient outcomes. For example, effective agents have been introduced to make complete response (CR) to induction therapy possible in almost half of multiple myeloma (MM) patients. However, complete disease eradication often remains elusive, creating conditions that strongly favor the persistence and evolution of therapy resistant malignant plasma cell clones. As a result the vast majority of the 130,000 people in the United States living with MM will ultimately die of progressive disease. High dose chemotherapy followed by autologous stem cell transplantation (ASCT) increases CR rates and prolongs disease-free survival, and the number of ASCTs for MM increases annually, emphasizing the importance of ASCT in current treatment paradigms. Yet disease almost invariably recurs even after ASCT.

30 Radioimmunotherapy (RIT) promises critical improvements in cancer therapy compared to strategies such as ASCT. RIT is less toxic to the patient than ASCT and provides the potential to completely eradicate disease. For illustration, in the context of MM the radiosensitivity of malignant plasma cells outside of the bone marrow (BM) is

well documented in clinical settings. Local recurrence of solitary extramedullary plasmacytomas occurs in less than 10% of cases after external beam radiotherapy (RT) alone, and sustained local disease control and durable symptom relief has been reported for 98% of lesions receiving >10 Gy. Furthermore, this excellent efficacy of external
5 beam RT for extramedullary plasmacytomas occurs even in patients with poor risk cytogenetics and active MM, suggesting that targeted RIT is agnostic to certain high risk features.

RIT selectively delivers radiation to target cells at disseminated disease sites, facilitating escalation to radiation doses not achievable through external beam RT. The
10 efficacy of RIT is well-established for several hematologic malignancies, and has been successfully integrated into ASCT-conditioning regimens with a significant improvement in progression free survival and overall survival among patients with non-Hodgkin lymphoma (NHL) and acute myeloid leukemia (AML) when targeting CD20 and CD45 antigens, respectively.

However, few studies have examined RIT in MM and none have explored
15 pretargeted radioimmunotherapy (PRIT), a two-step process shown in the clinic to be markedly superior to conventional, single-step RIT. In conventional RIT, a targeting antibody is directly labeled with a radioactive molecule. In two-step PRIT, a non-radioactive targeting antibody is administered first and allowed to localize to tumor sites.
20 The second step, after this "cold" antibody has maximally accumulated in the tumor, is to administer a low molecular weight radioactive moiety with a high affinity for the antibody. The small size of the second reagent facilitates rapid tumor penetration, rapid capture and retention by the pre-targeted antibody, and rapid clearance of unbound radioactive molecules from the blood. This two-step approach greatly decreases radiation
25 absorption by healthy tissues. The efficacy of PRIT can be further amplified by administering a "clearing agent" (CA) prior to the radioactive reagent. The CA accelerates clearance of any unbound antibody from the bloodstream, greatly reducing the chance of radioactive molecules attaching to unbound antibody and, thus reducing incidence of offsite toxicity.

CD38 is a transmembrane glycoprotein with high surface density and uniform
30 expression on MM and NHL cells, and relatively low expression on normal myeloid and lymphoid cells, and has proven a successful target for mAb-based immunotherapy in MM. Unlike many surface antigens, CD38 is stable on the cell surface, and this trait

combined with high density and uniform expression in MM and NHL make CD38 an excellent target for PRIT. The inventors recently documented striking therapeutic efficacy of PRIT in MM xenograft models using anti-CD38-streptavidin (OKT10-SA) and the β -emitter ^{90}Y labeled biotin. Objective remissions were observed within 7 days in
5 100% of mice treated with 800 to 1200 μCi of CD38-SA PRIT, including 100% complete remissions (i.e., having no detectable tumor in treated mice compared to tumors in control mice that were $2982 \pm 1002\%$ of initial tumor volume) by day 23. Despite these dramatic results obtained with the PRIT approach, concerns remain, including immunogenicity of bacterially-derived streptavidin used to confer high binding affinity between the antigen
10 targeting antibody reagent and the radioactive moiety. Furthermore, the presence of endogenous biotin can lower efficacy by blocking binding of radiolabeled biotin to the cancer-bound, streptavidin -labeled antibody reagent.

Accordingly, despite the advances in the art, a need remains for effective therapeutic reagents and methods that effectively target and eradicate cancers such as
15 hematological malignancies, while avoiding issues of immunogenicity, off-site toxicity, and endogenous interference of the radiolabel. The present disclosure addresses these and related needs.

SUMMARY

This summary is provided to introduce a selection of concepts in a simplified
20 form that are further described below in the Detailed Description. This summary is not intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

In one aspect, the disclosure provides a bispecific affinity reagent, comprising:
a first binding domain that specifically binds to a target antigen selected from
25 CD38, B cell maturation antigen (BCMA), Muc1, GPRC5D, and SlamF7; and a second binding domain that specifically binds to a radioactive ligand.

In another aspect, the disclosure provides a method of treating a hematological malignancy in a subject. The method comprises administering to the subject a therapeutically effective amount of a bispecific affinity reagent disclosed herein, and
30 thereafter administering to the subject a therapeutically effective amount of a radioactive ligand. In some embodiments, the method further comprises administering an enhancer, such as ATRA, GSI, or dexamethasone.

In another aspect, the disclosure provides a method of treating a hematological malignancy in a subject. The method comprises:

administering to the subject a therapeutically effective amount of a first bispecific affinity reagent and a therapeutically effective amount of a second bispecific affinity reagent, and thereafter

administering to the subject a therapeutically effective amount of a radioactive ligand;

wherein the first bispecific affinity reagent and the second bispecific affinity reagent each comprises a first binding domain that specifically binds to a cancer antigen and a second binding domain that specifically binds to the radioactive ligand,

wherein the first binding domain of the first bispecific affinity reagent and the first binding domain of the second bispecific affinity reagent specifically bind to different cancer antigens selected from CD38, BCMA, Muc1, SlamF7, GPRC5D, and CD20.

In another aspect, the disclosure provides a method of treating a malignancy characterized by expression of CD38. The method comprises:

administering to the subject an amount of all trans retinoic acid (ATRA), or functional derivatives or subunits thereof, sufficient to upregulate expression of CD38 in the malignant cells,

administering to the subject a therapeutically effective amount of a bispecific affinity reagent comprising a first binding domain that specifically binds to CD38 and a second binding domain that specifically binds to a radioactive ligand, and thereafter

administering to the subject a therapeutically effective amount of a radioactive ligand.

In another aspect, the disclosure provides a method of treating a malignancy characterized by expression of GPRC5D. The method comprises:

administering to the subject an amount of all trans retinoic acid (ATRA), or functional derivatives or subunits thereof, sufficient to upregulate expression of GPRC5D in the malignant cells,

administering to the subject a therapeutically effective amount of a bispecific affinity reagent comprising a first binding domain that specifically binds to GPRC5D and a second binding domain that specifically binds to a radioactive ligand, and thereafter

administering to the subject a therapeutically effective amount of a radioactive ligand.

In some embodiments, of the above aspects, the ATRA or functional derivatives or subunits thereof are administered in a liposomal formulation.

In another aspect, the disclosure provides a method of treating a malignancy characterized by expression of BCMA. The method comprises:

5 administering to the subject an amount of gamma secretase inhibitor (GSI) sufficient to upregulate expression of BCMA in the malignant cells,

administering to the subject a therapeutically effective amount of a bispecific affinity reagent comprising a first binding domain that specifically binds to BCMA and a second binding domain that specifically binds to a radioactive ligand, and thereafter

10 administering to the subject a therapeutically effective amount of a radioactive ligand.

In another aspect, the disclosure provides a method of treating a malignancy characterized by expression of Muc1. The method comprises:

15 administering to the subject an amount of gamma secretase inhibitor (GSI) sufficient to upregulate expression of Muc1 in the malignant cells,

administering to the subject a therapeutically effective amount of a bispecific affinity reagent comprising a first binding domain that specifically binds to Muc1 and a second binding domain that specifically binds to a radioactive ligand, and thereafter

20 administering to the subject a therapeutically effective amount of a radioactive ligand.

In some embodiments, the disclosed methods can further comprise administering an effective amount of a clearing agent (CA) after administering the bispecific affinity reagent and before administering the radioactive ligand. In some embodiments, the disclosed radioactive moiety comprises yttrium DOTA. In some embodiments, the disclosed affinity reagent is a fusion protein and the first binding domain and the second binding domain are separated by a hinge region. In some embodiments, the disclosed hinge region is or comprises a construct selected from an IgG1 Fc fragment, an IgG2 Fc fragment, and IgG3 Fc fragment, and an IgG4 Fc fragment. In some embodiments, the one or both of the disclosed first binding domain and the second binding domain are an antibody, a functional antibody fragment, functional antibody derivative. In some embodiments, the one or both of the disclosed first binding domain and the second binding domain comprise a variable light chain domain and variable heavy chain domain. In some embodiments, the disclosed variable light chain and variable heavy chain of the

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binding domain and/or the second binding are separated by a linker domain. In some embodiments, the one or both of the first binding domain and the second binding domain is an scFv. In some embodiments, the disclosed scFv is humanized.

DESCRIPTION OF THE DRAWINGS

5 The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

FIGURES 1A-1E. Structure and characterization of the CD38 bispecific protein.

10 (1A) Schematic of the 028-Fc-C825 bispecific (anti-CD38 x anti-Y-DOTA) Fc fusion gene. An anti-human CD38 028 scFv gene and an yttrium-DOTA capturing C825 disulfide-stabilized scFv (ds-scFv) gene were fused to the human IgG1 Fc fragment at the amino and carboxyl ends, respectively. An N-linked glycosylation containing linker (NLG) was incorporated between the Fc and C825 ds-scFv domains, as shown. Relevant
15 restriction enzymes for cloning and linearization are indicated. (Schematic not drawn to scale). (1B) SDS-PAGE analysis of the 028-Fc-C825 fusion protein. Bispecific 028-Fc-C825 fusion polypeptides were expressed in CHO-DG44 cells, where they spontaneously formed dimers via the hinge regions and were secreted into the growth medium. The purification fractions and the 028-Fc-C825 fusion protein (5µg) were analyzed by
20 electrophoresis on a 4-20% MES SDS PAGE gel (Invitrogen). Lane 1: SeeBlue Plus2 marker proteins in kDa (Invitrogen); Lane 2: Culture supernatant; Lane 3: Protein A column flow-through; Lane 4: wash; Lane 5: the non-reduced 028-Fc-C825 fusion protein (samples boiled); Lane 7: the monomeric 028-Fc-C825 fusion protein (samples boiled and reduced with 2-mercaptoethanol); Lane 6 is empty. The gel was stained with
25 Coomassie blue. (1C) Sandwich ELISA assay demonstrating concentration-dependent binding of the CD38 (028-Fc-C825) bispecific protein to the Y-DOTA ligand. A 96-well plate was coated with 70 µL of the BSA-Y-DOTA conjugate (1 µg/mL in PBS) and then blocked with 200 µL of 2% BSA in PBS buffer. After washing, the wells were treated with 100 µL of bispecific protein at 16 µg/mL followed by serial dilution as indicated.
30 The plate was further treated with HRP-anti-human Fc antibody followed by 3,3',5,5'-Tetramethylbenzidine (TMB). Controls demonstrate that binding to Y-DOTA is dependent on the C825 portion of the bispecific protein: the positive control, CD20 2H7-Fc-C825 bispecific, shows binding to Y-DOTA while the negative control, fusion

protein-Fc without C825, shows a lack of binding. (1D) and (1E) bifunctional binding assays of the CD38 (028-Fc-C825) bispecific protein demonstrate targeted binding to CD38+ cells and ligand capture of Y-DOTA-biotin. (1D) CD38+ multiple myeloma target cells (H929) or (E) CD38- control cells (U266) (0.5×10^6) were incubated in 40 μ L of HBSS-2%FBS buffer containing 1 μ g of biotin-Y-DOTA ligand and 2 μ g of either CD38 or CD20 bispecific proteins, or no protein for 30 min at 4°C. For CD38 blocking controls, cells were pre-incubated for 30 min in buffer containing 40 μ g anti-CD38 Ab. Cells were finally washed and resuspended in 40 μ L buffer plus 2 μ L of PE-streptavidin, incubated 30min at 4°C, washed 3 times, resuspended in 500 μ L of PBS buffer containing 1% formaldehyde, and analyzed by flow cytometry.

FIGURE 2. DOTAY-Dextran clearing agent (CA) effectively clears circulating CD38 bispecific protein from the bloodstream. Athymic nude mice ($n = 3-5$ per group) were injected at -24 hrs with 1.4 nM CD38 bispecific protein (028-C825), then at -1 hr with 5 μ g CA, and at 0 hrs with ^{90}Y -DOTA-Biotin. Controls received no CA. Percent injected dose per gram (%ID/g) was determined from retro-orbital venous samples taken at serial time points starting 5 min after the ^{90}Y -DOTA-Biotin injection. Error bars = 1 SEM.

FIGURES 3A-3C. Biodistribution and pharmacokinetics of ^{90}Y -DOTA-Biotin using CD38-Bispecific PRIT. Athymic nude mice ($n = 5$ per group) bearing H929 (MM) xenografts (10^7 cells injected in the right flank) were injected at -24 hrs with 2.8 nM pretargeting Ab (either CD38 bispecific = 028-C825, or control bispecific [targeting CD20] = 2H7-C825), then at -1 hr with CA, and at 0 hrs with ^{90}Y -DOTA-Biotin. (3A) Blood, tumor and normal organ specimens were taken 24 hrs after radioactivity injections. (3B) and (3C) Comprehensive tissue biodistributions were obtained at sequential time-points 6, 24, 48 and 120 hrs after ^{90}Y -DOTA-Biotin injection, using (3B) CD38 or (3C) control bispecific PRIT. Legend (3C) also applies to non-target tissues in (3B). Error bars = 1 SEM.

FIGURE 4. Dosimetry of ^{90}Y -DOTA-Biotin using CD38-Bispecific PRIT. Dosimetry, absorbed radiation dose per unit administered activity (Gy/mCi), was calculated for tumor and normal organs during the first 120 hrs after radioactivity injections (tissues per FIGURE 3B). Dosimetry includes organ self-dose absorbed fraction plus beta particle cross-organ absorbed fraction 46. Absorbed radiation dose to

tumor was 44 Gy/mCi, in contrast to 6, 3, and 3 Gy/mCi for liver, lung and kidney, respectively.

FIGURES 5A and 5B. Effect of CD38 bispecific PRIT on tumor growth rate and survival of mice bearing H929 (MM) xenografts. Athymic nude mice (n = 10 per group) with H929 xenografts were injected at -24hrs with a pretargeting protein (CD38 bispecific or control [anti-CD20] bispecific), then at -1 hr with CA, and at 0 hrs with 1200 μ Ci ^{90} Y-DOTA-Biotin. Tumor volume was monitored three times weekly and mice euthanized when tumor size reached IACUC mandated limits. For tumor volume graphics, data from euthanized mice were retained until all mice in a group died. (5A) Treatment with CD38 bispecific PRIT resulted in 100% CRs during days 15-30 and 80% long-term CRs. This contrasted with control mice, where 90% of untreated and 100% of control bispecific groups died of tumor progression by day 27 ($p \leq 0.0001$, CD38 bispecific vs. either control group, error bars = 1 SEM). One untreated mouse exhibited spontaneous tumor remission. (5B) Kaplan-Meier analysis indicates that CD38 bispecific PRIT significantly improved survival over controls ($p \leq 0.0001$), curing 80% of mice. Cure defined as no sign of tumor recurrence at day 150.

FIGURES 6A and 6B. Comparative effects of CD38 bispecific PRIT and CD38-SA PRIT on tumor growth and survival of mice bearing Namalwa (CD38+ BL) xenografts. Athymic nude mice (n = 8 per group) with Namalwa xenografts were injected at -24 hrs with a pretargeting protein (CD38 bispecific, CD38-SA, or control [anti-CD20] bispecific), then at -1 hr with CA, and at 0 hrs with 1200 μ Ci ^{90} Y-DOTA-Biotin. Tumor volumes were monitored three times weekly and mice euthanized when tumor size reached IACUC mandated limits. For tumor volume graphics, data for euthanized mice were retained until all mice in a group died. (6A) CD38 bispecific PRIT and CD38-SA PRIT each reduced tumor volumes to undetectable levels by day 21, followed by a single tumor recurrence in the CD38-SA group and no recurrences in the CD38 bispecific group. ($p < 0.0001$, either CD38 PRIT group vs. either control group, error bars = 1 SEM). (6B) Kaplan-Meier survival analysis. CD38 bispecific PRIT cured 75% of mice (all mortality due to early weight loss), while CD38-SA PRIT cured 88% of mice (all mortality due to tumor progression), demonstrating that at this 1200 μ Ci dose the two CD38 treatments each benefitted survival with high and equivalent efficacy ($p < 0.0001$ for either CD38 treatment vs. either control, $p = 0.48$ for CD38 bispecific vs. CD38-SA). Cure defined as the absence of tumor recurrence through day 150.

FIGURES 7A and 7B. Comparative dose-response effects of CD38 bispecific PRIT versus CD38-SA PRIT on tumor growth and survival of mice bearing CD38+ BL xenografts. These figures present data from two replicate experiments, each using 8-10 athymic nude mice per group. In total, n = 18 mice per treatment were injected at -24 hrs with a pretargeting protein (CD38 bispecific, CD38-SA, or control [anti-CD20] bispecific), then at -1 hr with CA, and at 0 hrs with 600 or 1000 μ Ci ^{90} Y-DOTA-Biotin. An additional n = 10 mice per group received only pretargeting protein (CD38 bispecific or CD38-SA) and CA, with no ^{90} Y-DOTA-Biotin. (7A) All control mice including untreated, pretargeting protein without ^{90} Y, and control bispecific 1000 μ Ci groups, experienced rapid tumor progression and died by day 14. All CD38 PRIT mice showed CR by day 11, and subsequent strongly reduced tumor progression relative to controls ($p < 0.0001$, any CD38 PRIT group vs. any control group). However, CD38 bispecific PRIT outperformed CD38-SA PRIT, the former showing later and fewer tumor progressions in the 600 and 1000 μ Ci treatment groups ($p < 0.003$, CD38 bispecific vs. CD38-SA). (7B) Kaplan-Meier survival analyses reflect the tumor volume results, showing greatly improved survival in each CD38 PRIT group relative to each control ($p < 0.0001$), and improved survival of CD38 bispecific PRIT mice relative to CD38-SA PRIT mice, across 600 and 1000 μ Ci levels ($p < 0.004$, CD38 bispecific vs. CD38-SA).

FIGURE 8. Flow cytometry demonstrates that the CD38 Ab OKT10, used in CD38-SA PRIT experiments, fails to block targeted binding of the CD38 bispecific to CD38+ cells (H929). CD38+ target cells (H929) (Red and Green) or CD38- control cells (U266) (Blue) (0.5×10^6) were incubated in 40 μ L of HBSS-2%FBS buffer containing 1 μ g of biotin-Y-DOTA ligand and 2 μ g of CD38 bispecific protein for 30 min at 4°C. For blocking test (Green), cells were pre-incubated for 30 min in buffer containing 40 μ g CD38 Ab OKT10. After bispecific incubation, cells were washed, resuspended in 40 μ L buffer plus 2 μ L of PE-streptavidin, incubated 30 min at 4°C, washed 3 times, resuspended in 500 μ L of PBS buffer containing 1% formaldehyde, and analyzed by flow cytometry.

FIGURES 9A-9C. Effect of CD38 bispecific PRIT on body weights of mice bearing H929 (MM), Namalwa (BL), or L363 (MM) xenografts. Athymic nude mice (n = 8-10 per group) with (9A) H929, (9B) Namalwa, or (9C) L363 xenografts were injected at -24 hrs with a pretargeting protein (CD38 bispecific, CD38-SA, or control [anti-CD20] bispecific), then at -1 hr with CA, and at 0 hrs with 600, 1000 or 1200 μ Ci ^{90} Y-DOTA-

Biotin. Body weights and tumor volumes were monitored three times weekly and mice euthanized when tumor size reached IACUC mandated limits.

FIGURES 10A-10D. (10A) Sandwich ELISA assay demonstrating concentration-dependent binding of the CD38 and BCMA bispecific FPs to microtiter wells coated with the Y-DOTA ligand. (10B) Flow Cytometric Analysis of Binding of purified CD38 bispecific fusion protein to H929 MM cells. (10C) Flow Cytometric Analysis of Binding of purified BCMA bispecific fusion protein to H929 MM cells with, or without, gamma secretase inhibition (GSI). A control bispecific fusion molecule not targeting BCMA was also tested. (10D) Comparison of survival of athymic mice bearing subcutaneous H929 (MM) xenografts treated with bispecific antibody PRIT.

FIGURES 11A-11D. Dose dependent effect of the gamma secretase inhibitor, RO4929097, on BCMA expression on 4 MM cell lines (11A, 11B) and 7 primary MM samples (11C, 11D). (11A) Surface BCMA expression by MM.1R cells cultured with the indicated concentrations of RO4929097; staining with anti-BCMA antibody (black lines) compared to isotype control (grey line). (11B) Fold-change in surface BCMA expression by MM cell lines when cultured with the indicated concentrations of RO4929097; fold change indicated relative to untreated MM cells of the same line. (11C) Surface BCMA expression by primary patient MM cells cultured with the indicated concentrations of RO4929097. Staining was as described in regard to FIGURE 11A. (11D) Fold change in BCMA on primary myeloma cells (n=7) cultured with vary amounts of GSI for 4 h. Primary and cell lines were cultured at 0.5×10^6 cells/mL. Fold change in BCMA is defined as $\text{Treated (MFIBCMA-MFI}_{\text{iso}}) / \text{Control (MFIBCMA-MFI}_{\text{iso}})$. Data is representative of 3 independent experiments with T cells derived from different donors.

FIGURE 12. To identify a physiologically relevant range of all-trans-retinoic acid (ATRA) doses capable of increasing CD38 receptor expression in MM cell lines, a series of in vitro experiments were performed and confirmed a dose dependent increase in CD38 receptor expression in three MM cell lines (U266, H929 and L363). These studies generated data to refine the process of manipulating CD38 receptor density for the purpose of increasing targeted delivery of radiolabeled DOTA-biotin after CD38 tumor cell pretargeting. A key outcome was that the effect on the U266 MM line was particularly notable because, while constitutive expression of CD38 is low on these cells, a striking dose dependent increase in expression is observed after incubation with solubilized ATRA.

FIGURE 13. Antibody Binding Capacity assay (QuantiBrite, Cat. No. 340495, BD Biosciences) was used according to manufacturer instructions to measure the absolute number of BCMA molecules on the self-surface and accessible to antibody binding following 17 hours incubation in the presence or absence of 5 μ M gamma secretase inhibitor (RO4929097) on three multiple myeloma cells lines. Data demonstrates that GSI upregulates surface BCMA target expression. This data supports BCMA as a viable target for bispecific radioimmunotherapy approaches through binding of BCMA.

FIGURE 14. Antibody Binding Capacity assay (QuantiBrite, BD Biosciences) was used according to manufacturer instructions to measure the absolute number of CD38 molecules on the self-surface and accessible to antibody binding following 65-72 hours treatment of ATRA on three multiple myeloma cells lines. Data demonstrates that ATRA upregulates surface CD38 target expression. This data supports CD38 as a viable target for bispecific radioimmunotherapy approaches through binding of CD38.

FIGURE 15. To ensure our clearing agent was able to effectively clear unbound agent from the blood stream, we conducted a time course experiment to test the impact of the clearing agent on the BCMA bispecific in the presence or absence of GSI. The percent of the injected dose (% ID) was monitored in the blood during a time course experiment by measuring the relative amount of Yttrium⁹⁰ in blood samples obtained from mice.

FIGURE 16. Biodistribution of ⁹⁰Y-DOTA-Biotin using BCMA-Bispecific PRIT. Athymic nude mice (n = 5 per group) bearing H929 (MM) xenografts (10⁷ cells injected in the right flank) were injected at -24 hrs with BCMA bispecific fusion then at -1 hr with CA, and at 0 hrs with ⁹⁰Y-DOTA-Biotin, Tumor and normal organ specimens were taken 24 hrs after radioactivity injections. Select tissue biodistributions were obtained 24 hrs after ⁹⁰Y-DOTA-Biotin injection.

DETAILED DESCRIPTION

Pretargeted radioimmunotherapy (PRIT) has demonstrated remarkable efficacy targeting tumor antigens, but immunogenicity and endogenous biotin blocking may limit clinical translation. Disclosed herein is a new PRIT approach for the treatment of hematological malignancies such as Multiple Myeloma (MM) and other B cell hyperproliferative disorders.

As described herein, the inventors developed bispecific antibody reagents for PRIT applications that avoids integration of biotin/streptavidin binding and, thus, eliminates endogenous biotin interference and immunogenic elements.

As described in more detail below, as a proof of concept, the inventors developed an anti-CD38 bispecific fusion protein that also specifically binds to radioactive ligands that can be administered separately. In murine xenograft models of MM and non-Hodgkin lymphoma (NHL), the CD38 bispecific construct demonstrated excellent blood clearance and tumor targeting. Dosimetry calculations showed a tumor absorbed dose of 43.8 Gy per mCi injected dose of yttrium-90, with tumor-to-normal organ dose ratios of 7:1 for liver and 15:1 for lung and kidney. In therapy studies, CD38 bispecific PRIT resulted in 100% complete remissions (CR) by day 12 in MM and NHL xenograft models, ultimately curing 80% of mice at optimal doses. In direct comparisons, efficacy of the CD38 bispecific proved equal or superior to streptavidin (SA)-biotin-based CD38-SA PRIT. Each approach cured at least 75% of mice at the highest radiation dose tested (1200 μ Ci), while at 600 and 1000 μ Ci doses the bispecific outperformed the SA approach, curing 35% more mice overall ($p < 0.004$). The high efficacy of the bispecific PRIT, combined with its' reduced risk of immunogenicity and endogenous biotin interference, make this design of bispecific affinity reagent an attractive candidate for clinical translation. Critically, CD38 PRIT can benefit patients with unresponsive, high-risk disease, because refractory disease typically retains radiation sensitivity.

Based on these positive results, the inventors applied this design to other B cell hyperproliferative associated antigens, such as B cell maturation antigen (BCMA) For example, as described in Example 2, a bispecific molecule that targets BCMA was developed as an alternative target for MM and other cancer cells for PRIT therapy. Experiments demonstrate the BCMA bispecific was able to target tumor cells with minimal off-tissue accumulation and toxicity. The enhancing agent of gamma secretase inhibitor (GSI) in combination with the BCMA bispecific fusion further facilitated blood clearance.

Based off the selective targeting of CD38 bispecific and BCMA bispecific on tumor cells enhancing agents were validated to upregulate surface target expression. In certain embodiments, gamma secretase inhibitors (GSIs) can be used to prevent cleavage of surface bound BCMA and therefore remove cleaved BCMA from the blood stream. In

particular embodiments, all-trans-retinoic acid (ATRA) can be used to upregulate CD38, Muc1, and GPRC5D on target cells.

Based on these results, PRIT utilizing the disclosed bispecific antibody compositions will serve as an effective treatment for various hematological disorders, including MM, NHL, and other B cell hyperproliferative diseases, as well as other solid tumor indications as further outlined below. Not to be bound by any particular theory, the PRIT bispecific antibody compositions described herein can be applied to any malignancy or neoplastic diseases in which CD38, BCMA, Muc1, GPRC5D, and/or SlamF7 is/are expressed.

Bispecific Affinity Reagent

In accordance with the foregoing, the disclosure provides a bispecific fusion protein comprising a first binding domain and a second binding domain. The protein is also referred to herein as a reagent or therapeutic agent. The first binding domain specifically binds to a target antigen selected from CD38, B cell maturation antigen (BCMA), Muc1, GPRC5D (G Protein-Coupled Receptor Class C Group 5 Member D), and SlamF7, and the second binding domain specifically binds to a radioactive ligand.

As used herein the term "binding domain" refers to a molecular domain, such as in a peptide, oligopeptide, polypeptide, or protein, that possesses the ability to specifically and non-covalently associate, unite, or combine with a target molecule (e.g., CD38, BCMA, Muc1, GPRC5D, SlamF7, or radioactive moiety). A binding domain includes any naturally occurring, synthetic, semi-synthetic, or recombinantly produced binding partner for the target biological molecule (e.g., CD38, BCMA, Muc1, GPRC5D, SlamF7, or radioactive moiety) or therapeutic compound (e.g., radioactive ligand, such as yttrium-DOTA (Y-DOTA)). In some embodiments, a binding domain is or comprises functional elements of an immunoglobulin or immunoglobulin-like molecule, such as an antibody or T cell receptor (TCR), which includes a functional binding domain or antigen-binding fragment thereof.

As used herein, the term "antibody" encompasses immunoglobulin molecules produced by or derived from any antibody-producing mammal (e.g., mouse, rat, rabbit, and primate including human), and which specifically bind to an antigen of interest. Exemplary antibodies include or are derived from polyclonal, monoclonal and recombinant antibodies. The antibodies can be human or humanized antibodies; murine

antibodies; chimeric, mouse-human, mouse-primate, primate-human monoclonal antibodies; and anti-idiotypic antibodies.

In some embodiments, one or both of the first and second binding domains is or comprises a functional antibody, or antigen binding fragment derivative thereof. An antibody fragment is a portion derived from or related to a full-length antibody, including the complementarity-determining regions (CDRs), antigen binding regions, or variable regions thereof. Illustrative examples of antibody fragments useful in the present disclosure include Fab, Fab', F(ab)₂, F(ab')₂ Fv fragment, VHH fragment, and VNAR fragment. Derivatives indicate that the domain incorporates further modification over mere selection of a portion or fragment of a source antibody. Derivatives can incorporate fusions of disparate parts of a source antibodies (or from multiple source antibodies) to provide a new, single protein domain that functions to bind the antigen of interest. Derivatives can include scFv fragments, single-chain Fab fragment (scFab), diabodies, linear antibodies, single-chain antibody molecules, multispecific antibodies formed from antibody fragments, and the like. A "single-chain Fv" or "scFv" antibody fragment comprises the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. The Fv polypeptide can further comprise a polypeptide linker between the V_H and V_L domains, which enables the scFv to form the desired structure for antigen binding.

Antibodies can be further modified to suit various uses. For example, a "chimeric antibody" is a recombinant protein that contains domains from different sources. For example, the variable domains and complementarity-determining regions (CDRs) can be derived from a non-human species (e.g., rodent) antibody, while the remainder of the antibody molecule is derived from a human antibody. A "humanized antibody" is a chimeric antibody that comprises a minimal sequence that conforms to specific complementarity-determining regions derived from non-human immunoglobulin that is transplanted into a human antibody framework. Humanized antibodies are typically recombinant proteins in which only the antibody complementarity-determining regions (CDRs) are of non-human origin.

Antibody fragments and derivatives that recognize specific epitopes can be generated by any technique known to those of skill in the art. For example, Fab and F(ab')₂ fragments of the invention can be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or

pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the variable region, the light chain constant region and the CHI domain of the heavy chain. Further, the antibodies of the present invention can also be generated using various phage display methods known in the art. Finally, the antibodies, or antibody fragments or derivatives, can be produced recombinantly according to known techniques.

In some embodiments, the binding proteins can include single chain antibody variable regions (e.g., domain antibodies, sFv, scFv, Fab), BCMA ligands (e.g., BAFF, APRIL and binding fragments thereof), antigen-binding regions of T cell receptors (TCRs), such as single chain TCRs (scTCRs), or synthetic polypeptides selected for the specific ability to bind to a biological molecule.

As used herein, "specifically binds" refers to an association or union of a binding domain, or a fusion protein containing the binding domain, to a target and bind to molecule with an affinity or K_a (i.e., an equilibrium association constant of a particular binding interaction with units of 1/M) equal to or greater than 10^5 M^{-1} , while not significantly associating or uniting with any other molecules or components in a sample. Binding domains (or fusion proteins thereof) can be classified as "high affinity" binding domains (or fusion proteins thereof) or "low affinity" binding domains (or fusion proteins thereof). "High affinity" binding domains refer to those binding domains with a K_a of at least 10^7 M^{-1} , at least 10^8 M^{-1} , at least 10^9 M^{-1} , at least 10^{10} M^{-1} , at least 10^{11} M^{-1} , at least 10^{12} M^{-1} , or at least 10^{13} M^{-1} . "Low affinity" binding domains refer to those binding domains with a K_a of up to 10^7 M^{-1} , up to 10^6 M^{-1} , up to 10^5 M^{-1} . Alternatively, affinity can be defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (e.g., 10^{-5} M to 10^{-13} M). In certain embodiments, a binding domain may have "enhanced affinity," which refers to a selected or engineered binding domain with stronger binding to a target antigen than a wild type (or parent) binding domain. For example, enhanced affinity may be due to a K_a (equilibrium association constant) for the target antigen that is higher than the wild type binding domain, or due to a K_d (dissociation constant) for the target antigen that is less than that of the wild type binding domain, or due to an off-rate (K_{off}) for the target antigen that is less than that of the wild type binding domain. A variety of assays are known for identifying binding domains of the present disclosure that specifically bind a particular target, as well as determining binding domain or fusion protein affinities, such as Western

blot, ELISA, and Biacore[®] analysis (see also, e.g., Scatchard et al., *Ann. N.Y. Acad. Sci.* 51:660, 1949; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

In some embodiments, one or both of the first binding domain and a second binding domain comprises a variable light chain domain and variable heavy chain domain, for example of an antibody. The variable light chain domain and variable heavy chain domain can be separated by a "linker domain". . The linker domain can be a five to about 35 amino acid sequence that connects the heavy chain immunoglobulin variable region to the light chain immunoglobulin variable region. In alternative embodiments where the first and/or second binding domain is a T cells receptor, the linker connects T cell receptor V α / β and C α / β chains (e.g., V α -C α , V β -C β , V α -V β) or connects each V α -C α , V β -C β , V α -V β pair to a hinge or hydrophobic domain. The linker domain provides a spacer function and flexibility sufficient for interaction of the two sub-binding domains so that the resulting single chain polypeptide retains a specific binding affinity to the same target molecule as an antibody or T cell receptor. In certain embodiments, a variable region linker comprises from about ten to about 30 amino acids or from about 15 to about 25 amino acids. In particular embodiments, a variable region linker peptide comprises from one to ten repeats of Gly_xSer_y, wherein x and y are independently an integer from 1 to 5 (e.g., Gly₄Ser, Gly₃Ser, Gly₂Ser, or (Gly₃Ser)_n(Gly₄Ser)₁, (Gly₃Ser)_n(Gly₄Ser)_n, or (Gly₄Ser)_n, wherein n is an integer of 1, 2, 3, 4, or 5) and wherein linked variable regions form a functional binding domain (e.g., scFv, scTCR). In particular embodiments, a linker domain may contain an N-linked glycosylation motif.

Exemplary first and second binding domains and bispecific affinity reagents are now described.

As indicated above, the defined first domains of the bispecific affinity reagents bind to one of CD38, BCMA, Muc1, GPRC5D, or SlamF7, which are antigens associated with the target cells in relevant hematological diseases, including B cell malignancies or hyperproliferative diseases. In some embodiments, the first binding domain comprises an amino acid sequence with at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the domains disclosed herein that specifically bind to the target antigens.

A "conservative substitution" is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are well known in the art (see, e.g., WO 97/09433 at page 10; Lehninger,

Biochemistry, 2nd Edition; Worth Publishers, Inc. NY, NY, pp. 71–77, 1975; and Lewin, Genes IV, Oxford University Press, NY and Cell Press, Cambridge, MA, p. 8, 1990).

"Sequence identity," as used herein, refers to the percentage of amino acid residues in one sequence that are identical with the amino acid residues in another reference polypeptide sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. The percentage sequence identity values can be generated using the NCBI BLAST 2.0 software as defined by Altschul, et al. (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs," Nucleic Acids Res. 25:3389-3402, with the parameters set to default values.

CD38 binding domains

CD38 is also expressed in a variety of malignant hematological diseases, including multiple myeloma, leukemias and lymphomas, such as B-cell chronic lymphocytic leukemia, T- and B-cell acute lymphocytic leukemia, Waldenstrom macroglobulinemia, primary systemic amyloidosis, mantle-cell lymphoma, pro-lymphocytic/myelocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, follicular lymphoma, Burkitt's lymphoma, large granular lymphocytic (LGL) leukemia, NK-cell leukemia and plasma-cell leukemia. Expression of CD38 has been described on epithelial/endothelial cells of different origin, including glandular epithelium in prostate, islet cells in pancreas, ductal epithelium in glands, including parotid gland, bronchial epithelial cells, cells in testis and ovary and tumor epithelium in colorectal adenocarcinoma. Other diseases, where CD38 expression could be involved, include, e.g., broncho-epithelial carcinomas of the lung, breast cancer (evolving from malignant proliferation of epithelial lining in ducts and lobules of the breast), pancreatic tumors, evolving from the β -cells (insulinomas), tumors evolving from epithelium in the gut (e.g. adenocarcinoma and squamous cell carcinoma), carcinoma in the prostate gland, and seminomas in testis and ovarian cancers. In the central nervous system, neuroblastomas express CD38. The present compositions and methods can encompass any of these disease indications characterized by expression of CD38. See, e.g., U.S. 9732154, incorporated herein by reference in its entirety.

In some embodiments, the first binding domain specifically binds to CD38. In some embodiments, the CD38 is a human CD38. (Gene ID: 952).

In certain embodiments, the first binding domain that specifically binds CD38 comprises a V_L region. For example, a V_L region in the first binding domain of the present disclosure is derived from or based on a V_L of a known monoclonal antibody that binds CD38 and may contain one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) insertions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) deletions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions (*e.g.*, conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_L of a known monoclonal antibody that binds CD38. An insertion, deletion, or substitution may be anywhere in the V_L region, including at the amino-terminus, carboxy-terminus, or both ends of the region, provided that each CDR comprises zero changes or at most one, two, three or four changes from a CDR of the V_L region of a known monoclonal antibody that binds CD38, and provided a binding domain containing the modified V_L region specifically binds its CD38 target with an affinity similar to the wild type binding domain. Similarly, in certain embodiments, the first binding domain that specifically binds CD38 comprises a V_H region. For example, a V_H region in the first binding domain of the present disclosure is derived from or based on a V_H of a known monoclonal antibody that binds CD38 and may contain one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) insertions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) deletions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions (*e.g.*, conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_H of a known monoclonal antibody that binds CD38. An insertion, deletion, or substitution may be anywhere in the V_H region, including at the amino-terminus, carboxy-terminus, or both ends of the region, provided that each CDR comprises zero changes or at most one, two, three or four changes from a CDR of the V_H region of a known monoclonal antibody that binds CD38, and provided a binding domain containing the modified V_H region specifically binds its CD38 target with an affinity similar to the wild type binding domain.

Exemplary CD38-specific antibodies include daratumumab, isatuximab, TAK-079, TAK-573, GBR-1342, AMG-424, MOR-202, MT-4019, MT-4019ND, MT-4019AS, A-145D, TAK-169, EDC-8, ABP-140, MT-4001V4, MT-4001, Xmab-13551, MT-4001V2, MT-4001V5, MT-4001V6, OSX-1750, Xmab-13243, DOM-1112, MT-4001V3, MT-4007ND.

Exemplary domains that bind to CD38 that can serve as (or part of) the first binding domain have at least 80% sequence identity (e.g., about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to a CD38 binding domain included in an amino acid sequence of a bispecific affinity reagent as set forth in one of SEQ ID NO:2, 4, and 6 (which are encoded by the nucleic acid sequences set forth in SEQ ID NOs:1, 3, and 5, respectively).

In some embodiments, the bispecific affinity reagent comprises an amino acid sequence as set forth in SEQ ID NOs:2, 4, or 6, or comprises an amino acid sequence with at least about 80% identity (e.g., about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to an amino acid sequence of as set forth in one of SEQ ID NO:2, 4, and 6. In some embodiments, the bispecific affinity reagent consists of or consists essentially of an amino acid sequence as set forth in SEQ ID NOs:2, 4, or 6. As used herein with respect to sequence identity, the term "consists essentially of" refers to a near exact identity but allowing for minor variation, such as conservative mutations or addition/deletions that do not otherwise impede or effect the ability of the bispecific molecule to function as described herein.

BCMA binding domains

In some embodiments, the first binding domain specifically binds to BCMA. In some embodiments, the BCMA is a human BCMA.

In certain embodiments, the first binding domain that specifically binds BCMA comprises a V_L region. For example, a V_L region in the first binding domain of the present disclosure is derived from or based on a V_L of a known monoclonal antibody that binds BCMA and may contain one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10) insertions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10) deletions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions (e.g., conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_L of a known monoclonal antibody that binds BCMA. An insertion, deletion, or substitution may be anywhere in the V_L region, including at the amino-terminus, carboxy-terminus, or both ends of the region, provided that each CDR comprises zero changes or at most one, two, three or four changes from a CDR of the V_L region of a known monoclonal antibody that binds BCMA, and provided a binding domain containing the modified V_L region specifically binds its BCMA target with an affinity similar to the wild type binding domain. Similarly, in certain embodiments, the first binding domain that specifically

binds BCMA comprises a V_H region. For example, a V_H region in the first binding domain of the present disclosure is derived from or based on a V_H of a known monoclonal antibody that binds BCMA and may contain one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) insertions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) deletions, one or more
5 (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions (*e.g.*, conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_H of a known monoclonal antibody that binds BCMA. An insertion, deletion, or substitution may be anywhere in the V_H region, including at the amino-terminus, carboxy-terminus, or both ends of the region, provided that each CDR comprises zero
10 changes or at most one, two, three or four changes from a CDR of the V_H region of a known monoclonal antibody that binds BCMA, and provided a binding domain containing the modified V_H region specifically binds its BCMA target with an affinity similar to the wild type binding domain.

Proteins that specifically bind to BCMA, their active binding sites, and
15 representative polynucleotides encoding the BCMA-specific binding proteins that are encompassed by the present disclosure are described in more detail in WO 2018/151836, which is incorporated herein by reference in its entirety.

Exemplary BCMA-specific antibodies include antibodies J22.0-xi, J22.9-xi, J6M0, J6M1, J6M2, J9M0, J9M1, J9M2, 11D5-3, CA8, A7D12.2, C11D5.3, C12A3.2,
20 C13F12.1, 13C2, 17A5, 83A10, 13A4, 13D2, 14B11, 14E1, 29B11, 29F3, 13A7, CA7, SGI, S307118G03, S332121F02, S332126E01, S322110D07, S336105A07, S335115G01, S335122F05, ET140-3, ET140-24, ET140-37, ET140-40, ET140-54, TBL-CLN1, C4.E2.1, Vicky-1, pSCHLI333, pSCHLI372, and pSCHLI373, and antigen-binding portions thereof. Various embodiments of BCMA-specific antibodies and
25 antigen-binding portions thereof, including humanized versions, are disclosed in, for example, PCT Publication Nos. WO 2002/066516, WO 2007/062090, WO 2010/104949, WO 2011/108008, WO 2012/163805, WO 2014/068079, WO 2015/166073, WO 2014/122143, WO 2014/089335, WO 2016/090327, and WO 2016/079177; Ryan et al., Mol. Cancer. Ther. 6(1):3009, 2007; and Abbas et al., Blood 725:1688, 2016, which
30 BCMA-specific antibodies, antigen-binding portions thereof and humanized versions are all incorporated herein by reference in their entireties. Variable domains and scFv molecules from these BCMA-specific antibodies, or binding domains with at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least

97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the binding domains therein, can be used as the first binding domain the bispecific affinity reagents described herein.

One exemplary scFv that can be incorporated into the first binding domain to specifically bind BCMA is set forth in SEQ ID NO:19. Thus, in some embodiments, the first binding domain is or comprises a sequence with at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:19.

Additional exemplary domains that bind to CD38 that can serve as (or part of) the first binding domain have at least 80% sequence identity (e.g., about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to a BCMA binding domain included in an amino acid sequence of a bispecific affinity reagent as set forth in one of SEQ ID NO:8 and 10 (which are encoded by the nucleic acid sequences set forth in SEQ ID NOs:7 and 9, respectively).

In some embodiments, the bispecific affinity reagent comprises an amino acid sequence as set forth in SEQ ID NOs:8 or 10, or comprises an amino acid sequence with at least about 80% identity (e.g., about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to an amino acid sequence of as set forth in one of SEQ ID NO:8 and 10. In some embodiments, the bispecific affinity reagent consists of or consists essentially of an amino acid sequence as set forth in one of SEQ ID NOs:8 and 10.

Muc1 binding domains

In some embodiments, the first binding domain specifically binds to Muc1. In some embodiments, the Muc1 is a human Muc1 isoform. In some embodiments, the human Muc1 isoform is a tumor associated isoform such as described in Nath, S. and Mukherjee, P., Muc1: a multifaceted oncoprotein with a key role in cancer progression, *Trends Mol Med.* 2014; 20(6):332–342, incorporated herein by reference in its entirety; see also GenBank accession nos. NP_001018016.1, NP_001018017.1, NP_001037855.1, NP_001037856.1, NP_001037857.1, NP_001037858.1, NP_001191214.1, NP_001191215.1, NP_001191216.1, NP_001191217.1, NP_001191218.1, NP_001191219.1, NP_001191220.1, NP_001191221.1, NP_001191222.1, NP_001191223.1, NP_001191224.1, NP_001191225.1, NP_001191226.1, NP_002447.4, each of which is incorporated herein by reference in its entirety. Muc1 is also known in

the literature as: ADMCKD, ADMCKD1, CA 15-3, CD227, EMA, H23AG, KL-6, MAM6, MCD, MCKD, MCKD1, MUC-1, MUC-1/SEC, MUC-1/X, MUC1/ZD, PEM, PEMT, and PUM. Mucins are O-glycosylated proteins that play an essential role in forming protective mucous barriers on epithelial surfaces. These proteins also play a role in intracellular signaling. This protein is expressed on the apical surface of epithelial cells that line the mucosal surfaces of many different tissues including lung, breast stomach and pancreas. This protein is proteolytically cleaved into alpha and beta subunits that form a heterodimeric complex. The N-terminal alpha subunit functions in cell-adhesion and the C-terminal beta subunit is involved in cell signaling. Overexpression, aberrant intracellular localization, and changes in glycosylation of this protein have been associated with carcinomas. Muc1 is associated with neoplastic progression and cellular adhesion. In addition to its expression in the hematopoietic lineage, it is also expressed in breast (including luminal, HER2+, and basal), ovarian, prostate, gastric, bile duct, liver, oral squamous cell carcinoma, thyroid, and pancreatic cancers in addition to leukemias, lymphomas, and MM. See, e.g., Horm, T.M., and Schroeder, J.A., MUC1 and Metastatic Cancer, *Cell Adh Migr.* 2013:7(2):187–198, incorporated herein by reference in its entirety.

In certain embodiments, the first binding domain that specifically binds Muc1 comprises a V_L region. For example, a V_L region in the first binding domain of the present disclosure is derived from or based on a V_L of a known monoclonal antibody that binds Muc1 and may contain one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10) insertions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10) deletions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions (e.g., conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_L of a known monoclonal antibody that binds Muc1. An insertion, deletion, or substitution may be anywhere in the V_L region, including at the amino-terminus, carboxy-terminus, or both ends of the region, provided that each CDR comprises zero changes or at most one, two, three or four changes from a CDR of the V_L region of a known monoclonal antibody that binds Muc1, and provided a binding domain containing the modified V_L region specifically binds its Muc1 target with an affinity similar to the wild type binding domain. Similarly, in certain embodiments, the first binding domain that specifically binds Muc1 comprises a V_H region. For example, a V_H region in the first binding domain of the present disclosure is derived from or based on a V_H of a known monoclonal

antibody that binds Muc1 and may contain one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) insertions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) deletions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions (*e.g.*, conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_H of a known monoclonal antibody that binds Muc1. An insertion, deletion, or substitution may be anywhere in the V_H region, including at the amino-terminus, carboxy-terminus, or both ends of the region, provided that each CDR comprises zero changes or at most one, two, three or four changes from a CDR of the V_H region of a known monoclonal antibody that binds Muc1, and provided a binding domain containing the modified V_H region specifically binds its Muc1 target with an affinity similar to the wild type binding domain.

Exemplary Muc1-specific antibodies include antibodies GO-2032c, gatipotuxumab, BrevaRex Mab-AR20.5, Seelomab-GEX, GO-3D1ADC, GO-203/NPs, TAB-004, BLSM-101, SPmAb-2.1, SPmAb-6, GO-3D1ADCC, clivatuzumab tetratextan, and sontuzumab.

Exemplary domains that bind to Muc1 that can serve as (or part of) the first binding domain have at least 80% sequence identity (*e.g.*, about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to a Muc1 binding domain included in an amino acid sequence of a bispecific affinity reagent as set forth in SEQ ID NO:18 (which is encoded by the nucleic acid set forth in SEQ ID NO:17).

Slam7 binding domains

In some embodiments, the first binding domain specifically binds to Slam7. In some embodiments, the Slam7 is a human Slam7. See, *e.g.*, GenBank accession nos. NP_001269517.1, NP_001269518.1, NP_001269519.1, NP_001269520.1, NP_001269521.1, NP_001269522.1, NP_001269523.1, NP_001269524.1, NP_001269525.1, and NP_067004.3, which are human isoforms of Slamf7 and are encompassed by the present disclosure). SlamF7 is also known as 19A24 protein, CD2 subset, CD2-like receptor activating cytotoxic cells, membrane protein FOAP-12, novel LY9 (lymphocyte antigen 9) like protein, and protein 19A SlamF7 is a signaling lymphocyte activation molecule F7 previously known as cell surface 1 CS1 (CCND3 subset 1, CD2-like receptor-activating cytotoxic cells [CRACC]).

In certain embodiments, the first binding domain that specifically binds Slam7 comprises a V_L region. For example, a V_L region in the first binding domain of the present disclosure is derived from or based on a V_L of a known monoclonal antibody that binds Slam7 and may contain one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) insertions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) deletions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions (*e.g.*, conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_L of a known monoclonal antibody that binds Slam7. An insertion, deletion, or substitution may be anywhere in the V_L region, including at the amino-terminus, carboxy-terminus, or both ends of the region, provided that each CDR comprises zero changes or at most one, two, three or four changes from a CDR of the V_L region of a known monoclonal antibody that binds Slam7, and provided a binding domain containing the modified V_L region specifically binds its Slam7 target with an affinity similar to the wild type binding domain. Similarly, in certain embodiments, the first binding domain that specifically binds Slam7 comprises a V_H region. For example, a V_H region in the first binding domain of the present disclosure is derived from or based on a V_H of a known monoclonal antibody that binds Slam7 and may contain one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) insertions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) deletions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions (*e.g.*, conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_H of a known monoclonal antibody that binds Slam7. An insertion, deletion, or substitution may be anywhere in the V_H region, including at the amino-terminus, carboxy-terminus, or both ends of the region, provided that each CDR comprises zero changes or at most one, two, three or four changes from a CDR of the V_H region of a known monoclonal antibody that binds Slam7, and provided a binding domain containing the modified V_H region specifically binds its Slam7 target with an affinity similar to the wild type binding domain.

Exemplary SlamF7-specific antibodies include antibodies elotuzumab, ABP-400, ABBV-838, and PDL-241. Exemplary SlamF7-specific antibodies (and their respective binding domains) are described in more detail in, *e.g.*, Friend, R., et al., Clinical potential of SLAMF7 antibodies – focus on elotuzumab in multiple myeloma, *Drug Des Devel Ther.* 2017; 11: 893–900; and WO2014055370, each of which is incorporated herein by reference.

Exemplary domains that bind to Slam7 that can serve as (or part of) the first binding domain have at least 80% sequence identity (e.g., about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to a Slam7 binding domain included in an amino acid sequence of a bispecific affinity reagent as set forth in SEQ ID NO:16 (which is encoded by the nucleic acid set forth in SEQ ID NO:15).

In one embodiment, the first domain that binds to SlamF7 comprises a variable heavy chain comprising the sequence set forth herein as SEQ ID NO:54. In one embodiment, the first domain that binds to SlamF7 comprises a variable light chain comprising the sequence set forth herein as SEQ ID NO:55. In a further embodiment, the first domain that binds to SlamF7 comprises the variable heavy domain of SEQ ID NO:54 and the variable light domain of SEQ ID NO:55. These are the heavy and light domains of elotuzumab. In other embodiments, the indicated sequence can incorporate modifications, as described above, so long as the domain retains SlamF7 binding capacity.

GPRC5D binding domains

GPRC5D mRNA is predominantly expressed in all malignant plasma cells from MM patients (Atamaniuk JA et al. *Eur J Clin Invest* 42(9) 953-960; 2012; Frigyesi-blood and Cohen, et al. *Hematology* 18(6): 348-35; 2013). GPRC5D expression is variable among the patients and correlate well with plasma cell burden and genetic aberrations such as Rb-1 deletion (Atamaniuk JA et al. *Eur J Clin Invest* 42(9) 953-960; 2012).

This exclusive expression of GPRC5D on the plasma-cell lineage designates it as an ideal target for therapies targeting multiple myeloma. In some embodiments, the first binding domain specifically binds to GPRC5D. In some embodiments, the GPRC5D is a human GPRC5D (see, e.g., GenBank AB099817.1 and BAC79169.1, incorporated herein by reference).

In certain embodiments, the first binding domain that specifically binds GPRC5D comprises a V_L region. For example, a V_L region in the first binding domain of the present disclosure is derived from or based on a V_L of a known monoclonal antibody that binds GPRC5D and may contain one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10) insertions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10) deletions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions (e.g., conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_L of a known

monoclonal antibody that binds GPRC5D. An insertion, deletion, or substitution may be anywhere in the V_L region, including at the amino-terminus, carboxy-terminus, or both ends of the region, provided that each CDR comprises zero changes or at most one, two, three or four changes from a CDR of the V_L region of a known monoclonal antibody that binds GPRC5D, and provided a binding domain containing the modified V_L region specifically binds its GPRC5D target with an affinity similar to the wild type binding domain. Similarly, in certain embodiments, the first binding domain that specifically binds GPRC5D comprises a V_H region. For example, a V_H region in the first binding domain of the present disclosure is derived from or based on a V_H of a known monoclonal antibody that binds GPRC5D and may contain one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) insertions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) deletions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions (*e.g.*, conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_H of a known monoclonal antibody that binds GPRC5D. An insertion, deletion, or substitution may be anywhere in the V_H region, including at the amino-terminus, carboxy-terminus, or both ends of the region, provided that each CDR comprises zero changes or at most one, two, three or four changes from a CDR of the V_H region of a known monoclonal antibody that binds GPRC5D, and provided a binding domain containing the modified V_H region specifically binds its GPRC5D target with an affinity similar to the wild type binding domain.

Exemplary GPRC5D antibodies, and thus GPRC5D binding domains, encompassed by the present disclosure include are described in WO 2018/147245, WO 2018/017786A2, and WO 2016/090329, each of which is incorporated herein by reference in its entirety.

Exemplary binding domains that specifically bind GPRC5D comprise a variable heavy (VH) chain region selected from the group consisting of SEQ ID NOs: 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, or 71.

Exemplary binding domains that specifically bind GPRC5D comprise a variable light (VL) chain region selected from the group consisting of SEQ ID NOs: 72, 73, 74, 75, 76, or 77.

In some embodiments, the first binding domain that specifically binds GPRC5D comprises a variable heavy (VH) chain region selected from the group consisting of SEQ ID NOs: 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, or 71 and a variable light (VL)

region selected from the group consisting of SEQ ID NOs: 72, 73, 74, 75, 76, or 77. In further embodiments, the first binding domain that specifically binds GPRC5D comprises a variable heavy (VH) chain region selected from the group consisting of SEQ ID NOs: 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, or 71 respectively paired with a variable
5 light (VL) region selected from the group consisting of SEQ ID NOs: 72, 73, 74, 75, 76, or 77.

In further embodiments, the first binding domain that specifically binds GPRC5D comprises a SEQ ID NO: 59, 60, or 64 paired with a VL chain region comprising SEQ ID NO: 72. In some embodiments, the first binding domain that specifically binds GPRC5D
10 comprises a VH chain region that comprises SEQ ID NO: 61, 65, 66, 67, or 71 paired with a VL chain region comprising SEQ ID NO: 73. In some embodiments, the first binding domain that specifically binds GPRC5D comprises a VH chain region that comprises SEQ ID NO: 62 or 69 paired with a VL chain region comprising SEQ ID NO: 74. In some embodiments, the first binding domain that specifically binds GPRC5D
15 comprises a VH chain region that comprises SEQ ID NO: 63 or 68 paired with a VL chain region comprising SEQ ID NO: 75. In some embodiments, the first binding domain that specifically binds GPRC5D comprises a VH chain region comprising SEQ ID NO: 70 paired with a VL chain region comprising SEQ ID NO: 76. In some embodiments, the first binding domain that specifically binds GPRC5D comprises a VH chain region
20 comprising SEQ ID NO: 71 paired with a VL chain region comprising SEQ ID NO: 77.

Exemplary binding domains that specifically bind GPRC5D comprise a variable heavy (VH) chain region selected from the group consisting of SEQ ID NOs: 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108.

25 Exemplary binding domains that specifically bind GPRC5D comprise a variable light (VL) chain region selected from the group consisting of SEQ ID NOs: 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139.

In some embodiments, the first binding domain that specifically bind GPRC5D
30 comprise a variable heavy (VH) chain region selected from the group consisting of SEQ ID NOs: 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108 and further comprises variable light (VL) chain region selected from the group consisting of SEQ ID NOs: 109, 110, 111, 112, 113,

22, 115, 30, 117, 38, 119, 120, 50, 122, 123, 62, 125, 126, 127, 128, 82, 130, 131, 94, 133, 134, 135, 311, 137, 335, 139. In some embodiments, the first binding domain that specifically bind GPRC5D comprise a variable heavy (VH) chain region set forth in SEQ ID NOs: 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108 respectively paired with a variable light (VL) chain region set forth in SEQ ID NOs: 109, 110, 111, 112, 113, 22, 115, 30, 117, 38, 119, 120, 50, 122, 123, 62, 125, 126, 127, 128, 82, 130, 131, 94, 133, 134, 135, 311, 137, 335, 139.

Radioactive ligand binding domains

10 As indicated above, the second binding domain in the bispecific affinity reagent specifically binds to a radioactive ligand. The radioactive ligand can be any ligand, typically a small molecule, which is radioactive and configured for administration into a subject. The radioactive ligand can be or comprise a radioactive ion or radionuclide. In one embodiment, the radioactive ligand is or comprises a radioactive ion or radionuclide complexed with a chelator.

15 In certain embodiments, the second binding domain that specifically binds to a radioactive ligand comprises a V_L region. For example, a V_L region in the first binding domain of the present disclosure is derived from or based on a V_L of a known monoclonal antibody that binds to a radioactive ligand and may contain one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) insertions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) deletions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions (*e.g.*, conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_L of a known monoclonal antibody that binds to a radioactive ligand. An insertion, deletion, or substitution may be anywhere in the V_L region, including at the amino-terminus, carboxy-terminus, or both ends of the region, provided that each CDR comprises zero changes or at most one, two, three or four changes from a CDR of the V_L region of a known monoclonal antibody that binds to a radioactive ligand, and provided a binding domain containing the modified V_L region specifically binds its to a radioactive ligand target with an affinity similar to the reference binding domain. Similarly, in certain embodiments, the first binding domain that specifically binds to a radioactive ligand comprises a V_H region. For example, a V_H region in the first binding domain of the present disclosure is derived from or based on a V_H of a known monoclonal antibody that binds to a radioactive ligand and may contain one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9,

or 10) insertions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) deletions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions (*e.g.*, conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_H of a known monoclonal antibody that binds to a radioactive ligand. An insertion, deletion, or substitution may be anywhere in the V_H region, including at the amino-terminus, carboxy-terminus, or both ends of the region, provided that each CDR comprises zero changes or at most one, two, three or four changes from a CDR of the V_H region of a known monoclonal antibody that binds to a radioactive ligand, and provided a binding domain containing the modified V_H region specifically binds its to a radioactive ligand target with an affinity similar to the reference type binding domain.

The radionuclide can be, *e.g.*, a beta emitter, an alpha emitter, or a low-energy electron emitter.

An exemplary chelator encompassed by the disclosure is DOTA, also known as tetraxetan. DOTA includes a central 12-membered tetraaza ring that can hold metal ions and radionuclides. DOTA is a macrocyclic chelating agent that forms stable complexes with metals that are essentially irreversible under physiological conditions. DOTA has a molecular weight of 405 Daltons, diffuses very rapidly, and exhibits rapid renal clearance. DOTA. A variant of DOTA that has a structure that differs to a certain limited extent from the structure of DOTA and that retains the ability to function (*e.g.*, retains sufficient activity to be used for one or more of the purposes described herein) is an active variant of DOTA.

The chelated ion can be radionuclide, which confers radioactivity to the ligand. The radionuclide can be a β emitter, *e.g.*, ⁸⁶Y, ⁹⁰Y, ¹⁸⁶Re, ¹⁸⁸Re, ¹³¹I, ¹⁷⁷Lu, or ⁶⁷Cu, an α emitter, *e.g.*, ²¹³Bi, ²¹¹At, ²²⁵Ac, or a low-energy electron emitter, *i.e.*, an Auger-emitter, *e.g.*, ¹²⁵I, ¹¹¹In or ⁶⁷Ga. In general, useful radionuclides include, for example, but are not limited to, ⁹⁰Y, ¹¹¹In, ⁶⁷Ga, ⁶⁸Ga, ¹⁷⁷Lu, ¹⁸⁸Re, ²²³Ra, ⁵⁷Gd, ⁶⁴Cu, ⁶⁷Cu, ⁸⁹Zr, ⁴⁷Sc, ¹⁵³Sm, ¹⁶⁶Tb, ¹⁶⁶Tb, ¹⁶⁶Ho, ²¹²Pb, ²¹²Bi, ²¹³Bi, ²²⁵Ac, ²²⁷Th, ²¹¹At and ²²⁷Ac. In one embodiment, the radionuclide is Yttrium (⁹⁰Y). Thus, in some embodiments, the radioactive ligand is or comprises ⁹⁰Y-DOTA.

U.S. 8,648,176, incorporated herein by reference in its entirety, describes in more detail exemplary second binding domains that specifically binds to a metal chelate, such as DOTA. The second binding domain, *e.g.*, an scFv domain, that binds a metal chelate with a radionuclide, can have a sequence at least or about 70% (*e.g.*, at least or about

75%, 80%, 85%, 90%, 95% or 98%) identical to SEQ ID NO:20, exclusive of the linker at residues 120-134. Residues 1-119 of SEQ ID NO:20 represent the variable heavy chain of the antibody designated 2D 12.5 (Corneillie et al., J. Am. Chem. Soc. 125:15039-15048, 2003); residues 120-134 represent a linker; and residues 135-244
5 represent the variable light chain of 2D 12.5. These embodiments of the second domain can include a mutation at a position corresponding to one or more of the following positions within SEQ ID NO:20: 29, 30, 31, 32, 33, 34, 36, 37, 47, 48, 49, 51, 54, 55, 56, 57, 58, 60, 69, 71, 73, 94, 95, 96, 97, 102, 103, 105, 106, 107, 164, 165, 166, 167, 169, 171, 172, 184, 185, 188, 189, 223, 224, 225, 226, 228, 229, 230, 231, 233, and 234.
10 Alternatively, or in addition, the second binding domain can include a mutation at a position corresponding to one or more of the following positions within SEQ ID NO:20: 60, 61, 63, 71, 80, 88, 108, 139, 157, 165, 187, 230, and 234. Alternatively, or in addition, the second binding domain can include a mutation at a position corresponding to one or more of the following positions within SEQ ID NO:20: 100, 187, and 227. More
15 specifically, the present second binding domain can include the sequence of residues 1-244 of mutant C8.2-1 (SEQ ID NO:21); C8.2-2 (SEQ ID NO:22); C8.2-3 (SEQ ID NO:23); C8.2-4 (SEQ ID NO:24); C8.2-5 (SEQ ID NO:25); C8.2-6 (SEQ ID NO:26); C7.3 1 (SEQ ID NO:27); C7.3 2 (SEQ ID NO:28); C7.3 3 (SEQ ID NO:29); C7.3 4 (SEQ ID NO:30); C7.3 5 (SEQ ID NO:31); C7.3 6 (SEQ ID NO:32); C7.3 7 (SEQ ID NO:33);
20 C7.3 8 (SEQ ID NO:34); C7.3 9 (SEQ ID NO:35); or C7.3 10 (SEQ ID NO:36).

In some embodiments, the bispecific affinity reagent is a fusion protein comprising both the first binding domain and the second binding domain separated by a hinge region. As used herein, a "hinge region" or a "hinge" refers to a region that provides sufficient space and flexibility between the first and second binding domains to
25 facilitate the binding of each binding domain to its respective specific antigen without mutual interference. Exemplary hinge domains that are encompassed by this disclosure, and detailed discussions thereof, are provided in WO 2018/151836, which is incorporated herein by reference in its entirety. In some embodiments, the hinge region can comprise
(a) an immunoglobulin hinge sequence (made up of, for example, upper and core regions)
30 or a functional fragment or variant thereof, (b) a type II C-lectin interdomain (stalk) region or a functional fragment or variant thereof, or (c) a cluster of differentiation (CD) molecule stalk region or a functional variant thereof. In some embodiments, the immunoglobulin hinge region can be a naturally occurring upper and middle hinge amino

acid sequences interposed between and connecting the CH₁ and CH₂ domains (for IgG, IgA, and IgD) or interposed between and connecting the CH₁ and CH₃ domains (for IgE and IgM) found in the heavy chain of an antibody. In certain embodiments, the hinge region is human, and in particular embodiments, comprises a human IgG hinge region. In some embodiments, the IgG hinge region comprises a human IgG1 Fc hinge. In some
5 embodiments, the IgG hinge region comprises a human IgG2 Fc hinge. In some embodiments, the IgG hinge region comprises a human IgG3 Fc hinge. In some embodiments, the IgG hinge region comprises a human IgG4 Fc hinge.

Methods of Treatment

10 The bispecific affinity reagents (e.g., fusion proteins with first and second binding domains) described herein are useful to pretarget cells for radiotimmunoherapy. Accordingly, in another aspect, the disclosure provides a method of treating a hematological or other applicable malignancy. The method comprises administering to the subject a therapeutically effective amount of the bispecific affinity reagent as
15 described herein, and administering a therapeutically effective amount of a radioactive ligand.

The hematological malignancy can include any hematological hyperproliferative disorder, including hematological cancer. In some embodiments, the hematological disorder is a B cell hyperproliferative disorder or malignancy. The term B cell
20 malignancy can include multiple myeloma (MM), a leukemia or lymphoma.

Leukemias encompassed by this disclosure include acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML). Subtypes of ALL include precursor B acute lymphoblastic leukemia, precursor T acute lymphoblastic leukemia, Burkitt's leukemia,
25 and acute biphenotypic leukemia. Subtypes of CLL include B-cell prolymphocytic leukemia, which is a more aggressive disease. Subtypes of AML include acute promyelocytic leukemia, acute myeloblastic leukemia, and acute megakaryoblastic leukemia. Subtypes of CML include chronic myelomonocytic leukemia. Other types of leukemias include Hairy cell leukemia (HCL), T-cell prolymphocytic leukemia (T-PLL),
30 large granular lymphocytic leukemia. Any of these types of leukemias are encompassed by the present disclosure to the extent that the associated transformed cells express CD38, BCMA, Muc1, GPRC5D, and/or SlamF7.

Lymphomas are any neoplasms of the lymphatic tissues. Lymphomas encompassed by this disclosure include the Hodgkin's lymphomas (HL) and the non-Hodgkin lymphomas (NHL). Subtypes of lymphomas include B cell small cell lymphoma, splenic marginal zone lymphoma, extranodal marginal zone B cell lymphoma, also called MALT lymphoma, nodal marginal zone B cell lymphoma, extranodal marginal zone B cell lymphoma, also called MALT lymphoma, follicular lymphoma, primary cutaneous follicle center lymphoma, mantle cell lymphoma, diffuse large B cell lymphoma, Epstein-Barr virus-positive DLBCL of the elderly, lymphomatoid granulomatosis, primary mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, ALK+ large B-cell lymphoma, plasmablastic lymphoma, primary effusion lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman's disease, and Burkitt lymphoma. Any of these types of lymphoma are encompassed by the present disclosure to the extent that the associated transformed cells express CD38, BCMA, Muc1, GPRC5D, and/or SlamF7.

In addition to hematologically relevant malignancies, a person of ordinary skill in the art would understand that, while this disclosure is generally framed in the context of hematological malignancies, the disclosed compositions and methods can also be applied to other tumor and cancers, such as breast (including luminal, HER2+, and basal), ovarian, prostate, gastric, bile duct, liver, oral squamous cell carcinoma, thyroid, and pancreatic cancers. Such applications are also encompassed by the present disclosure.

The hematological malignancy and/or hyperproliferative disorder, or other relevant tumors and cancers contemplated in this aspect can be characterized by the expression of, or in some embodiments an increased expression of, at least one of CD38, BCMA, Muc1, GPRC5D, and SlamF7 in the malignant cell of the subject. The status of these other hematological malignancies and/or hyperproliferative disorders, or other relevant tumors or cancers with respect to expression of CD38, BCMA, Muc1, GPRC5D, and/or SlamF7 can be readily determined by persons of ordinary skill in the art using, e.g., immunoassays that incorporate affinity reagents with domains such as the first domains described herein.

As used herein, the term "treat" refers to medical management of a disease, disorder, or condition of a subject (e.g., a human or non-human mammal, such as a primate, horse, dog, mouse, rat, and the like). For example, an appropriate dose or treatment regimen comprising bispecific fusion protein that bind CD38, BCMA, Muc1,

GPRC5D, or SlamF7 in combination administration of a corresponding radioactive moiety (e.g., in PRIT) is administered to elicit a therapeutic or prophylactic benefit. Therapeutic or prophylactic/preventive benefit includes improved clinical outcome; lessening or alleviation of symptoms associated with a disease; decreased occurrence of symptoms; improved quality of life; longer disease-free status; diminishment of extent of disease, stabilization of disease state; delay of disease progression; remission; survival; prolonged survival; or any combination thereof. For example, the replication rate of a proportion of targeted CD38, BCMA, Muc1, GPRC5D, and/or SlamF7 expressing cells is slowed or stopped. In other outcomes, a proportion of CD38, BCMA, Muc1, GPRC5D, and/or SlamF7 expressing cells is killed.

The bispecific affinity reagent in this aspect can encompass any bispecific affinity reagent described herein comprising a first binding domain that specifically binds to one of CD38, BCMA, Muc1, GPRC5D, and SlamF7, and a second binding domain that specifically binds a radioactive ligand.

Effective doses of the bispecific affinity reagent can be readily determined by persons of ordinary skill in the art. As described below, murine xenograft experiments utilized single injections of 2.8 nmol (210 to 420 μ g) bispecific reagent for successful effect on the grafted tumors. Preferred embodiments for dosing are equivalent dosing regimens that can be readily established for human subjects adjusting for body mass. For example, doses can range from 0.05 mg/kg to 100 mg/kg, 0.1 mg/kg to 75 mg/kg, 0.1 mg/kg to 50 mg/kg, 0.1 mg/kg to 25 mg/kg, 1 mg/kg to 30 mg/kg, 2 mg/kg to 25 mg/kg, 5 mg/kg to 25 mg/kg, 10 mg/kg to 20 mg/kg, or 15 mg/kg to 20 mg/kg. Exemplary doses include 0.05 mg/kg, 0.1 mg/kg, 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, and 100 mg/kg.

The radioactive ligand of this aspect is described above. In an illustrative, non-limiting embodiment, the radioactive ligand comprises yttrium-DOTA. The radioactive ligand is administered to the subject after the bispecific affinity reagent is administered. In some embodiments, the radioactive ligand is administered to the subject about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 24, 30, 35, or 40 hours after the administration of the bispecific affinity reagent. In some embodiments, the radioactive ligand is administered to the subject about 10-to about 30, about 15 to about 25 hours, or about 20 to about 25 hours after the administration of the bispecific affinity reagent.

Effective doses of the radioactive ligand can be readily determined by persons of ordinary skill in the art to provide for sufficient ligand to specifically bind cell the present cell-bound bispecific affinity reagent in the subject. As described below, murine xenograft experiments utilized single injections of about 1.2 nM (2 μ g) DOTA-biotin
5 labeled with 20 to 40 μ Ci (0.74–1.48 MBq) of ^{90} Y for successful effect on the grafted tumors. Equivalent dosing regimens can be readily established for human subjects adjusting for body mass.

In some embodiments, the method further comprises administering an effective amount of a clearing agent (CA). The CA accelerates the clearance of any unbound
10 antibody from the subject's bloodstream to reduce the likelihood that the radioactive moiety will bind to bispecific fusion protein that is not bound to CD38, BCMA, Muc1, GPRC5D, or SlamF7. Accordingly, the CA is typically administered after administering the bispecific fusion protein but before administering the radioactive moiety. In some embodiments, the CA is administered 8 hours, 7 hours, 6 hours, about 5 hours, 4 hours, 3
15 hours, 2 hours, 1 hour, or just prior to administration of the radioactive ligand. In some embodiments, the CA is administered between about 0.5 and 4 hours or about 1-2 hours before administration of the radioactive ligand.

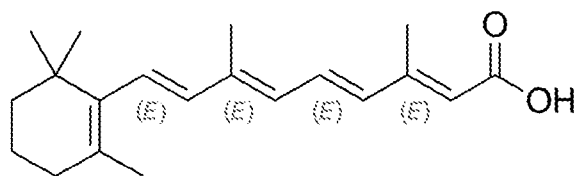
Any clearing agent effective to facilitate removal of unbound bispecific affinity reagent from circulation is encompassed by the disclosure. An exemplary CA is a
20 DOTAY-Dextran clearing agent (Orcutt KD, et al., *Molecular cancer therapeutics*. 2012;11(6):1365-1372), incorporated herein by reference in its entirety.

Effective doses of the CA can be readily determined by persons of ordinary skill in the art to provide for at least some clearance of unbound bispecific affinity reagent. As described below, murine xenograft models utilized approximately 0.5 to 10 μ g per
25 individual mouse. Equivalent dosing regimens can be readily established for human subjects adjusting for body mass.

In some embodiments, the method further comprises administering an agent that enhances expression of the target antigen, referred to herein as an "enhancing agent."

An exemplary enhancing agent encompassed by the disclosure is all-trans retinoic acid (ATRA), which is a physiologic derivative of vitamin A (retinol) and can be
30 represented by formula I. ATRA has been shown to increase CD38 expression levels in cells, such as target MM cells. See, e.g., Nijhof, I.S., et al., Upregulation of CD38 expression on multiple myeloma cells by all-trans retinoic acid improves the efficacy of

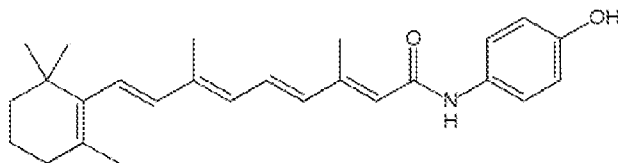
daratumumab, *Leukemia*, 2015; 29(10):2039-49, incorporated herein by reference in its entirety. Considering that CD38 exhibits high surface density and uniform expression on MM and NHL cells, and relatively low expression on normal myeloid and lymphoid cells, expression enhancement induced by ATRA administration can serve to further distinguish the transformed cells from healthy cells. Furthermore, ATRA has been shown to increase GPRC5D expression levels in cells. See, e.g., Inoue, S., et al., The RAIG Family Member, GPRC5D, Is Associated with Hard-Keratinized Structures, *Journal of Investigative Dermatology*, 2004; 122(3):565-573, incorporated herein by reference in its entirety. Thus, like CD38, expression enhancement of GPRC5D induced by ATRA administration can serve to further distinguish the transformed cells from healthy cells and provide for enhanced targeting of diseased cells.



I

15

Functional derivatives or analogs of ATRA have also been identified to enhance CD38 or GPRC5D expression in diseased cells, such as MM cells, and can be used to similar effect as ATRA. Such derivatives or analogs are also encompassed by the present disclosure. For example, one analog is fenretinide (chemical name: (2E,4E,6E,8E)-N-(4-hydroxyphenyl)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenamide), which is a semi-synthetic retinoid derivative and can be represented by formula I:



II

25

Use of these ATRA enhancers and functional analogs or derivatives thereof can contribute to enhanced therapeutic attack of transformed CD38 or GPRC5D positive cells, including MM and NHL cells from the subject using the bispecific affinity reagent

PRIT approach described herein that incorporates a first binding domain that specifically binds to CD38 or GPRC5D.

5 ATRA and its derivatives and analogs can be administered concurrently with prior to the bispecific affinity reagent comprising a first binding domain that specifically binds CD38 or GPRC5D. In some embodiments, the ATRA (or derivative or analog thereof) enhancing agent is administered prior to the administration of the bispecific affinity reagent comprising a first binding domain that specifically binds CD38 or GPRC5D. For example, the ATRA (or derivative or analog thereof) enhancing agent can be administered between about 1 hour to about 3 days prior to the administration of the bispecific affinity reagent comprising a first binding domain that specifically binds CD38 or GPRC5D, such as about 2, 5, 10, 15, 20, 24 hours before the administration of the bispecific affinity reagent comprising a first binding domain that specifically binds CD38 or GPRC5D. In other embodiments, the ATRA (or derivative or analog thereof) enhancing agent is administered about 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, or 3.5 days prior to the administration of the bispecific affinity reagent comprising a first binding domain that specifically binds CD38 or GPRC5D.

The ATRA (or derivative or analog thereof) can be formulated for any form of administration, such as systemic (e.g., I.V., parenteral) or oral administration (e.g., in a solid matrix or liquid solution suitable for ingestion). In some embodiments, the ATRA (or derivative or analog thereof) is formulated in liposomal form, which has provided advantages for delivery, bioavailability, and reducing toxicity. See, e.g., WO2001074384A1, Grace, V.M., et al, Liposome encapsulated all trans retinoic acid (ATRA) has enhanced immunomodulatory and inflammation reducing activities in mice model, *Anticancer Agents Med Chem.* 2015;15(2):196-205; Ozpolat, B. and Lopez-Berestein, G., Liposomal-all-trans-retinoic acid in treatment of acute promyelocytic leukemia, *Leuk Lymphoma.* 2002 May;43(5):933-41; and Ozpolat, B. and Lopez-Berestein, G., Pharmacokinetics of intravenously administered liposomal all-trans-retinoic acid (ATRA) and orally administered ATRA in healthy volunteers, *J Pharm Pharmaceut Sci*, 6(2):292-301, 2003, each of which is incorporated herein by reference in its entirety.

The ATRA (or derivative or analog thereof) can be administered at any dose effective to induce upregulation of CD38 or GPRC5D in target cells (e.g., MM cells). Exemplary, non-limiting doses are between 1 mg/m² to about 400 mg/m², such as about 1

mg/m², about 5 mg/m², about 10 mg/m², about 15 mg/m², about 20 mg/m², about 25 mg/m², about 30 mg/m², about 35 mg/m², about 40 mg/m², about 45 mg/m², about 50 mg/m², about 55 mg/m², about 60 mg/m², about 65 mg/m², about 70 mg/m², about 75 mg/m², about 80 mg/m², about 85 mg/m², about 90 mg/m², about 95 mg/m², about 100 mg/m², about 110 mg/m², about 120 mg/m², about 130 mg/m², about 140 mg/m², about 150 mg/m², about 160 mg/m², about 170 mg/m², about 180 mg/m², about 190 mg/m², about 200 mg/m², about 225 mg/m², about 250 mg/m², about 275 mg/m², about 300 mg/m², about 350 mg/m², or about 400 mg/m².

Another exemplary enhancing agent encompassed by the disclosure is a γ -secretase inhibitor (GSI). Administration of GSIs is associated with enhanced expression of surface BCMA and Muc1 antigens on hematological cells, such as MM cells. By preventing the cleavage of BCMA and Muc1 from the cell surface, less soluble antigen is present to serve as a decoy for the bispecific agent. GSIs are described in more detail in WO 2018/151836, which is incorporated herein by reference in its entirety. Exemplary γ -secretase inhibitors (GSIs) include small molecules, peptidomimetic compounds or γ -secretase-specific binding proteins. A GSI can target any one or more of the γ -secretase complex proteins, including presenilin 1 (PS1), presenilin 2 (PS2), nicastrin (NCT), anterior pharynx-defective 1 (APH-1), and presenilin enhancer 2 (PEN-2), provided that the γ -secretase cleavage activity is reduced compared to uninhibited γ -secretase. In certain embodiments, the γ -secretase activity is reduced at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or 100%. Assays for measuring γ -secretase activity are known in the art (see, e.g., Laurent et al., 2015). For example, the level of soluble BCMA can be a surrogate measure for γ -secretase activity. Representative small molecule GSIs, for use with a BCMA-targeted immunotherapy to treat a proliferative or autoimmune disease or disorder, include avagacestat, DAPT, BMS-906024, BMS-986115, LY411575, LY3039478, MK-0752, PF-03084014, RO4929097, semagacestat, YO-01027, and any combination thereof. Other GSIs are γ -secretase-specific binding proteins, such as antibodies or antigen binding portions thereof that a γ -secretase complex or a γ -secretase complex protein, such as presenilin 1 (PS1), presenilin 2 (PS2), nicastrin (NCT), anterior pharynx-defective 1 (APH-1), and presenilin enhancer 2 (PEN-2). An exemplary γ -secretase-specific binding protein is a nicastrin-specific binding protein, such as antibodies scFvG9, A5226A, 2H6, 10C11, and antigen binding fragments thereof.

This can contribute to enhanced therapeutic attack of transformed BCMA positive and/or Muc1 positive cells, including MM cells from the subject using the bispecific affinity reagent PRIT approach described herein that incorporates a first binding domain that specifically binds to BCMA and/or Muc1.

5 GSI can be administered concurrently with or prior to the bispecific affinity reagent. In some embodiments, the bispecific affinity reagent comprises a first binding domain that specifically binds BCMA. In other embodiments, the bispecific affinity reagent comprises a first binding domain that specifically binds Muc1. In some
10 embodiments, the GSI enhancing agent is administered prior to the administration of the bispecific affinity reagent comprising a first binding domain that specifically binds BCMA or Muc1. For example, the GSI enhancing agent can be administered between about 1 hour to about 3 days prior to the administration of the bispecific affinity reagent comprising a first binding domain that specifically binds BCMA or Muc1, such as about
15 2, 5, 10, 15, 20, 24 hours before the administration of the bispecific affinity reagent comprising a first binding domain that specifically binds BCMA or Muc1. In other
embodiments, the GSI enhancing agent is administered about 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, or 3.5 days prior to the administration of the bispecific affinity reagent comprising a first binding domain that specifically binds BCMA or Muc1.

 GSI can be formulated for any appropriate routes of administration. In some
20 embodiments, the GSI is administered orally, intravenously, parentally, and the like.

 The GSI can be administered at any dose effective to induce upregulation of BCMA or Muc1 in target cells (e.g., MM cells). Exemplary, non-limiting doses include about 1 mg/kg to about 200 mg/kg, for example about 5 mg/kg, about 10 mg/kg, about
25 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 55 mg/kg, about 60 mg/kg, about 65 mg/kg, about 70 mg/kg, about 75 mg/kg, about 80 mg/kg, about 85 mg/kg, about 90 mg/kg, about 95 mg/kg, about 100 mg/kg, about 125 mg/kg, about 150 mg/kg, about 175 mg/kg, or about 200 mg/kg.

 In another embodiment, the enhancing agent can be a corticosteroid, such as
30 dexamethasone.

 To illustrate the general sequence of administration steps for certain embodiments, non-limiting embodiments for administration in the disclosed method include administering an enhancing agent, followed within about 24 hours (e.g., 6 to 30

hours) with administration of the bispecific affinity reagent, followed within about 24 hours (e.g., 6 to 48 hours) of administration of the CA, followed within about 2 hours (e.g., 30 minutes to about 3 hours) of the administration of the radioactive ligand.

Combination Therapies

5 The disclosure also encompasses methods that incorporate administration of two or more bispecific affinity reagents to target the hematological malignant or hyperproliferative cells in a subject for treatment by specifically targeting multiple cellular antigens.

10 The therapeutic strategies and affinity reagents described above can be combined together (or with a bispecific affinity reagent that can specifically bind CD20) to utilize (e.g., administer) two or more of the described bispecific affinity reagents in combination with separate administration of at least one radioactive ligand. The combination strategies can optionally include administering at least one clearing agent prior to administering the at least one radioactive ligand, as described in more detail above. The
15 combination strategies can also optionally include administering at least one enhancing agent to increase the expression of the target antigen in the malignant or hyperproliferative cells, as described in more detail above.

 Accordingly, the disclosure provides a method of treating a hematological malignancy in a subject. The method comprises administering to the subject a
20 therapeutically effective amount of a first bispecific affinity reagent and a therapeutically effective amount of a second bispecific affinity reagent. Thereafter, the method further comprises administering to the subject a therapeutically effective amount of a radioactive ligand. The first bispecific affinity reagent and the second bispecific affinity reagent each comprises a first binding domain that specifically binds to an antigen and a second
25 binding domain that specifically binds to the radioactive ligand. The antigen can be associated with a cancer or hyperproliferative disorder, such as a hematological malignancy or hyperproliferative disease as described herein. The first binding domain of the first bispecific affinity reagent and the first binding domain of the second bispecific affinity reagent specifically bind to different antigens selected from CD38, BCMA,
30 Muc1, GPRC5D, SlamF7, and CD20, as described herein.

 CD20 is a B cell specific surface antigen found on several transformed hematological cells, such as in non-Hodgkin lymphoma cells, and can serve as an additional target in combination therapies addressing hematological malignancies as

described herein. CD20 is also known as B1; MS4A1; S7; Bp35; CVID5; MS4A2; and LEU-16. CD20 remains an appealing antigen, however, due to its extensive clinical record as a successful immunotherapy target, as demonstrated in trials using rituximab, a monoclonal antibody targeting CD20 (Coiffier *et al.*, *N Engl J Med* 2002;346(4):235-42; Lenz *et al.*, *J Clin Oncol* 2005;23(9):1984-92; Marcus R, *et al.*, *J Clin Oncol* 2008;26(28):4579-86; Pfreundschuh *et al.*, *Lancet Oncol* 2011;12(11):1013-22).

The anti-CD20 bispecific affinity reagent of this aspect has the same structural design as the other bispecific affinity reagents, as described in more detail above. Briefly, the first binding domain can comprise any antibody or a fragment or derivative thereof that binds CD20. The anti-CD20 bispecific affinity reagent also comprises a second binding domain that specifically binds a radioactive ligand, as described in more detail above. Finally, the first and the second binding domains can be linked by a hinge region, as described above.

In some embodiments, the CD20 is a human CD20, such as represented by GenBank Gene ID: 931).

In certain embodiments, the first binding domain that specifically binds CD20 comprises a V_L region. For example, a V_L region in the first binding domain of the present disclosure is derived from or based on a V_L of a known monoclonal antibody that binds CD20 and may contain one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) insertions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) deletions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions (*e.g.*, conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_L of a known monoclonal antibody that binds CD20. An insertion, deletion, or substitution may be anywhere in the V_L region, including at the amino-terminus, carboxy-terminus, or both ends of the region, provided that each CDR comprises zero changes or at most one, two, three or four changes from a CDR of the V_L region of a known monoclonal antibody that binds CD20, and provided a binding domain containing the modified V_L region specifically binds its CD20 target with an affinity similar to the wild type binding domain. Similarly, in certain embodiments, the first binding domain that specifically binds CD20 comprises a V_H region. For example, a V_H region in the first binding domain of the present disclosure is derived from or based on a V_H of a known monoclonal antibody that binds CD20 and may contain one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) insertions, one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) deletions, one or more (*e.g.*, 2, 3,

4, 5, 6, 7, 8, 9, or 10) amino acid substitutions (*e.g.*, conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_H of a known monoclonal antibody that binds CD20. An insertion, deletion, or substitution may be anywhere in the V_H region, including at the amino-terminus, carboxy-terminus, or both ends of the region, provided that each CDR comprises zero changes or at most one, two, three or four changes from a CDR of the V_H region of a known monoclonal antibody that binds CD20, and provided a binding domain containing the modified V_H region specifically binds its CD20 target with an affinity similar to the wild type binding domain.

10 Anti-CD20 antibodies, or functional fragments or derivatives thereof can serve as the first binding domain. Exemplary anti-CD20 antibodies suitable for use in the therapeutic methods described herein include 2H7, 1.5.3, 1F5, Leu16, rituximab, ofatumumab, veltuzumab, and ocrelizumab.

15 In certain embodiments, a binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to an amino acid sequence of a light chain variable region (V_L); *e.g.*, to a V_L from 1.5.3 (SEQ ID NO.:39), 1F5 (SEQ ID NO.:41), Leu16 (SEQ ID NO.:40), rituximab, ofatumumab, veltuzumab, or ocrelizumab.

20 In further embodiments, a binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to an amino acid sequence of a heavy chain variable region (V_H); *e.g.*, to a V_H from 1.5.3 (SEQ ID NO.:42), 1F5 (SEQ ID NO.:44), Leu16 (SEQ ID NO.:43), rituximab, ofatumumab, veltuzumab, or ocrelizumab.

25 In still further embodiments, a binding domain comprises (a) an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to an amino acid sequence of a V_L ; *e.g.*, to a V_L from 1.5.3 (SEQ ID NO.:39), 1F5 (SEQ ID NO.:42), Leu16 (SEQ ID NO.:40), rituximab, ofatumumab, veltuzumab, or ocrelizumab; and (b) an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to an amino acid sequence of a V_H ; *e.g.*, to a

V_H from 1.5.3 (SEQ ID NO.:42), 1F5 (SEQ ID NO.:44), Leu16 (SEQ ID NO.:43), rituximab, ofatumumab, veltuzumab, or ocrelizumab. In any of the aforementioned embodiments, each CDR of the V_L, V_H, or both comprises zero changes or at most one, two, three, four, five or six changes, as compared to a parent monoclonal antibody or
5 fragment or derivative thereof that specifically binds to CD20, provided that a binding domain containing the modified V_L, V_H, or both region specifically binds CD20 with an affinity similar to the wild type binding domain.

In certain embodiments, a binding domain comprises an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least
10 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to an amino acid sequence of a scFv, *e.g.*, a scFv from an antibody of 1.5.3 (SEQ ID NO.:56), 1F5 (SEQ ID NO.:58), Leu16 (SEQ ID NO.:57), rituximab, ofatumumab, veltuzumab, or ocrelizumab, wherein each CDR of the scFv comprises zero changes or at most one, two, three, four, five or six changes, as compared to the corresponding CDR of a parent
15 monoclonal antibody or fragment or derivative thereof that specifically binds to CD20, provided that scFv containing one or more modified CDRs specifically binds CD20 with an affinity similar to the wild type scFv or corresponding antibody.

In certain embodiments, a binding domain is encoded by a polynucleotide that is at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least
20 95%, or 100% identical to a polynucleotide sequence encoding a light chain variable region (V_L); *e.g.*, to a V_L-encoding polynucleotide from 1.5.3 (SEQ ID NO.:48), 1F5 (SEQ ID NO.:50), Leu16 (SEQ ID NO.:49), rituximab, ofatumumab, veltuzumab, or ocrelizumab.

In further embodiments, a binding domain comprises a polynucleotide that is at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least
25 95%, or 100% identical to a polynucleotide sequence encoding a heavy chain variable region (V_H); *e.g.*, to a V_H-encoding polynucleotide from 1.5.3 (SEQ ID NO.:51), 1F5 (SEQ ID NO.:53), Leu16 (SEQ ID NO.:52), rituximab, ofatumumab, veltuzumab, or ocrelizumab.

30 In still further embodiments, a binding domain comprises (a) a polynucleotide that is at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to a polynucleotide sequence encoding a V_L; *e.g.*, to a V_L-encoding polynucleotide from 1.5.3 (SEQ ID NO.:48), 1F5 (SEQ ID NO.:50), Leu16

(SEQ ID NO.:49), rituximab, ofatumumab, veltuzumab, or ocrelizumab; and (b) a polynucleotide that is at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to a polynucleotide sequence encoding a V_H; *e.g.*, to a V_H-encoding polynucleotide from 1.5.3 (SEQ ID NO.:51), 1F5 (SEQ ID NO.:53), Leu16 (SEQ ID NO.:52), rituximab, ofatumumab, veltuzumab, or ocrelizumab. In any of the aforementioned embodiments, polynucleotides encoding each CDR of the V_L, V_H, or both comprises zero changes or at most one to six nucleotide changes, as compared to a polynucleotide encoding a parent monoclonal antibody or fragment or derivative thereof that specifically binds to CD20, provided that a binding domain containing the modified V_L, V_H, or both regions specifically binds CD20 with an affinity similar to the wild type binding domain.

In certain embodiments, a binding domain comprises a polynucleotide that is at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to a polynucleotide sequence encoding a scFv, *e.g.*, an encoded scFv comprising variable domains from an antibody of 1.5.3 (SEQ ID NO.:45), 1F5 (SEQ ID NO.:47), Leu16 (SEQ ID NO.:46), rituximab, ofatumumab, veltuzumab or ocrelizumab. In each of the aforementioned embodiments, polynucleotide sequences encoding each CDR of a scFv comprises zero changes or at most one to six nucleotide changes, as compared to a polynucleotide encoding a parent scFv from a monoclonal antibody that specifically binds to CD20, provided that scFv containing one or more modified CDRs specifically binds CD20 with an affinity similar to the wild type scFv or corresponding antibody.

In any of the embodiments described herein, a binding domain may consist, comprise, be based on or be derived from a V_H, a V_L, or both, from rituximab (*see, e.g.*, US 2014/0004037), ocrelizumab (*see, e.g.*, US 8,679,767), ofatumumab (*see, e.g.*, US 2009/0169550), or veltuzumab (*see, e.g.*, US 2009/0169550), the nucleotide and amino acid sequences of which are herein incorporated by reference in their entirety. Additionally, in any of the methods described herein, a CD20 binding molecule may comprise rituximab, ofatumumab, veltuzumab, or ocrelizumab, or any combination thereof.

For purposes of illustration only, a non-limiting example includes the anti-human CD20 antibody 2H7, or antigen binding domains thereof, as described in more detail in Green, D.J., et al., Comparative Analysis of Bispecific Antibody and Streptavidin-

Targeted Radioimmunotherapy for B-cell Cancers, *Cancer Res* 2016 76(22):6669-6679, incorporated herein by reference in its entirety. In some embodiments, the bispecific affinity reagent that specifically binds CD20 (via the first binding domain) is a fusion protein described in Green, D.J., et al., *Cancer Res* 2016.

5 Exemplary domains that bind to CD20 that can serve as (or part of) the first (anti-CD20) binding domain of the anti-CD20 bispecific affinity reagent have at least 80% sequence identity (e.g., about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to a CD20 binding domain included in an amino acid sequence of a bispecific affinity reagent as set forth in one of SEQ ID NO:12 and 14
10 (which are encoded by the nucleic acid sequences set forth in SEQ ID NOs:11 and 13, respectively).

In some embodiments, the anti-CD20 bispecific affinity reagent comprises an amino acid sequence as set forth in SEQ ID NOs:12 or 14 or comprises an amino acid sequence with at least about 80% identity (e.g., about 80%, 85%, 90%, 91%, 92%, 93%,
15 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to an amino acid sequence of as set forth in one of SEQ ID NO: 12 and 14. In some embodiments, the bispecific affinity reagent consists of or consists essentially of an amino acid sequence as set forth in SEQ ID NOs:12 or 14.

The first and second bispecific affinity reagents can be administered together in a
20 single formulation or separately in individual formulations. If administered separately, the first and second bispecific affinity reagents are typically administered within about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 24 hours of each other. Dosing and formulations are described above.

The method can further comprise administering an effective amount of a clearing agent (CA) after administering the bispecific affinity reagents and before administering the radioactive ligand. The CA is described herein. The selection of CA should be appropriate for the type of bispecific affinity reagent used in the first administration step such that the bispecific affinity reagents unbound to target cellular antigens are removed from circulation before the radioactive ligand is administered.

30 The at least one radioactive ligand is described above. In some embodiments, the second domains of the first and second bispecific affinity reagents each specifically bind the same radioactive ligand and, thus, only the single radioactive ligand is administered in a sufficient amount to allow the different cell-bound affinity reagents to bind radioactive

ligand. In other embodiments, the second binding domains of the first and second bispecific affinity reagents each specifically bind distinct radioactive ligands, in which embodiments both radioactive ligands are administered together or separately, but each in an amount such that each of the respective cell-bound first and second bispecific affinity reagents can also bind a radioactive ligand.

The hematological malignancy in this aspect directed to combination therapies can be a B cell malignancy or other B cell hyperproliferative disorder, such as MM, a lymphoma, or a leukemia. See discussion above, which applies to this aspect.

All pairwise combinations of anti-CD38, anti-BCMA, anti-Muc1, anti-GPRC5D, anti-SlamF7, and anti-CD20 bispecific affinity reagents are contemplated in this aspect of the disclosure.

In one embodiment of the method, the first binding domain of the first bispecific affinity reagent specifically binds CD38 and the first binding domain of the second bispecific affinity reagent specifically binds BCMA. In another embodiment, the first binding domain of the first bispecific affinity reagent specifically binds CD38 and the first binding domain of the second bispecific affinity reagent specifically binds Muc 1. In another embodiment, the first binding domain of the first bispecific affinity reagent specifically binds CD38 and the first binding domain of the second bispecific affinity reagent specifically binds SlamF7. In another embodiment, the first binding domain of the first bispecific affinity reagent specifically binds CD38 and the first binding domain of the second bispecific affinity reagent specifically binds GPRC5D. In another embodiment, the first binding domain of the first bispecific affinity reagent specifically binds CD38 and the first binding domain of the second bispecific affinity reagent specifically binds CD20. In a further embodiment, where the first binding domain of the first bispecific affinity reagent specifically binds CD38 and the first binding domain of the second bispecific affinity reagent specifically binds CD20, the hematological malignancy is a lymphoma. In a further embodiment, the lymphoma is an NHL.

In any of the above embodiments, wherein the first binding domain of the first bispecific affinity reagent specifically binds CD38, the method can also further comprise administering ATRA or a functional analog or derivative thereof in an amount sufficient to upregulate expression of CD38 in the malignant cells. More detailed descriptions of the ATRA enhancer, or functional analogs or derivatives thereof, and their use in connection with the steps of the method, are provided above and are equally applicable in

these embodiments of the combination method aspect. For example, in some instances, the ATRA or functional analog or derivative thereof can be in liposomal formulation and/or can be administered prior to administration of the two bispecific affinity reagents.

In one embodiment of the method, the first binding domain of the first bispecific
5 affinity reagent specifically binds BCMA and the first binding domain of the second
bispecific affinity reagent specifically binds Muc 1. In another embodiment, the first
binding domain of the first bispecific affinity reagent specifically binds BCMA and the
first binding domain of the second bispecific affinity reagent specifically binds SlamF7.
In another embodiment, the first binding domain of the first bispecific affinity reagent
10 specifically binds BCMA and the first binding domain of the second bispecific affinity
reagent specifically binds GPRC5D. In another embodiment, the first binding domain of
the first bispecific affinity reagent specifically binds BCMA and the first binding domain
of the second bispecific affinity reagent specifically binds CD20.

In one embodiment of the method, the first binding domain of the first bispecific
15 affinity reagent specifically binds Muc 1 and the first binding domain of the second
bispecific affinity reagent specifically binds SlamF7. In another embodiment, the first
binding domain of the first bispecific affinity reagent specifically binds Muc1 and the
first binding domain of the second bispecific affinity reagent specifically binds GPRC5D.
In another embodiment, the first binding domain of the first bispecific affinity reagent
20 specifically binds Muc 1 and the first binding domain of the second bispecific affinity
reagent specifically binds CD20.

In any of the above embodiments, wherein the first binding domain of the first
bispecific affinity reagent specifically binds BCMA or Muc1, the method can also further
comprise administering a GSI in an amount sufficient to upregulate expression of the
25 BCMA or Muc1 in the malignant cells. More detailed descriptions of the GSI enhancer
and its use in connection with the steps of the method, are provided above and are equally
applicable in these embodiments of the combination method aspect.

In one embodiment, the first binding domain of the first bispecific affinity reagent
specifically binds SlamF7 and the first binding domain of the second bispecific affinity
30 reagent specifically binds CD20. In another embodiment, the first binding domain of the
first bispecific affinity reagent specifically binds SlamF7 and the first binding domain of
the second bispecific affinity reagent specifically binds GPRC5D.

Exemplary Therapeutic Combinations

Illustrative, non-limiting embodiments of methods of this disclosure are described. Further definition for various elements is provided above.

In one embodiment, the disclosure provides a method of treating a malignancy characterized by expression of CD38. The method comprises administering to the subject
5 an amount of all trans retinoic acid (ATRA), or functional derivatives or analogs thereof, sufficient to upregulate expression of CD38 in the malignant cells, as described above. The method also comprises administering to the subject a therapeutically effective amount of a bispecific affinity reagent comprising a first binding domain that specifically binds to CD38 and a second binding domain that specifically binds to a radioactive
10 ligand. After administering the bispecific affinity reagent, the method comprises administering to the subject a therapeutically effective amount of a corresponding radioactive ligand.

In another embodiment, the disclosure provides a method of treating a malignancy characterized by expression of GPRC5D. The method comprises administering to the
15 subject an amount of ATRA, or functional derivatives or analogs thereof, sufficient to upregulate expression of GPRC5D in the malignant cells, as described above. The method also comprises administering to the subject a therapeutically effective amount of a bispecific affinity reagent comprising a first binding domain that specifically binds to GPRC5D and a second binding domain that specifically binds to a radioactive ligand.
20 After administering the bispecific affinity reagent, the method comprises administering to the subject a therapeutically effective amount of a corresponding radioactive ligand.

The ATRA or functional derivatives or analogs thereof for the above embodiments can be administered before or concurrently with the bispecific affinity reagent. In some embodiments, the ATRA or functional derivatives or analogs thereof is
25 administered prior to the bispecific affinity reagent by about 24 hours or less. In further embodiments, the ATRA or functional derivatives or subunits thereof are administered in a liposomal formulation.

In another embodiment, the disclosure provides a method of treating a malignancy characterized by expression of BCMA. The method comprises administering to the
30 subject an amount of gamma secretase inhibitor (GSI) sufficient to upregulate expression of BCMA in the malignant cells, as described above. The method also comprises administering to the subject a therapeutically effective amount of a bispecific affinity reagent comprising a first binding domain that specifically binds to BCMA and a second

binding domain that specifically binds to a radioactive ligand. The method comprises thereafter administering to the subject a therapeutically effective amount of a radioactive ligand.

5 The GSI can be administered before, concurrently with, or closely after (within up to 3 hours after) the bispecific affinity reagent. In some embodiments, the GSI is administered prior to the bispecific affinity reagent by about 24 hours or less.

In another embodiment, the disclosure provides a method of treating a malignancy characterized by expression of Muc1. The method comprises administering to the subject an amount of gamma secretase inhibitor (GSI) sufficient to upregulate expression of
10 Muc1 in the malignant cells, as described above. The method also comprises administering to the subject a therapeutically effective amount of a bispecific affinity reagent comprising a first binding domain that specifically binds to Muc1 and a second binding domain that specifically binds to a radioactive ligand. The method comprises thereafter administering to the subject a therapeutically effective amount of a radioactive
15 ligand.

In any of these illustrative embodiments, the methods can further comprise administering an effective amount of a clearing agent (CA) after administering the bispecific affinity reagent and before administering the radioactive ligand.

In any of these illustrative embodiments, the radioactive moiety comprises yttrium
20 DOTA.

In any of these illustrative embodiments, the affinity reagent is a fusion protein and the first binding domain and the second binding domain are separated by a hinge region. The hinge region can comprise a construct selected from an IgG1 Fc fragment, an IgG2 Fc fragment, and IgG3 Fc fragment, and an IgG4 Fc fragment. One or both of the
25 first binding domain and the second binding domain can be an antibody, a functional antibody fragment, functional antibody derivative, as described above. In some embodiments, one or both of the first binding domain and the second binding domain comprise a variable light chain domain and variable heavy chain domain. The variable light chain and variable heavy chain of the binding domain and/or the second binding are
30 separated by a linker domain. In specific embodiments, one or both of the first binding domain and the second binding domain is an scFv. In some embodiments, the scFv is humanized.

Unless specifically defined herein, all terms used herein have the same meaning as they would to one skilled in the art of the present invention. Practitioners are particularly directed to Sambrook J., et al. (eds.) *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Press, Plainsview, New York (2001); Ausubel, F.M., et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, New York (2010); and Coligan, J.E., et al. (eds.), *Current Protocols in Immunology*, John Wiley & Sons, New York (2010) for definitions and terms of art.

The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."

Following long-standing patent law, the words "a" and "an," when used in conjunction with the word "comprising" in the claims or specification, denotes one or more, unless specifically noted.

Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise," "comprising," and the like, are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to indicate, in the sense of "including, but not limited to." Words using the singular or plural number also include the plural and singular number, respectively. Additionally, the words "herein," "above," and "below," and words of similar import, when used in this application, shall refer to this application as a whole and not to any particular portions of the application. Unless stated otherwise, the term "about" implies minor variation around the stated value of no more than 10% (above or below), such as up to 10% variation above or below the reference sequence, up to 9% variation above or below the reference sequence, up to 8% variation above or below the reference sequence, up to 7% variation above or below the reference sequence, up to 6% variation above or below the reference sequence, up to 5% variation above or below the reference sequence, up to 4% variation above or below the reference sequence, up to 3% variation above or below the reference sequence, up to 2% variation above or below the reference sequence, or up to 1% variation above or below the reference sequence.

Disclosed are materials and compositions that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods and compositions. It is understood that, when combinations, subsets, interactions, groups, etc., of these materials are disclosed, each of various individual and collective

combinations is specifically contemplated, even though specific reference to each and every single combination and permutation of these compounds may not be explicitly disclosed. This concept applies to all aspects of this disclosure including, but not limited to, steps in the described methods. Thus, specific elements of any foregoing
5 embodiments can be combined or substituted for elements in other embodiments. For example, if there are a variety of additional steps that can be performed, it is understood that each of these additional steps can be performed with any specific method steps or combination of method steps of the disclosed methods, and that each such combination or subset of combinations is specifically contemplated and should be considered disclosed.
10 Additionally, it is understood that the embodiments described herein can be implemented using any suitable material such as those described elsewhere herein or as known in the art.

Publications cited herein and the subject matter for which they are cited are hereby specifically incorporated by reference in their entireties.

15 EXAMPLES

The following examples are provided for the purpose of illustrating, not limiting, the disclosure.

EXAMPLE 1

This Example describes the production and characterization of a new anti-CD38
20 bispecific protein that entirely removes biotin binding and SA from PRIT, while maintaining high CD38 binding affinity and high avidity for the radiolabeled second step. We also directly compare therapeutic efficacy of the new CD38-bispecific with the CD38-SA approach. Several PRIT technologies have been developed to facilitate avidity of the radiolabeled second step reagent for the pretargeted tumor-bound antibody. These
25 include the streptavidin-biotin and bispecific antibody approaches used here; complementary hybridization (Watson–Crick pairing) of phosphorodiamidate morpholino DNA oligomers; and cyclooctene-modified Abs binding to radiolabeled tetrazine ligands. While prior studies show that all PRIT methods are superior to single-step RIT, no head-to-head comparisons have examined which PRIT method is most promising for clinical
30 development of CD38 targeting. We present a comparative analysis of the biodistribution and therapeutic efficacy of the two most popular PRIT strategies. Our findings demonstrate that efficacy, reduced immunogenicity, and absence of interference from

endogenous biotin all make anti-CD38 bispecific PRIT an excellent candidate for clinical translation.

Methods

Construction of anti-CD38 bispecific Ab and DOTAY-Dextran clearing agent

5 Methods detailing the construction of the 028-Fc-C825 bispecific (anti-CD38 x anti-Y-DOTA) fusion gene, isolation of CHO cell clones stably expressing the 028-Fc-C825 bispecific Ab, purification of the bispecific Ab, and synthesis of the DOTAY-Dextran (DYD) clearing agent (CA) are described herein. Construction of the control, 2H7-Fc-C825 (anti-CD20 x anti-Y-DOTA) bispecific was described previously in Green
10 DJ, et al. Comparative Analysis of Bispecific Antibody and Streptavidin-Targeted Radioimmunotherapy for B-cell Cancers. *Cancer Res.*, 2016, incorporated herein by reference in its entirety.

1) Construction of the 028-Fc-C825 bispecific (Anti-CD38 x anti-Y-DOTA) fusion gene

15 A plasmid harboring a C825 ds-scFv gene, an affinity-improved 2D12.5 antibody, was generated (Orcutt KD, et al. Engineering an antibody with picomolar affinity to DOTA chelates of multiple radionuclides for pretargeted radioimmunotherapy and imaging. *Nucl Med Biol.* 2011;38(2):223-233; and Orcutt KD, et al. Effect of small-molecule-binding affinity on tumor uptake in vivo: a systematic study using a pretargeted
20 bispecific antibody. *Mol Cancer Ther.* 2012;11(6):1365-1372). Fragments of an anti-CD38 human antibody (028) variable regions (Vl and Vh) were obtained by PCR using a set of overlapped oligonucleotides designed based on published data by GenMab (Copenhagen, DK) (de weers MW, T.; et al., Antibodies against human CD38. In: Office UPaT ed. USPTO. Vol. US 2013/0209355 A1. United States: GENMAB A/S; 2013.).
25 The fragments were assembled using an scFv containing a 25-mer Gly4Ser linker between Vl and Vh to generated a plasmid Q100-3. The 028 scFv fragment (HindIII-XhoI) was prepared by PCR from Q100-3 using oligos YL835 (AGACCCAAGCTTGCCGCCATGGATTTTCAAGTGCAGATTT) (SEQ ID NO:37) and YL827 (TTTGGGCTCGAGTGAAGAGACGGTGACCATTGTCCC) (SEQ ID
30 NO:38) followed by restrictions with HindIII and XhoI. The fragment (HindIII-XhoI) was cloned into the expression vector O89-1-6 at the same sites resulting in an R6-1 construct carrying the 028-Fc-C825 bispecific anti-CD38 and the anti-Y-DOTA fusion gene.

2) Isolation of CHO cell clones stably expressing the 028-Fc-C825 bispecific Ab

R6-1 plasmid DNA was prepared using an endotoxin-free maxi preparation kit (#12362, QIAGEN). The plasmid DNA (250µg) was linearized with the AscI restriction enzyme at the 5' nonessential region of the CMV promoter. The DNA was purified by phenol extraction and NaOAc/ethanol precipitation. The linearized DNA was re-suspended in 400µl and maintained in logarithmic growth in Excell 302 complete medium (CM) supplemented with glutamine (4mM), pyruvate, recombinant insulin (#12585-014, Invitrogen) and penicillin-streptomycin including 1x HT supplement (#11067-030, Invitrogen). For each transfection 2×10^7 cells were harvested and resuspended in 400 µl complete medium with HT supplement. The AscI-linearized DNA was added to CHO cells in a total volume of 0.8ml and transferred into a cuvette (4mm gap) for electroporation using the Gene Pulser Xcell transfection apparatus (BioRad) at 280 volts, 950 microFarads. Transfected cells were incubated in non-selective media overnight and plated in 96-well flat bottom plates (Costar) at various dilutions. 50nM methotrexate (#045K1335, Sigma) without HT supplement. Plated cells were fed every five days until colonies appeared. Culture supernatants from master wells were screened for expression of fusion protein using an anti-human Fc sandwich ELISA. Clones with the highest expression of the bispecific fusion protein were expanded using progressively increasing concentrations of methotrexate, from 50-500nM. Supernatants were measured for Fc fusion protein expression using sandwich ELISA assay.

3) Expression and purification of 028-Fc-C825 bispecific Ab

Cells (10^7) of a high expressing clone, 38G11/500, were thawed, washed with RPMI-10% FBS medium, re-suspended in 10ml Excell 302 CM containing 50nM MTX (CM+MTX) in a T25 flask, and incubated at 37°C with 5% CO₂ overnight. Cells were pelleted and transferred to a T75 flask containing 30 ml CM+MTX, then expanded by passaging every 3-4 days in T175 flasks with 100 ml CM+MTX. Expanded cells were diluted into 40 T175 flasks with 100 ml per flask CM+MTX at a density of 1×10^5 cells/ml and incubated 14 days. Supernatants were collected and filtered through 0.22 µm Millipore PES membrane filter units. The pH of the supernatant was adjusted to 8.0 with 1M Na₂CO₃ and sodium azide was added to a final concentration of 0.1%. Conditioned supernatant was loaded on a 12-ml protein A-agarose (IPA 400HC crosslinked agarose) column (#10-2500-03, RepliGen Bio Processing) and washed with 10-column volumes of PBS (~120 ml) by gravity flow. The 028-Fc-C825 fusion protein

was eluted with 0.1M sodium citrate buffer at pH 3.6. Concentration of the eluted protein in each fraction (~1 ml size) was measured at 280nm using a Nanodrop spectrometer. Fractions containing the fusion protein were pooled and dialyzed against PBS overnight at room temperature. The final fusion protein was sterile filtered through 0.1 µm PVDF filter units and stored at 4°C.

4) Synthesis of a DOTAY-Dextran (DYD) clearing agent (CA) for the 028-Fc-C825 bispecific Ab

Amino dextran 500kD, 30.5mg, (Life Technologies) was reacted with 6.1 mg of DOTA-SCN (mw=697) in 6 mL DMSO with 11.4 µL of triethylamine overnight at room temperature, as previously described (Orcutt KD, et al., *Mol Cancer Ther.*, 2012;11(6):1365-1372). The mixture was then diluted with 84 mL 0.4 M sodium acetate pH 5.2, and 100 eq of yttrium nitrate (336 mgMW 383.01) was added and incubated overnight at 37°C with gentle tumbling, then concentrated in a Vivaspin 20 unit. The mixture was placed in a Slide-a-Lyzer and dialyzed against 2L of water for 3 days before drying on a Biotage evaporator. The dried material was dissolved in 2 mL PBS and passed over a BioRad EconoPak 10DG column, then again reduced to 2 mL on the evaporator and run over another 10 DG column in PBS. Dextran fractions were combined, dialyzed against water for 9 buffer changes over 5 days, again dried on the evaporator and exposed to high vacuum overnight (23 mg). The dried compound was weighed, re-suspended in saline at 4 mg/mL and sterile filtered.

Streptavidin-biotin pretargeting reagents

Conjugates of the OKT10 anti-CD38 monoclonal antibody and streptavidin were synthesized, purified and characterized as previously published. See, e.g., Press OW, et al., A comparative evaluation of conventional and pretargeted radioimmunotherapy of CD20-expressing lymphoma xenografts. *Blood*. 2001;98(8):2535-2543; and Pagel JM, et al. Comparison of anti-CD20 and anti-CD45 antibodies for conventional and pretargeted radioimmunotherapy of B-cell lymphomas. *Blood*. 2003;101(6):2340-2348. A synthetic, dendrimeric CA containing 16 *N*-acetylgalactosamine residues and a single biotin residue per molecule (NAGB) was obtained from Aletheon Pharmaceuticals (Seattle, WA) for use with the OKT10-SA conjugate.

Radiolabeling of DOTA-biotin with Yttrium-90

⁹⁰Y (PerkinElmer, Seattle, WA) labeling of DOTA-Biotin was performed using 12mg/mL DOTA-Biotin, 500 mmol/L ammonium acetate pH 5.3 and ⁹⁰Y heated for 60

minutes at 84°C. After cooling to room temperature, 100 mmol/L DTPA was added and labeling efficiency determined using avidin-agarose beads (Press OW, et al., *Blood*. 2001).

Cell culture

5 The human multiple myeloma cell lines H929, U266B1 and RPMI-8226 were obtained from the American Type Culture Collection (ATCC, Manassas, VA). These lines and the CD38⁺, CD20⁻ human Burkitt lymphoma (BL) cell line Namalwa were authenticated by DNA profiling (ATCC kit 135-XV), tested for mycoplasma, and maintained in log-phase growth at >95% viability (trypan-blue exclusion) in RPMI 1640
10 media supplemented with 10% fetal bovine serum, 50 U/ml penicillin G, and 50 µg/ml streptomycin sulfate, for no more than 6 weeks after thawing.

Flow cytometry-based bifunctional binding assay

Log-phase growth H929 cells (0.5 x 10⁶/group) were harvested and washed once with 1 ml of HBSS-2%FBS (HBSS) buffer. For CD38 blocking groups, cells were
15 resuspended in 40 µl of HBSS buffer containing 40 µg of daratumumab (Janssen R&D, Raritan, NJ) and incubated at 4°C for 30 min. Then 2 µg of bispecific fusion protein and 1 µg of Y-DOTA-biotin were added to all groups and cells incubated at 4°C for 30 min, followed by two washes. Recovered cells were resuspended in 40 µl of HBSS buffer containing 2 µl of PE-labeled streptavidin (#60669, AnaSpec Inc., Fremont CA),
20 incubated at 4°C for 30 min, washed three times, resuspended in 500 µl of PBS buffer containing 1% formaldehyde and analyzed on a Guava EasyCyte mini cytometer.

Mouse xenograft models

Female FoxN1Nu athymic nude mice (Envigo, Hayward, CA) were maintained under standard protocols approved by the FHCRC Institutional Animal Care and Use
25 Committee (IACUC). CD38⁺ tumor cells (10⁷) were injected subcutaneously in the right flank 7-11 days prior to experiments to produce 50-80 mm³ tumor xenografts. To attenuate tumor rejection due to natural killer cell activity, mice were injected intraperitoneally with anti-asialoGM1 antibody (986-10001, Wako, Richmond, VA) 1 day prior to tumor implantation, 5 days later and weekly thereafter. All mice were placed
30 on a biotin-deficient diet (23979, TestDiet, Richmond, IN) 7 days prior to PRIT studies.

Blood clearance and biodistribution studies

Groups of 3-5 mice bearing H929 flank tumors were injected via the tail vein (i.v.) with 2.8 nmol (210 to 420 µg) of 028-Fc-C825 (CD38 bispecific) or 2H7-Fc-C825

(CD20 control bispecific). Optimal Ab dosing was determined in prior experiments. Green DJ, et al. A preclinical model of CD38-pretargeted radioimmunotherapy for plasma cell malignancies. *Cancer Res.* 2014;74(4):1179-1189; Green DJ, et al. Comparative Analysis of Bispecific Antibody and Streptavidin-Targeted
5 Radioimmunotherapy for B-cell Cancers. *Cancer Res.* 2016. Twenty-three hours later mice were injected with 5 µg of DYD CA, followed 1 hour later by 1.2 nM (2µg) DOTA-biotin labeled with 20 to 40 µCi (0.74–1.48 MBq) of ⁹⁰Y. For blood clearance studies, retro-orbital blood sampling was performed at serial time points up to 24 hours after ⁹⁰Y injection. For biodistribution studies, blood samples, tumors, and body organs were
10 harvested at 6 to 120 hrs. ⁹⁰Y dose in each tissue sample was counted on a gamma counter and the percent of injected dose per gram (%ID/g) calculated. Hui TE, et al. A mouse model for calculating cross-organ beta doses from yttrium-90-labeled immunoconjugates. *Cancer.* 1994;73(3 Suppl):951-957.

Dosimetry: estimating absorbed doses of radioactivity in tissues

15 Mean absorbed doses to organs and tissues were calculated from the activity over time curves generated in biodistribution experiments. The calculations estimate organ self-dose plus cross-organ dose by accounting for organ mass, specific absorbed energy fraction, the emission spectrum of the radionuclides, and the beta particle absorbed fractions for small organs (Hui TE, et al., *Cancer.* 1994). The results were expressed as
20 radiation absorbed dose (gray) per unit administered activity (per millicurie).

Therapy studies

Therapeutic efficacy of CD38 bispecific and CD38-SA PRIT were assessed in groups of 8-10 mice (sample size determined by power analysis) bearing flank H929 or Namalwa xenografts. Mice were randomized into groups with equivalent mean tumor
25 volumes, and treatments administered as in biodistribution studies (above), with the addition of a CD38-SA (OKT10-SA) Ab treatment group that received 50 µg of NAGB as CA. All groups received second step DOTA-biotin labeled at 600, 1000 or 1200 µCi of ⁹⁰Y (22.2, 37 or 44.4 MBq). Tumor size and body weight were measured three times a week following treatment. Mice were euthanized when they experienced excessive
30 weight loss, hind limb paralysis, or exceeded tumor volume limits per IACUC requirements.

Statistical analyses

In murine xenograft studies, treatment effects on tumor growth rate were determined by first calculating tumor growth rate for each mouse as area under the curve (AUC) of tumor volume over time, standardized for number of days the mouse was alive. Treatment effects on standardized tumor AUC were then determined using analysis of variance. Treatment effects on mouse survival were determined by log-rank comparisons of Kaplan-Meier survival functions. Analyses were performed using JMP 12.2.0 (SAS Institute, Cary, NC) and GraphPad Prism 7 (GraphPad software, La Jolla, CA).

Results

Engineering, expression, purification and in vitro testing of the anti-CD38, 028-Fc-C825 bispecific protein

The 028-Fc-C825 bispecific antibody fusion protein, designed to recognize the CD38 surface antigen and the yttrium-DOTA ligand, was constructed by fusing DNA fragments encoding scFv of the 028 anti-CD38 human antibody and the ds-scFv of the affinity-enhanced C825 antibody to both sides of a human IgG1 Fc containing a NLG linker (FIGURE 1A). CHO-DG44 cells were transfected with the fusion construct DNA and high-expressing clones were selected using methotrexate. The monomeric bispecific protein spontaneously dimerized to form a 160-KDa molecule, which was purified from culture supernatants by affinity chromatography and characterized by SDS-PAGE (FIGURE 1B) ELISA showed that the bispecific captured the Y-DOTA ligand in a concentration-dependent fashion (FIGURE 1C). Bifunctional flow cytometry analysis demonstrated that the bispecific bound only to cells expressing CD38, that binding was blocked by unmodified anti-CD38 monoclonal antibody (mAb) binding the same epitope, and that the bispecific captured the Y-DOTA ligand (FIGURES 1D, 1E). The anti CD38 mAb OKT10 did not block cell binding of the CD38 bispecific, indicating that the binding epitopes did not overlap (FIGURE 8).

In vivo pharmacokinetics (PK) and blood clearance of the CD38 bispecific protein

PK analysis of the CD38 bispecific protein required our standard, 2-step PRIT protocol, because direct radioiodination of the bispecific protein impaired binding. For PRIT, mice were injected first with the unlabeled protein, followed 23 hours later with CA, then one hour later with ^{90}Y -DOTA-Biotin. Blood samples were taken at 5 min, 30 min, and 1, 4 and 24 hrs after the ^{90}Y injection. FIGURE 2 demonstrates that without CA, blood clearance of ^{90}Y was 72.8% ID/g after 30min and 86.8% after 24hr. With 5 μg CA, clearance was 97.8% ID/g at 30 min and 99.3% after 24hr. We additionally tested

increasing doses of CA; all doses produced virtually identical results (e.g., clearance using 32 µg CA was 98.2% IG/g at 30 min and 99.8% after 24hr, data not shown). We therefore used the 5 µg dose for further experiments.

5 *In vivo* biodistributions of radioactivity demonstrate favorable tumor targeting and retention using CD38 bispecific PRIT

Biodistributions of radioactivity in blood, tumors (H929 xenografts), and normal organs were compared between CD38 bispecific PRIT and control (CD20-targeted) bispecific PRIT (FIGURE 3A). Tumor bearing mice were injected at -24 hr with unlabeled protein, at -1 hr with CA, then at 0hr with ⁹⁰Y-DOTA-Biotin. Assessed 24 hrs after ⁹⁰Y-DOTA-Biotin injection, tumor-to-normal tissue ratios of absorbed radiation were 16:1 for blood, 14:1 for lung, 12:1 for liver and 19:1 for kidney for the CD38 bispecific group (FIGURE 3A). For the control bispecific group, ratios were <2:1 for the same tissues (FIGURE 3A). We also evaluated radioactivity biodistributions over time, taking tissues 6, 24, 48 and 120 hrs after ⁹⁰Y-DOTA-Biotin injections. These studies confirmed the tumor specificity of CD38 bispecific PRIT, and further demonstrated high retention of radiation in tumors over time (FIGURE 3B). Control bispecific PRIT showed no tumor targeting or retention (FIGURE 3C).

Dosimetry

We estimated radiation-absorbed doses to tumors, whole body, and 10 normal tissues from time-activity curves generated in CD38 bispecific biodistribution experiments, using a dosimetry method that calculates both organ self-dose absorbed fractions and β-particle cross-organ dose contributions, per unit of administered activity (FIGURES 3B and 4) (see, e.g., Hui TE, et al. *Cancer*. 1994). Absorbed radiation dose to tumor was 43.8 Gy/mCi, and tumor-to-normal organ ratios of absorbed dose were 51:1 for the femur, 23:1 for the whole body, 15.4:1 for the lung, 15:1 for the kidneys, 10:1 for the blood, and 7.4:1 for the liver (FIGURE 4).

Therapy studies

We studied the efficacy of CD38 bispecific PRIT in two xenograft models, and found that optimized PRIT dosing cured at least 75% of mice in every experiment. Athymic mice (n = 8-10 per group) bearing H929 MM or Namalwa NHL xenografts were injected first with cold Ab, then 23 hrs later with CA, and then in one more hour with ⁹⁰Y-DOTA-Biotin. Previous PRIT studies by our group indicated that 1200 µCi of ⁹⁰Y provides the most favorable therapeutic ratio, and we first examined efficacy of the CD38

bispecific Ab at 1200 μ Ci. In the H929 model, treatment with CD38 bispecific PRIT dramatically reduced tumor growth (FIGURE 5A) and increased survival (FIGURE 5B) relative to treatment with bispecific control PRIT (1200 μ Ci) and untreated control groups ($p \leq 0.0002$, CD38 bispecific vs. either control). In total, 19 of 20 mice in the 2 control groups died of tumor progression within 30 days (1 untreated mouse showed spontaneous tumor regression), while in the CD38 bispecific group 8 mice sustained complete remissions through the end of the study (150 days) and 2 died of tumor progression, both after day 50. In a second CD38⁺ tumor xenograft model (Namalwa), we added head-to-head comparisons of the CD38 bispecific Ab versus the CD38-SA (OKT10-SA) Ab, each labeled with 1200 μ Ci of ⁹⁰Y. In this experiment, both CD38 treatments resulted in 100% CRs by day 21, followed by one subsequent relapse in the CD38-SA group and zero relapses in the CD38 bispecific group (FIGURES 6A and 6B). Thus at this dose the two CD38 treatments reduced tumor growth and increased survival with high and equivalent efficacy ($p < 0.0001$ for either CD38 treatment vs. either control, $p = 0.48$ for CD38 bispecific vs. CD38-SA). To further characterize the dose-response relationship of CD38 PRIT, we also evaluated 600 and 1000 μ Ci of ⁹⁰Y pretargeted to xenograft tumors. These reduced dose experiments compared CD38 bispecific PRIT versus CD38-SA PRIT, and both anti-CD38 systems reduced tumor growth (FIG 7A) while improving survival (FIG 7B) in a marginally dose dependent manner ($p < 0.062$, PRIT 600 μ Ci vs. PRIT 1000 μ Ci). Importantly, at these reduced doses the CD38 bispecific Ab strongly outperformed CD38-SA, resulting in long-term survival of 61% versus 22%, respectively, with 600 μ Ci, and 78% versus 47%, respectively, with 1000 μ Ci ($p < 0.004$, CD38 bispecific vs. CD38-SA). In contrast, all controls, including mice treated with cold (no radiolabel) CD38 bispecific, cold CD38-SA, or control bispecific at 1000 μ Ci, died of tumor progression by day 15 (FIGURES 7A and 7B, $p < 0.0001$, any CD38 PRIT group vs. any control group).

Toxicity

PRIT using all Abs was well tolerated, with minimal weight loss and recovery to starting weight within 14 days of treatment (FIGURES 9A-9C). Over all studies (FIGURES 5A-7B), 2 of 18 mice treated with CD38 bispecific at 1200 μ Ci and 2 of 18 treated with CD38 bispecific at 1000 μ Ci died due to low body weight before day 18. All other deaths in all groups resulted from tumor progression. Anecdotal data suggests that early weight loss and mortality may be ameliorated as follows. Between our final two studies we implemented husbandry changes to reduce external radiation exposure from

cage mates and bedding; mortality before the changes was 2 of 8 mice and mortality after was 0 of 10 mice for 1000 uCi CD38 bispecific treatments (groups combined in FIGURES 7A and 7B).

Discussion

5 Combinations of immunomodulatory and proteasome inhibitor based therapies frequently induce remission in MM patients, but relapse is nearly inevitable and the need for development of potentially curative treatments remains critical. Our results demonstrate 75-88% cure rates using PRIT in two CD38 expressing murine xenograft tumor models. These results are consistent with the steep dose-response relationship
10 between radiation and hematologic malignancies. In MM, external beam radiation can cure isolated plasmacytomas and provide sustained local disease control in 98% of lesions receiving >10 Gy *and* in follicular non-Hodgkin lymphoma, another generally incurable B cell malignancy, external beam radiation can also eradicate disease that is limited to a single site of involvement.

15 RIT offers a delivery model designed to parlay the unique anti-tumor potency of radiation demonstrated in localized disease to a broader population of patients with multi-focal disease by sparing healthy tissues while delivering a targeted radiation payload directly to malignant cells. Single-step RIT has demonstrated some promise in MM, yet clinical applications have been very limited. Similar to CD20 single-step RIT, enthusiasm
20 may be limited by low tumor-to-normal ratios (e.g. <2.4:1 for kidney, lung and liver in NHL). PRIT methods can greatly improve these therapeutic ratios. In MM models, we previously demonstrated that CD38-SA PRIT provides tumor-to-normal tissue dosimetry ratios of 6:1 for kidney, lung, and liver, and here show that CD38 bispecific PRIT provides ratios of 15:1 for kidney and lung and 7:1 for liver. To our knowledge, we have
25 performed the only studies of PRIT in MM.

Recent clinical successes with unmodified anti-CD38 Mab therapy in MM have motivated ongoing clinical studies of this therapy in B-cell NHL. High density CD38 expression is a common feature of malignant B cells and the predictable growth kinetics associated with NHL cell lines have led to their frequent use in research models for the
30 development of CD38 targeted MM therapy. Despite advances in the management of NHL, nearly 30% of these patients die of progressive disease within 5 years of diagnosis. As in MM, treatment-refractory NHL typically retains sensitivity to radiation, making CD38 PRIT a potentially effective treatment for such patients as well. Beyond tumors

that share a common B-cell lineage, CD38 is expressed in most natural killer (NK)/T-cell lymphomas, where 50% of patients die within 5 years and overexpression of CD38 predicts poor outcomes for NK/T-cell lymphoma patients, presenting another potential translational application for CD38 PRIT. A broad range of potential indications for CD38 PRIT increases the probability of successful translation into a commercially viable radioimmunotherapeutic. To further evaluate CD38 as a target for RIT, we are conducting a clinical trial using a CD38 mAb directly conjugated to the alpha-emitter astatine-211 (single-step RIT). We are also developing bispecific fusion protein constructs for alpha emitter delivery which will facilitate head-to-head comparisons of alpha and beta emitter PRIT.

While the preclinical efficacy of PRIT appears clear, concerns have been raised regarding clinical translation. SA is a bacterial protein, and PRIT using SA results in immunogenicity and thus limits the ability to administer multiple cycles of therapy, although two factors may mitigate this concern. First, the immunocompromised status of many patients with hematological malignancies limits immunogenic responses, and second, PRIT is designed to be efficacious following a single dose, as demonstrated in both this manuscript and our previous studies. To reduce these concerns, several methods of modifying SA have been developed, but approaches that eliminate SA would obviate the issue entirely. To achieve this we developed the CD38 bispecific fusion protein, which exploits the same principles as the SA-biotin system, but replaces SA with a humanized yttrium-DOTA capturing C825 disulfide-stabilized scFv (FIGURE 1A). A bispecific construct harboring the same anti-yttrium-DOTA (C825) in an immunocompetent murine model (Orozco JJ. et al, unpublished) has demonstrated no evidence of toxicity. Reduced immunogenicity may allow for repeat dosing of CD38 bispecific PRIT, and fractionation may offer the opportunity to improve the therapeutic ratio in clinical settings. Our data here however suggest that PRIT is effective and well tolerated as a single-dose therapeutic.

A second concern for SA-PRIT is the potential for endogenous biotin in patient blood and tissues to occupy and block SA binding sites, preventing subsequent binding of the second-step radio-DOTA-biotin reagents. The bispecific approach obviates this concern, as binding of the C825 portion of the bispecific to the DOTA portion of the second-step reagent precludes any possible interference from endogenous biotin.

Our data suggest that bispecific PRIT has superior efficacy when compared with CD38-SA PRIT (FIGURES 7A and 7B). The anti-CD38 region of the bispecific protein binds a different epitope of CD38 than the OKT10-SA protein, as demonstrated by the absence of OKT10 mAb blocking of the bispecific CD38 in cell binding assays (FIGURE 8). The binding of separate epitopes raises the possibility that the first step CD38 targeting constructs have differing therapeutic effects, but we demonstrated that neither construct has anti-tumor efficacy when administered without radiolabeling (FIGURES 7A and 7B). Alternatively the therapeutic differences may be a consequence of differing *in vivo* binding efficiencies. The bispecific and SA treatments were designed such that all mice received an equivalent injected protein dose carrying an identical radiolabel dose, but subsequent tumor binding might differ for two reasons. First, it is feasible that CD38-SA binding, and thus PRIT efficacy, was reduced by very low level blocking by residual endogenous biotin in mouse tissues, despite institution of a biotin-free diet. Second, differences in antibody-ligand interaction may be a function of each construct's avidity for its respective epitope, resulting in kinetics that may favor the bispecific.

In conclusion, we have characterized a new CD38 bispecific targeting protein for PRIT of MM and NHL. The bispecific Ab is relatively easy to produce, exhibits excellent blood clearance using an inexpensive clearing agent, and shows excellent tumor-to-normal organ ratios of absorbed activity reflected by favorable dosimetry in murine models. Moreover, the CD38 bispecific rapidly reduced disease burden, and at optimal doses ultimately cured 75-80% of mice in each of two xenograft models with minimal toxicity. CD38-SA was equally effective at the highest radiation dose, but the bispecific's superiority over a range of doses, combined with its reduced risk of immunogenicity and lack of endogenous biotin interference, make the CD38 bispecific a prime candidate for clinical translation.

EXAMPLE 2

This Example describes an expansion of the findings of Example 1 with the development of a bispecific protein that uses an alternative cancer marker, B cell maturation antigen (BCMA) as the targeting antigen.

As described above, multiple myeloma (MM) is generally incurable and autologous stem cell transplantation (ASCT) remains a standard of care approach to disease management, yet no modification to ASCT conditioning has substantively

improved transplant outcome in over 25 years. In Example 1, development of an anti-CD38 bispecific fusion protein is described that resulted in 100% complete remissions (CR) by day 12 in preclinical MM xenograft models, ultimately curing 80% of mice at optimal doses. The high efficacy of bispecific PRIT, combined with its reduced risk of immunogenicity and endogenous biotin interference, make this bispecific approach a very attractive candidate for clinical translation in both transplant, and ultimately non-transplant settings. This Example addresses development of additional bi-specific compositions useful for PRIT that target other cancer antigens to optimize the bispecific PRIT strategy for MM. The project is intended to (1) optimize a B cell maturation antigen (BCMA) Bi-specific FP construct, evaluate biodistribution and therapeutic efficacy in a mouse model, and demonstrate that upregulation of the target further enhances the efficacy of the BCMA FP.

Background

The vast majority of the 130,000 patients in the United States living with multiple myeloma (MM) will ultimately die of progressive disease despite high rates of initial response to novel agents. Disease recurrence is presumably a function of malignant plasma cell clones that persist in spite of available therapies. Novel anti-myeloma agents introduced over the past decade have made complete response (CR) possible in a significant subset of patients. Unfortunately, almost all of these individuals ultimately relapse. High dose chemotherapy followed by ASCT increases CR rates and prolongs disease free survival, but relapse remains virtually inevitable and recurrence remains a major shortcoming of all available treatment strategies. The development of bispecific pretargeted radioimmunotherapy to improve outcomes after high dose therapy and ASCT represents an entirely novel approach to the management of MM. ASCT is a standard of care for eligible MM patients, yet over the past two decades there has been no modification to high dose therapy conditioning regimens that has improved post-transplant outcome. Despite the widespread administration of "novel agents", the numbers of ASCTs for MM continues to increase every year, emphasizing the importance of transplantation. The radiosensitivity of malignant plasma cells outside of the bone marrow has been well documented in clinical settings. Local recurrence of solitary extramedullary plasmacytomas occurs in less than 10% of cases after external beam radiation alone. Radiation therapy is also effective as a palliative measure in patients experiencing pain or other sequelae resulting from MM-induced osteolysis. Sustained

local disease control and durable symptom relief has been reported for 98% of lesions receiving >10Gy. Steep dose response relationships have been demonstrated for most hematological malignancies, and the impact of radiation dose escalation may be of particular importance in the case of MM. Further, the poor prognosis associated with high risk bone marrow cytogenetics in active MM are not predictive of a decrement in the very high rates of local control and cure after external beam radiation therapy is used to treat solitary extramedullary plasmacytomas with the same cytogenetic derangements. This suggests that the unique attributes associated with the targeted delivery of radiation may augur clinical efficacy even among patients classified as "high risk".

Radioimmunotherapy (RIT) selectively delivers radiation to target cells at multifocal disease sites and facilitates escalation to radiation doses not achievable through external beam therapy. The efficacy of RIT in the treatment of hematologic malignancies is well established. RIT has successfully been integrated into stem cell transplant conditioning regimens with a significant improvement in PFS and OS among patients with B cell lymphoma and acute myelogenous leukemia. A limited number of radionuclide based therapies have been explored in the clinical treatment of MM. While each of these radionuclide based approaches has theoretical promise, none have directly targeted radiation to the CD38 antigen on MM cells. We have demonstrated striking therapeutic efficacy with anti-CD38 (OKT10) pretargeted radioimmunotherapy (PRIT) using the β -emitter ^{90}Y directed against MM cells. Objective remissions were observed within 7 days in 100% of the mice treated with 800 μCi to 1200 μCi of anti-CD38 pretargeted ^{90}Y -DOTA-biotin, including 100% complete remissions (no detectable tumor in treated mice compared to tumors that were $2982 \pm 1002\%$ of initial tumor volume in control animals) by day 23. Furthermore, 100% of animals bearing H929 MM tumor xenografts achieved longterm myeloma-free survival (>70 days) after an optimal radiation dose, compared to none (0%) of the control animals.

As described in Example 1, a new PRIT approach was developed for the treatment of MM. Specifically an anti-CD38 bispecific fusion protein was developed that eliminates endogenous biotin interference and immunogenic elements. In murine xenograft models of MM, the CD38 bispecific construct demonstrated excellent blood clearance and tumor targeting. In therapy studies, CD38 bispecific PRIT resulted in 100% complete remissions (CR) by day 12 in MM and NHL xenograft models, ultimately curing 80% of mice at optimal doses. In direct comparisons, efficacy of the CD38

bispecific proved equal or superior to streptavidin (SA)-biotin-based CD38-SA PRIT. Each approach cured at least 75% of mice at the highest radiation dose tested (1200 μ Ci), while at 600 and 1000 μ Ci doses the bispecific outperformed the SA approach, curing 35% more mice overall ($p < 0.004$). The high efficacy of bispecific PRIT, combined with its reduced risk of immunogenicity and endogenous biotin interference, make the CD38 bispecific an attractive candidate for clinical translation. Critically, CD38 PRIT may benefit patients with unresponsive, high-risk disease, because refractory disease typically retains radiation sensitivity. We posit that PRIT might not only prolong survival, but possibly cure MM. Moreover, we have demonstrated that the anti-CD38 bispecific fusion protein is very effective in eliminating disease in non-Hodgkin lymphoma (NHL) tumor models and commercialization of this approach offers potential therapeutic application in the management of NHL. The bispecific CD38 construct has been vetted in our preclinical models and is ready for comprehensive characterization and scale up to support IND submission.

15 **Approach**

To provide an alternative target for therapeutic application of the bispecific PRIT approach, another bispecific fusion molecule was generated that targeted B cell maturation antigen (BCMA) instead of CD38. Exemplary proteins that specifically bind to BCMA, their active binding sites, and representative polynucleotides encoding the BCMA-specific binding proteins are described in more detail in U.S. Provisional Application No. 62/460612, incorporated herein by reference in its entirety.

Pre-clinical data using BCMA bispecific construct demonstrates efficacy and represents an alternative path to commercialization. Both BCMA and CD38 agents hold significant translational promise. Whereas the restricted nature of BCMA expression allows theoretical advantage for commercial translation specifically in MM, the CD38 bispecific molecule may have broader application in other B cell malignancies.

Antigen targeting in multiple myeloma

An extensive analysis of potential MM surface antigen targets for RIT led to the selection of CD38 and BCMA based consistency of expression on clonal plasma cells and other favorable features. We evaluated BCMA expression in bone marrow obtained from 50 consecutive MM patients at various times prior to, during, and following therapy. We detected variable levels of BMCA on MM cells in 100% of cases, a finding confirmed by others. As described above, several PRIT methods have been developed, each has been

shown to be markedly superior to conventional RIT with directly radiolabeled antibodies. All of these strategies administer a derivatized tumor-reactive antibody in a non-radioactive form, allowing it to localize to tumor sites and accumulate without subjecting the rest of the body to nonspecific irradiation. After maximal accumulation of antibody in the tumor, a low molecular weight radioactive moiety with a high affinity for the derivatized tumor-reactive antibody is administered. The small size of the second reagent facilitates rapid tumor penetration, capture and retention by the pre-targeted antibody. Unbound molecules of the radioactive second reagent are so small that they are rapidly cleared from the blood and excreted in the urine. In some PRIT approaches, a "clearing agent" (CA) is injected shortly before the radiolabeled small molecule to accelerate removal of residual unbound antibody from the bloodstream, preventing it from complexing with the radiolabeled second step reagent. Our findings to date suggest biodistribution of radioactivity favors the bispecific antibody approach. We have demonstrated that both streptavidin-biotin and bispecific antibody based PRIT methods are capable of curing 70-100% of animals bearing tumor xenografts when used under optimal conditions, but the expected reduced immunogenicity and absence of potential interference from endogenous biotin-blocking argue in favor of the bispecific antibody approach over SA-biotin PRIT for future clinical trials. In addition, we have demonstrated that the bispecific antibody construct appears to produce less hematologic toxicity than SA-biotin PRIT when studied in a lymphoma model.

Results

Construction of bispecific 028-Fc-C825 (Anti-CD38 x anti-Y-DOTA) and C11-Fc-C825 (Anti-BCMA x anti-Y-DOTA) fusion genes

pDG mammalian expression vectors containing anti-CD38 028-hIgG1-hRNase and anti-BCMA C11-hIgG1-hRNase genes under the control of CMV promoters were generated. A plasmid harboring a C825 ds-scFv gene, an affinity-improved 2D12.5 antibody, was generated in the Wittrup lab (MIT). (Orcutt KD, et al. Engineering an antibody with picomolar affinity to DOTA chelates of multiple radionuclides for pretargeted radioimmunotherapy and imaging. *Nuclear medicine and biology*. 2011;38(2):223-233; and Orcutt KD, et al., Effect of small-molecule-binding affinity on tumor uptake *in vivo*: a systematic study using a pretargeted bispecific antibody. *Molecular cancer therapeutics*. 2012;11(6):1365-1372, both incorporated herein by reference in their entireties). A C825 ds-scFv fragment was obtained by PCR from its

template plasmid and cloned into a TOPO TA vector (Invitrogen) to generate the O86-2 plasmid. An EcoRV-XbaI fragment was excised from the plasmid O86-2 containing the C825 ds-scFv gene and cloned into the plasmid O22-3-9 at EcoRI-XbaI sites resulting in an O89-1-6 construct carrying either the 028-Fc-C825 bispecific anti-CD38 or C11-Fc-C825 bispecific anti-BCMA (encoding the amino acid sequence set forth in SEQ ID NO:19) and the anti-Y-DOTA Fc-fusion gene (FIGURE 1A).

Cell binding analysis of antibody constructs

H929 cells (0.5×10^6 each) were incubated in 100 μ l of HBSS buffer containing 2% FBS and treated with 1.8 μ g of the bispecific FP for 30 min at 4°C. After washing, the cells were mixed with 2 μ l of PE-anti-human Fc antibody in 40 μ l of HBSS-2% FBS buffer for 30 min at 4°C. Washed, resuspended and analyzed on a Guava cytometer (FIGURES 10B and 10C). ^{90}Y -DOTA capture by C825 binding domain was demonstrated using a sandwich ELISA assay in which a 96-well plate was coated with 70 μ l of the BSA-Y-DOTA conjugate (1 μ g/ml in PBS) and then blocked with 200 μ l of 2% BSA in PBS buffer. After washing, the wells were treated with 100 μ l of fusion proteins at a concentration of 16 μ g/ml followed by serial dilution as indicated. The plate was further treated with HRP-anti-human Fc antibody followed by TMB. A control fusion protein shows no binding to Y-DOTA (FIGURE 10A).

Therapy studies

We have assessed the therapeutic efficacy of ^{90}Y -PRIT using bispecific PRIT methods, groups of 10 mice bearing flank H929 MM xenograft tumors received 2.8 nmol of 028-Fc-C825 or the nonbinding, negative control 2H7-FC-C825 followed by 5 μ g DYD 23 hours later. A single dose of 1.2 nmol of DOTA-biotin labeled with 1200 μ Ci ^{90}Y was administered 1 hour after the CA. Mice were assessed every 2 days for tumor volume measurements, weight changes, and general appearance. Mice were euthanized if xenografts exceeded 1200 mm³, caused obvious discomfort or impaired ambulation, or if mice lost more than 30% of their baseline body weight. The treatment was very well tolerated and the efficacy of the bispecific approach is evident (FIGURE 10D).

Gamma Secretase Inhibitors (GSI) upregulate BCMA expression on malignant plasma cells

The effect of GSI on BCMA expression was evaluated by incubating four MM cell lines (RPMI8226, U266B1, MM1.R, and H929) and primary MM samples in media containing 0 (DMSO), 0.01, 0.1, and 1.0 μ M of GSI and measuring surface BCMA at 5

hr. Exposure to the drug at these concentrations did not affect cell viability and we observed a marked increase in the MFI of BCMA staining maximal at the 1.0 μ M concentration in all 4 cell lines (FIGURES 11A and 11B) and in primary MM cells (FIGURES 11C and 11D). These concentrations are 3-30 fold below the serum levels that are achieved by well tolerated oral dosing regimens of GSI in phase 1 trials in cancer patients. A time course revealed upregulation of BCMA occurred within 1 hr of GSI exposure with the peak MFI of BCMA staining at 5 to 24 hrs. Consistent with the upregulation of surface BCMA, we also observed a decline in soluble BCMA levels at 0.1 and 1.0 μ M of GIS.

Methods and Strategies for Additional Studies

Biodistribution studies

Groups of 3-5 mice with similar-sized tumors receive 2.8 nmol of C11-Fc-C825 or 2H7-Fc-C825 (isotype control) reagents. Twenty-three hours later, mice receive 5 μ g of DYD CA, followed 1 hour later by 1.2 nmol DOTA-biotin labeled with 20 to 40 μ Ci (0.74–1.48 MBq) of ^{90}Y . Blood samples, tumors, and body organs are obtained and ^{90}Y activity are measured using a calibrated system and demonstrate the specificity of the approach.

In vivo targeting of MM xenografts

Biodistribution studies are conducted in xenografted mice to compare the BCMA bispecific construct with CD38 bispecific PRIT. Mice are injected IV with saline, anti-CD38 bispecific FPs, or control bispecific reagents. After 20 h, clearing agent (CA) is administered at optimal doses for each construct (Table 1), followed 2 h later by the appropriate radiolabeled metal chelate (e.g., ^{90}Y -DOTA or ^{90}Y -DOTA-biotin). A DOTAY-Dextran clearing agent is used instead (Orcutt KD, et al., *Molecular cancer therapeutics*. 2012;11(6):1365-1372, incorporated herein by reference in its entirety). First-step reagents are trace-labeled with ^{125}I to assess their content in tumors and organs independent of the ^{90}Y -chelate using a double label method (Pressman D. *Radiolabeled antibodies*. Ann NY Acad Sci. 1957;69:644-650, incorporated herein by reference in its entirety). Groups of 5 mice each are bled from the retro-orbital venous plexus 1, 4, 24, 48, and 120 h after ^{90}Y -ligand administration, euthanized, and tumors and normal organs excised, weighed, and γ counted for ^{125}I and ^{90}Y (correcting for ^{90}Y crossover into the ^{125}I channel). Each experiment is optimally performed at least 3 times (using H929, L363 and RPMI 8226).

Pharmacokinetics

PK data are analyzed and best-fit curves plotted using Win-NonLin (Pharsight) to determine serum $t_{1/2}$, volume of distribution, blood clearance, etc. The modeling capabilities of Win-NonLin guide decisions to modulate reagent doses and time intervals, if deemed advisable.

Radiation dosimetry

Absorbed doses to organs and tumors are estimated for 400, 800, and 1200 μCi of administered ^{90}Y activity are calculated for each preselected organ or tissue and time-activity curves are constructed for serially sacrificed mice to determine the total number of nuclear transformations to infinity (proportional to the area under the biodistribution curves). (Fisher DR, et al. Energy Distribution and the Relative Biological Effects of Internal Alpha Emitters. *Rad Prot Dosimetry*. 1985;13:223-227; Humm JL, et al. Internal dosimetry using data derived from autoradiographs. *Journal of nuclear medicine*. 1993;34(10):1811-1817; Sgouros G, et al. MIRD Pamphlet No. 22 (abridged): radiobiology and dosimetry of alphaparticle emitters for targeted radionuclide therapy. *J Nucl Med*. 2010;51(2):311-328; Sgouros G, et al. Modelling and dosimetry for alpha-particle therapy. *Current radiopharmaceuticals*. 2011;4(3):261-265; each of which is incorporated herein by reference in its entirety). The mathematical approach β -emitter dosimetry in animal tissues is well established. Fisher DR, et al. *Rad Prot Dosimetry*. 1985;13:223-227; Fisher DR and Harty R. The microdosimetry of lymphocytes irradiated by alpha-particles. *Int J Radiat Biol Relat Stud Phys Chem Med*. 1982;41(3):315-324; Fisher DR. The Microdosimetry of Monoclonal Antibodies Labeled with Alpha Emitters. Oak Ridge, Tennessee: Oak Ridge Associated Universities; 1986; each of which is incorporated herein by reference in its entirety), β -particle or γ -ray Monte Carlo dosimetry [MCNP]) is used to calculate the dose to specific target tissues (Fisher DRaX-MCT. MCNP--A general Monte Carlo N-particle transport code, version 5, volume I: overview and theory LA-UR-03-1987. Los Alamos, New Mexico: Los Alamos National Laboratory; 2005; incorporated herein by reference in its entirety).

Therapy experiments

Therapy experiments can use the bispecific constructs followed by 400, 800, or 1200 μCi of ^{90}Y -labeled ligand in groups of 10 xenograft-bearing mice (Table 1). At least 3 experiments are conducted with different myeloma cell lines. Because some

investigators criticize subcutaneous xenograft MM tumors in athymic mouse models, disseminated MM tumors in a NRG mouse model can also be addressed.

Table 1: Groups for Biodistribution and Therapy Experiments with Bispecific Antibodies (see FIGURE 1 for construct)				
Group	1st step (1.4, 2.8 nmol)	Clearing Agent	2nd step Radiolabeled Ligand (1-2 nmol)	Times of eval.*
1	none (control)	NONE	⁹⁰ Y-DOTA or ⁹⁰ Y-DOTA-biotin	1,4, 24, 48, 120 h
2	¹²⁵ I-Control Bispecific x C825	DOTAY-Dextran	⁹⁰ Y-DOTA	1,4, 24, 48, 120 h
3	¹²⁵ I-Anti-BCMA × C825 (bispecific BCMA)-construct in FIGURE 1	DOTAY-Dextran	⁹⁰ Y-DOTA	1,4, 24, 48, 120 h
4	Anti-CD38 × C825 (bispecific CD38)**	DOTAY-Dextran	⁹⁰ Y-DOTA	N/A
*Biodistribution studies **Therapy studies only				

5 Discussion

The restricted nature of BCMA expression in MM is a theoretical advantage for commercial translation. BCMA targeting is also being explored as a target in B cell malignancies. If those findings remain promising, the results could extend potential application of our approach to patients with non-Hodgkin lymphoma (NHL). A broader role for CD38 bispecific PRIT is also feasible and we have already demonstrated efficacy using this agent in preclinical models of NHL. As in MM, treatment-refractory NHL typically retains sensitivity to radiation, making CD38 PRIT a potentially effective treatment for such patients as well. Beyond tumors that share a common B-cell lineage, CD38 is expressed in most natural killer (NK)/T-cell lymphomas, where 50% of patients die within 5 years 55 and overexpression of CD38 predicts poor outcomes for NK/T-cell

lymphoma patients, presenting another potential translational application for CD38 PRIT. Despite impressive efficacy and safety profiles that led to FDA approval of two radioimmunoconjugates for the treatment of B cell lymphoma (^{131}I -tositumomab and ^{90}Y -ibritumomab tiuxitan), these agents have rarely been incorporated into clinical care.

5 RIT targeting CD20 remains in the National Comprehensive Cancer Network Guidelines (NCCN) as a first-line therapy for elderly or infirm patients with follicular lymphoma and as a recommended approach to consolidation or second-line therapy for follicular NHL. Nonetheless, overall utilization remains low and RIT is administered disproportionately within the confines of academic centers. Limited use is likely a consequence of multiple

10 factors which include the availability and ease of administration associated with other novel targeting agents and concerns about radiation toxicity, particularly to the bone marrow. Concerns regarding reimbursement to community oncologists cannot be trivialized, however the absolute cost of RIT for consolidation is lower than the cost of maintenance rituximab (\$46,000; and \$54,000 to \$72,000 [12 - 16 courses]) respectively.

15 RIT may also offer a quality of life advantage to patients because administration involves a single patient infusion visit as compared to frequent infusions during rituximab maintenance.

Innovations that improve targeting, diminish toxicity and highlight the unique favorable attributes associated with RIT will help to overcome a history of limited

20 adoption. We have demonstrated that bispecific antibodies targeting CD38 on myeloma cells work as well as the CD20 antibodies published in Cancer Research and that BCMA is a viable alternative target for the bispecific PRIT approach. Ultimately, superior efficacy provides the most compelling argument for the adoption of PRIT.

25 While illustrative embodiments have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

CLAIMS

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A bispecific affinity reagent, comprising:
a first binding domain that specifically binds to a target antigen selected from CD38, B cell maturation antigen (BCMA), Muc1, GPRC5D, and SlamF7; and
a second binding domain that specifically binds to a radioactive ligand.
2. The bispecific affinity reagent of Claim 1, wherein the affinity reagent is a fusion protein and the first binding domain and the second binding domain are separated by a hinge region.
3. The bispecific affinity reagent of Claim 2, wherein the hinge region comprises a construct selected from an IgG1 Fc fragment, an IgG2 Fc fragment, and IgG3 Fc fragment, and an IgG4 Fc fragment.
4. The bispecific affinity reagent of Claim 1, wherein the radioactive ligand comprises yttrium-DOTA (Y-DOTA).
5. The bispecific affinity reagent of Claim 1, wherein one or both of the first binding domain and the second binding domain are an antibody, a functional antibody fragment, or functional antibody derivative.
6. The bispecific affinity reagent of Claim 1, wherein one or both of the first binding domain and the second binding domain comprise a variable light chain domain and a variable heavy chain domain.
7. The bispecific affinity reagent of Claim 6, wherein the variable light and variable heavy chain of the first binding domain and/or the second binding are separated by a linker domain.
8. The bispecific affinity reagent of Claim 7, wherein one or both of the first binding domain and the second binding domain is an scFv.
9. The bispecific affinity reagent of Claim 8, wherein the scFv is humanized.

10. The bispecific affinity reagent of Claim 1, wherein the first binding domain specifically binds to CD38.

11. The bispecific affinity reagent of Claim 10, wherein the first binding domain comprises an amino acid sequence with at least 95% sequence identity to a CD38 binding domain included in an amino acid sequence of a bispecific affinity reagent as set forth in SEQ ID NO:2, 4, or 6.

12. The bispecific affinity reagent of Claim 10, wherein the bispecific affinity reagent comprises an amino acid sequence as set forth in SEQ ID NO:2, 4, or 6, or comprises an amino acid sequence with at least 90% identity to an amino acid sequence as set forth in SEQ ID NO:2, 4, or 6.

13. The bispecific affinity reagent of Claim 1, wherein the first binding domain specifically binds to BCMA.

14. The bispecific affinity reagent of Claim 13, wherein the first binding domain comprises an amino acid sequence with at least 95% sequence identity to a BCMA binding domain included in an amino acid sequence of a bispecific affinity reagent as set forth in SEQ ID NO:8 or 10.

15. The bispecific affinity reagent of Claim 13, wherein the first binding domain comprises an amino acid sequence with at least 95% sequence identity to an amino acid sequence as set forth in SEQ ID NO:19.

16. The bispecific affinity reagent of Claim 13, wherein the bispecific affinity reagent comprises an amino acid sequence as set forth in SEQ ID NO:8 or 10, or comprises an amino acid sequence with at least 90% identity to an amino acid sequence as set forth in SEQ ID NO:8 or 10.

17. The bispecific affinity reagent of Claim 1, wherein the first binding domain specifically binds to Muc1.

18. The bispecific affinity reagent of Claim 17, wherein the first binding domain comprises an amino acid sequence with at least 95% sequence identity to a Muc1

binding domain included in an amino acid sequence of a bispecific affinity reagent as set forth in SEQ ID NO:18.

19. The bispecific affinity reagent of Claim 1, wherein the first binding domain specifically binds to SlamF7.

20. The bispecific affinity reagent of Claim 19, wherein the first binding domain comprises an amino acid sequence with at least 95% sequence identity to a SlamF7 binding domain included in an amino acid sequence of a bispecific affinity reagent as set forth in SEQ ID NO:16.

21. The bispecific affinity reagent of Claim 1, wherein the first binding domain specifically binds to GPRC5D.

22. The bispecific affinity reagent of Claim 1, wherein the first binding domain comprises an amino acid sequence with at least 95% sequence identity to a GPRC5D binding domain comprising or included in an amino acid sequence selected from SEQ ID NOS:59-139.

23. The bispecific affinity reagent of Claim 1, wherein the second binding domain comprises an amino acid sequence with at least 95% sequence identity to a DOTA binding domain comprising or included in an amino acid sequence selected from SEQ ID NOS:20-36.

24. A method of treating a hematological malignancy in a subject, comprising:
administering to the subject a therapeutically effective amount of the bispecific affinity reagent of any one of Claims 1-23, and thereafter
administering to the subject a therapeutically effective amount of a radioactive ligand.

25. The method of Claim 24, further comprising administering an effective amount of a clearing agent (CA) after administering the bispecific affinity reagent and before administering the radioactive ligand.

26. The method of Claim 24, wherein the radioactive ligand comprises yttrium-DOTA.

27. The method of Claim 24, wherein the hematological malignancy is a B cell malignancy.

28. The method of Claim 27, wherein the B cell malignancy is multiple myeloma (MM), a leukemia, or lymphoma.

29. The method of Claim 24, wherein the hematological malignancy exhibits expression of CD38, and wherein the first binding domain of the bispecific affinity reagent specifically binds to CD38.

30. The method of Claim 29, wherein the hematological malignancy is a lymphoma and the method further comprises administering to the subject therapeutically effective amount of a second bispecific affinity reagent comprising:

- a first binding domain that specifically binds to CD20; and
- a second binding domain that specifically binds to the radioactive ligand.

31. The method of Claim 30, wherein the first binding domain of the second bispecific affinity reagent comprises an amino acid sequence with at least 95% sequence identity to a CD20 binding domain included in an amino acid sequence of a bispecific affinity reagent as set forth in SEQ ID NO:12 or 14.

32. The method of Claim 30, wherein the second bispecific affinity reagent comprises an amino acid sequence as set forth in SEQ ID NO:12 or 14, or comprises an amino acid sequence with at least 90% identity to an amino acid sequence as set forth in SEQ ID NO:12 or 14.

33. The method of Claim 30, wherein the second bispecific affinity reagent is administered prior to administration of the radioactive ligand.

34. The method of Claim 29, further comprising administering to the subject an amount of all trans retinoic acid (ATRA), or functional derivatives or subunits thereof, sufficient to upregulate expression of CD38 or GPRC5D in the malignant cells.

35. The method of Claim 34, wherein the ATRA or functional derivatives or subunits thereof are administered in a liposomal formulation.

36. The method of Claim 24, wherein the hematological malignancy exhibits expression of BCMA, and wherein the first binding domain specifically binds to BCMA.

37. The method of Claim 36, further comprising administering to the subject an amount of a gamma secretase inhibitor (GSI) sufficient to upregulate expression of BCMA in the malignant cells.

38. The method of Claim 24, wherein the hematological malignancy exhibits elevated expression of Muc1 compared to healthy cells, and wherein the first binding domain specifically binds to Muc1.

39. The method of Claim 38, further comprising administering to the subject an amount of a gamma secretase inhibitor (GSI) sufficient to upregulate expression of Muc1 in the malignant cells.

40. The method of Claim 24, wherein the hematological malignancy exhibits elevated expression of SlamF7 compared to healthy cells, and wherein the first binding domain specifically binds to SlamF7.

41. A method of treating a hematological malignancy in a subject, comprising:
administering to the subject a therapeutically effective amount of a first bispecific affinity reagent and a therapeutically effective amount of a second bispecific affinity reagent, and thereafter

administering to the subject a therapeutically effective amount of a radioactive ligand;

wherein the first bispecific affinity reagent and the second bispecific affinity reagent each comprises a first binding domain that specifically binds to a cancer antigen and a second binding domain that specifically binds to the radioactive ligand,

wherein the first binding domain of the first bispecific affinity reagent and the first binding domain of the second bispecific affinity reagent specifically bind to different cancer antigens selected from CD38, BCMA, Muc1, SlamF7, GPRC5D, and CD20.

42. The method of Claim 41, further comprising administering an effective amount of a clearing agent (CA) after administering the bispecific affinity reagent and before administering the radioactive ligand.

43. The method of Claim 41, wherein the radioactive ligand comprises yttrium-DOTA (Y-DOTA).

44. The method of Claim 41, wherein the first affinity reagent and the second affinity reagent are each fusion proteins with their respective first binding domains and second binding domains separated by a hinge region.

45. The method of Claim 44, wherein the hinge region of the first and/or second affinity reagent is comprises a construct selected from an IgG1 Fc fragment, an IgG2 Fc fragment, and IgG3 Fc fragment, and an IgG4 Fc fragment.

46. The method of Claim 41, wherein one or both of the first binding domain and the second binding domain of the first and/or second bispecific affinity reagents are an antibody, a functional antibody fragment, or functional antibody derivative.

47. The method of Claim 41, wherein one or both of the first binding domain and the second binding domain of the first and/or second bispecific affinity reagents comprise a variable light chain domain and variable heavy chain domain.

48. The method of Claim 47, wherein the variable light and variable heavy chain of the binding domain and/or the second binding are separated by a linker domain.

49. The method of Claim 41, wherein one or both of the first binding domain and the second binding domain of the first and/or second bispecific affinity reagents is an scFv.

50. The method of Claim 49, wherein the scFv is humanized.

51. The method of Claim 41, wherein the first binding domain of the first bispecific affinity reagent specifically binds CD38 and the first binding domain of the second bispecific affinity reagent specifically binds one of BCMA, Muc1, SlamF7, GPRC5D, and CD20.

52. The method of Claim 51, further comprising administering to the subject an amount of all trans retinoic acid (ATRA), or functional derivatives or subunits thereof, sufficient to upregulate expression of CD38 in the malignant cells.

53. The method of Claim 52, wherein the ATRA or functional derivatives or subunits thereof are administered in a liposomal formulation.

54. The method of Claim 41, wherein the first binding domain of the first bispecific affinity reagent specifically binds BCMA and the first binding domain of the second bispecific affinity reagent specifically binds one of CD38, Muc1, SlamF7, GPRC5D, and CD20.

55. The method of Claim 54, further comprising administering to the subject an amount of a gamma secretase inhibitor (GSI) sufficient to upregulate expression of BCMA in the malignant cells.

56. The method of Claim 41, wherein the first binding domain of the first bispecific affinity reagent specifically binds Muc1 and the first binding domain of the second bispecific affinity reagent specifically binds one of CD38, BCMA, SlamF7, GPRC5D, and CD20.

57. The method of Claim 56, further comprising administering to the subject an amount of a gamma secretase inhibitor (GSI) sufficient to upregulate expression of Muc1 in the malignant cells.

58. The method of Claim 39, wherein the first binding domain of the first bispecific affinity reagent specifically binds SlamF7 and the first binding domain of the second bispecific affinity reagent specifically binds one of CD38, BCMA, Muc1, GPRC5D, and CD20.

59. The method of Claim 41, wherein the first binding domain of the first bispecific affinity reagent specifically binds GPRC5D and the first binding domain of the second bispecific affinity reagent specifically binds one of CD38, BCMA, Muc1, SlamF7, and CD20.

60. The method of Claim 59, further comprising administering to the subject an amount of all trans retinoic acid (ATRA), or functional derivatives or subunits thereof, sufficient to upregulate expression of GPRC5D in the malignant cells.

61. The method of Claim 41, wherein the hematological malignancy is a B cell malignancy.

62. The method of Claim 61, wherein the B cell malignancy is multiple myeloma (MM), a leukemia, or lymphoma.

63. A method of treating a malignancy characterized by expression of CD38, comprising

administering to the subject an amount of all trans retinoic acid (ATRA), or functional derivatives or subunits thereof, sufficient to upregulate expression of CD38 in the malignant cells,

administering to the subject a therapeutically effective amount of a bispecific affinity reagent comprising a first binding domain that specifically binds to CD38 and a second binding domain that specifically binds to a radioactive ligand, and thereafter

administering to the subject a therapeutically effective amount of a radioactive ligand.

64. A method of treating a malignancy characterized by expression of GPRC5D, comprising

administering to the subject an amount of all trans retinoic acid (ATRA), or functional derivatives or subunits thereof, sufficient to upregulate expression of GPRC5D in the malignant cells,

administering to the subject a therapeutically effective amount of a bispecific affinity reagent comprising a first binding domain that specifically binds to GPRC5D and a second binding domain that specifically binds to a radioactive ligand, and thereafter

administering to the subject a therapeutically effective amount of a radioactive ligand.

65. The method of Claim 53 or Claim 64, wherein the ATRA or functional derivatives or subunits thereof are administered in a liposomal formulation.

66. A method of treating a malignancy characterized by expression of BCMA, comprising

administering to the subject an amount of gamma secretase inhibitor (GSI) sufficient to upregulate expression of BCMA in the malignant cells,

administering to the subject a therapeutically effective amount of a bispecific affinity reagent comprising a first binding domain that specifically binds to BCMA and a second binding domain that specifically binds to a radioactive ligand, and thereafter

administering to the subject a therapeutically effective amount of a radioactive ligand.

67. A method of treating a malignancy characterized by expression of Muc1, comprising

administering to the subject an amount of gamma secretase inhibitor (GSI) sufficient to upregulate expression of Muc1 in the malignant cells,

administering to the subject a therapeutically effective amount of a bispecific affinity reagent comprising a first binding domain that specifically binds to Muc1 and a second binding domain that specifically binds to a radioactive ligand, and thereafter

administering to the subject a therapeutically effective amount of a radioactive ligand.

68. The method of any one of Claims 63 to 67, further comprising administering an effective amount of a clearing agent (CA) after administering the bispecific affinity reagent and before administering the radioactive ligand.

69. The method of any one of Claims 63 to 67, wherein the radioactive moiety comprises yttrium-DOTA.

70. The method of any one of Claims 63 to 67, wherein the affinity reagent is a fusion protein and the first binding domain and the second binding domain are separated by a hinge region.

71. The method of Claim 70, wherein the hinge region is comprises a construct selected from an IgG1 Fc fragment, an IgG2 Fc fragment, and IgG3 Fc fragment, and an IgG4 Fc fragment.

72. The method of any one of Claims 63 to 67, wherein one or both of the first binding domain and the second binding domain are an antibody, a functional antibody fragment, functional antibody derivative.

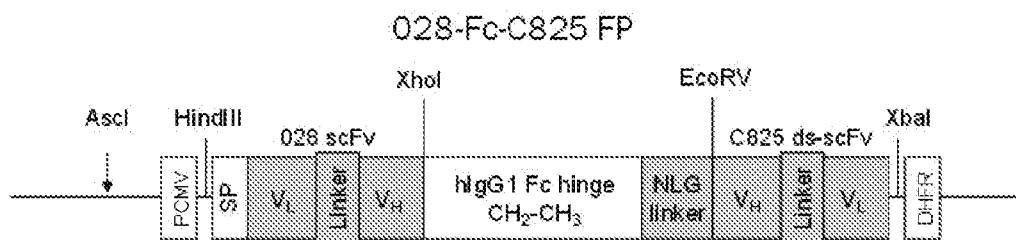
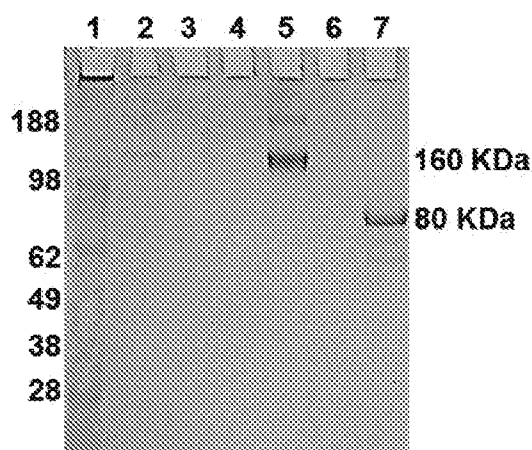
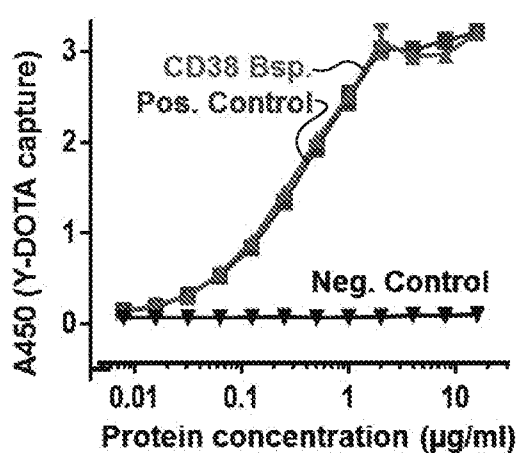
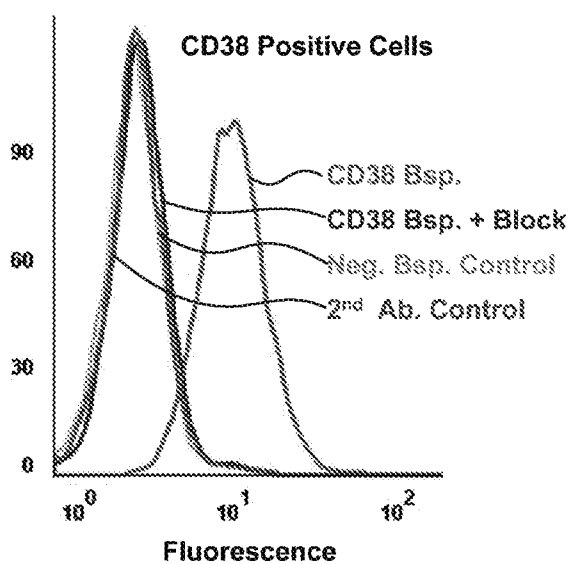
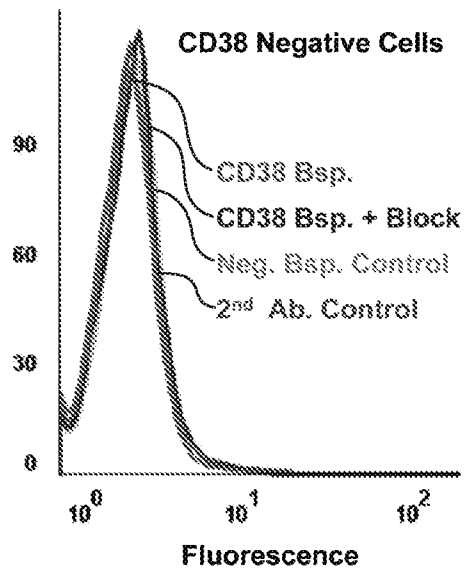
73. The method of any one of Claims 63 to 67, wherein one or both of the first binding domain and the second binding domain comprise a variable light chain domain and variable heavy chain domain.

74. The method of Claim 73, wherein the variable light chain and variable heavy chain of the binding domain and/or the second binding are separated by a linker domain.

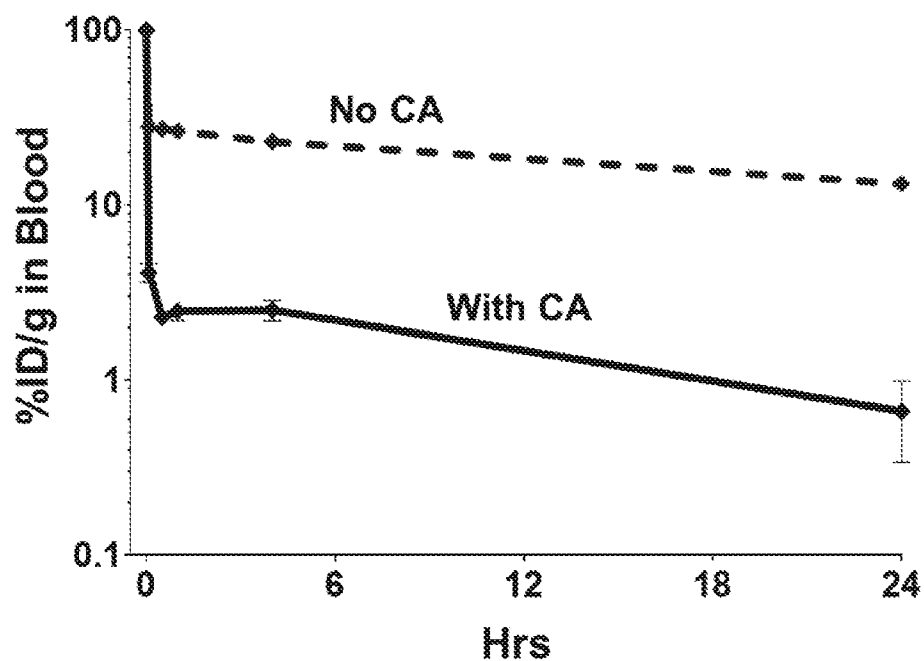
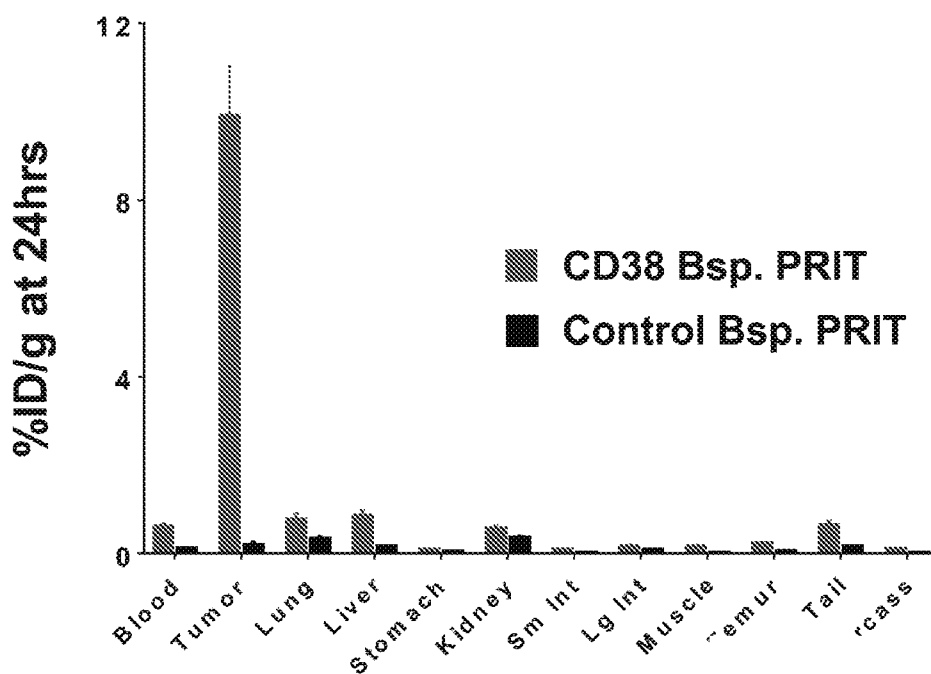
75. The method of Claim 74, wherein one or both of the first binding domain and the second binding domain is an scFv.

76. The method of Claim 75, wherein the scFv is humanized.

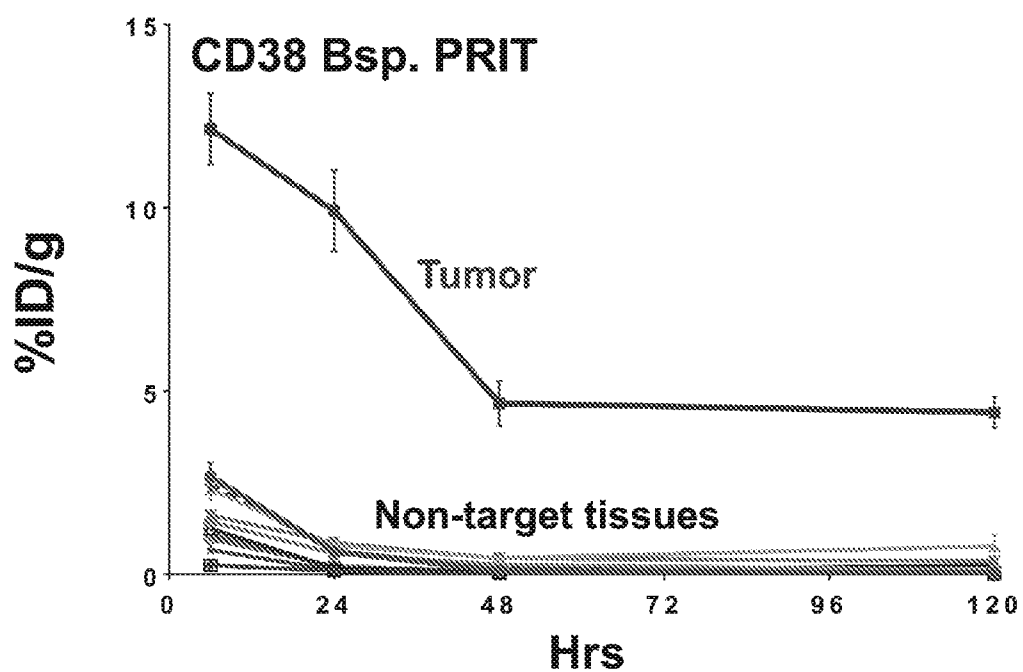
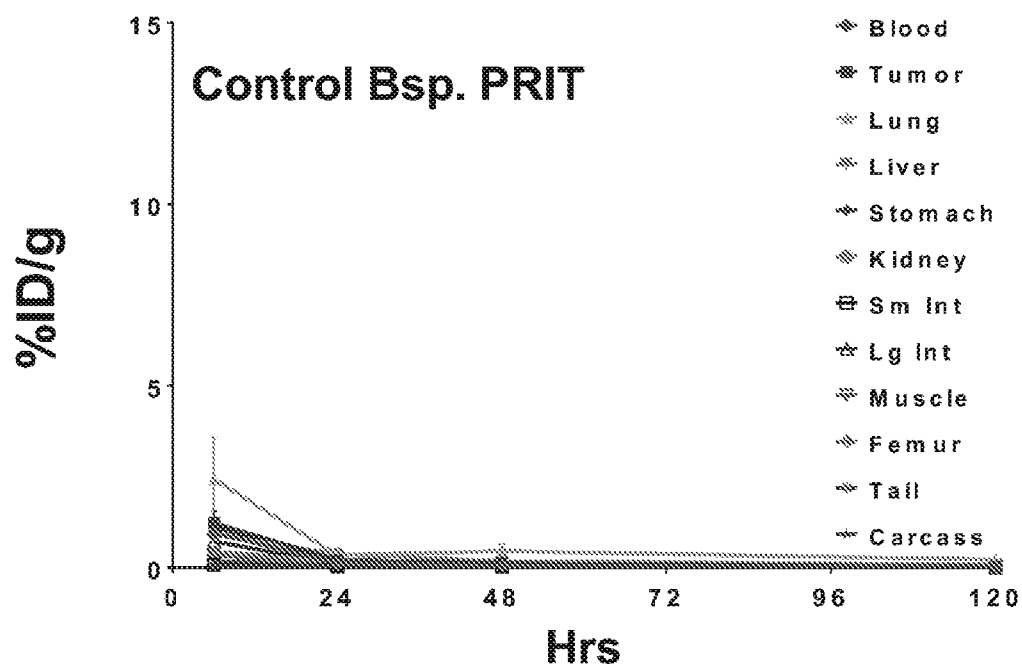
1/18

**FIG. 1A****FIG. 1B****FIG. 1C****FIG. 1D****FIG. 1E**

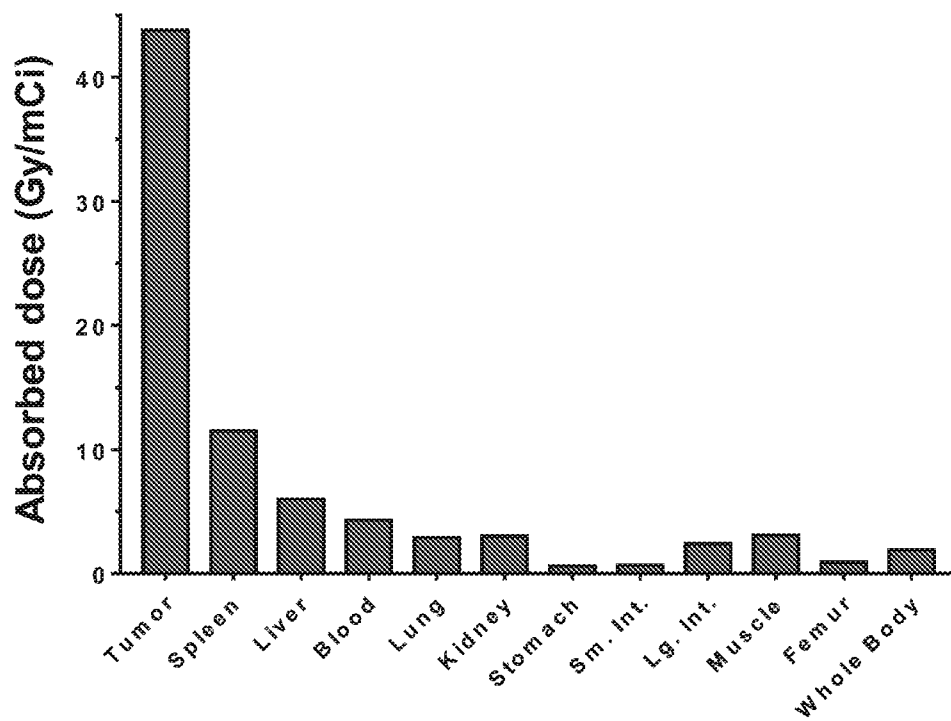
2/18

**FIG. 2****FIG. 3A**

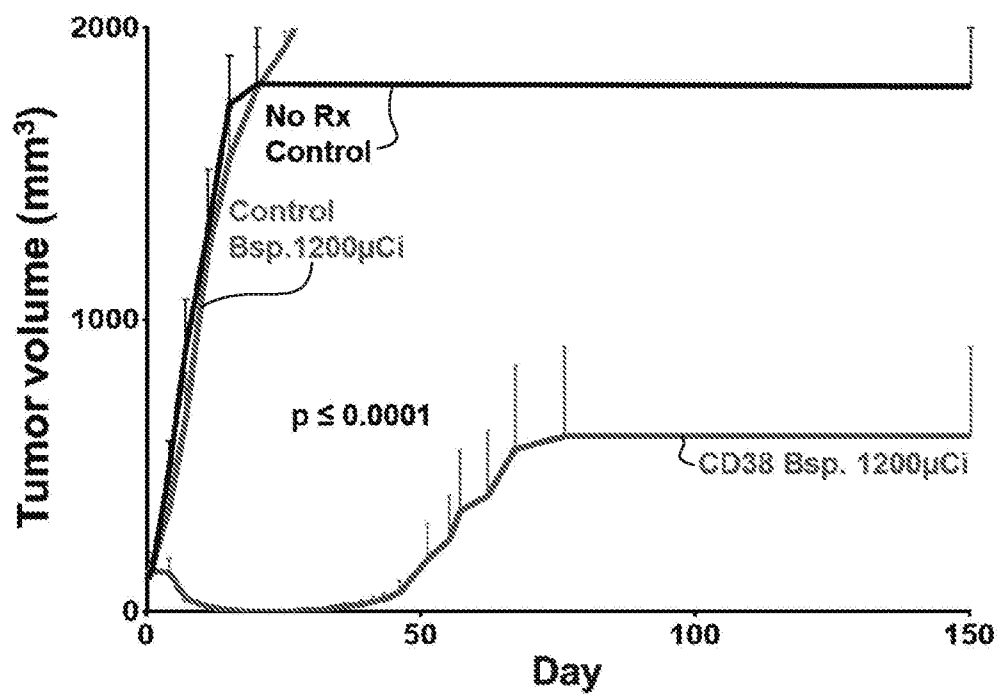
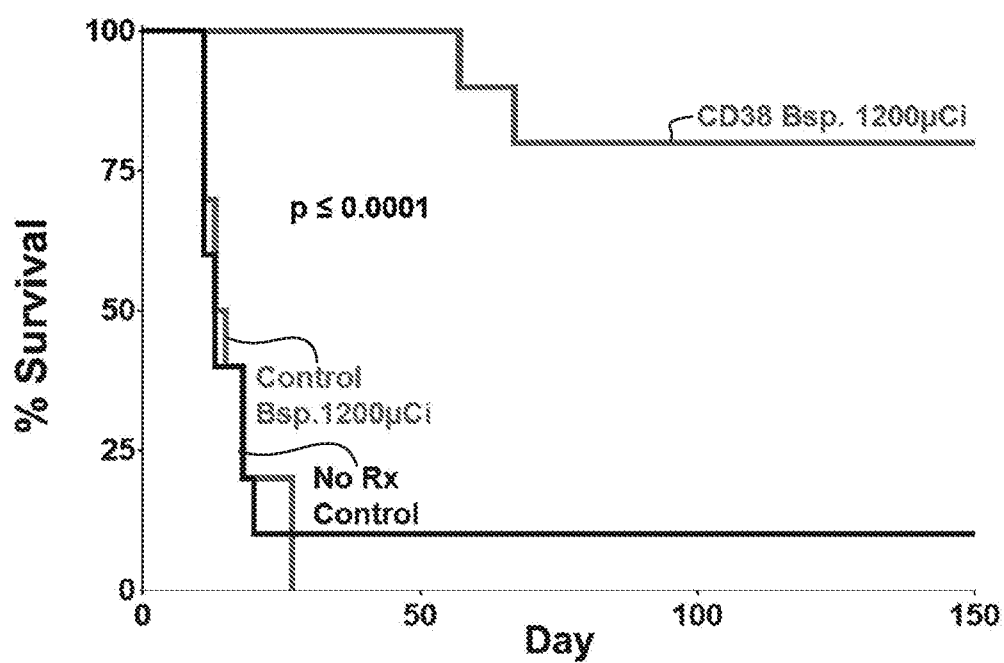
3/18

**FIG. 3B****FIG. 3C**

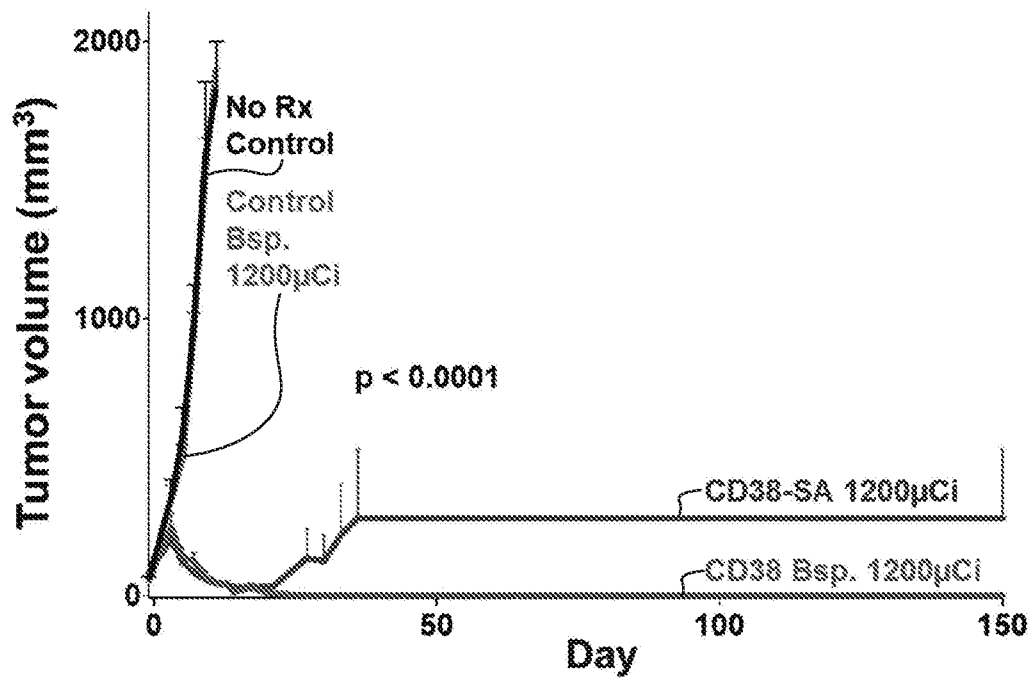
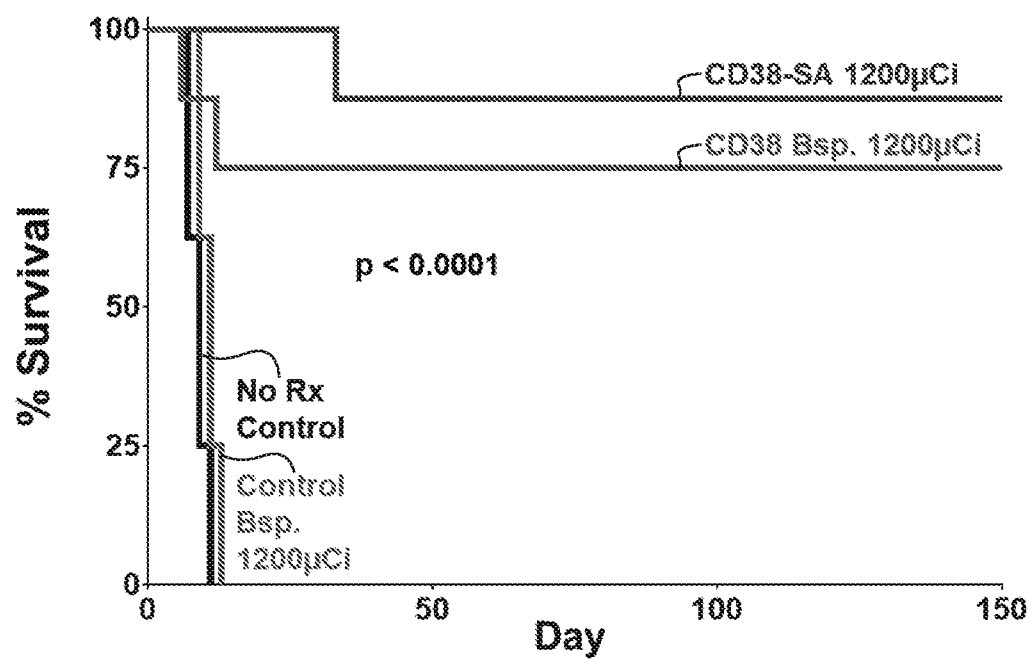
4/18

**FIG. 4**

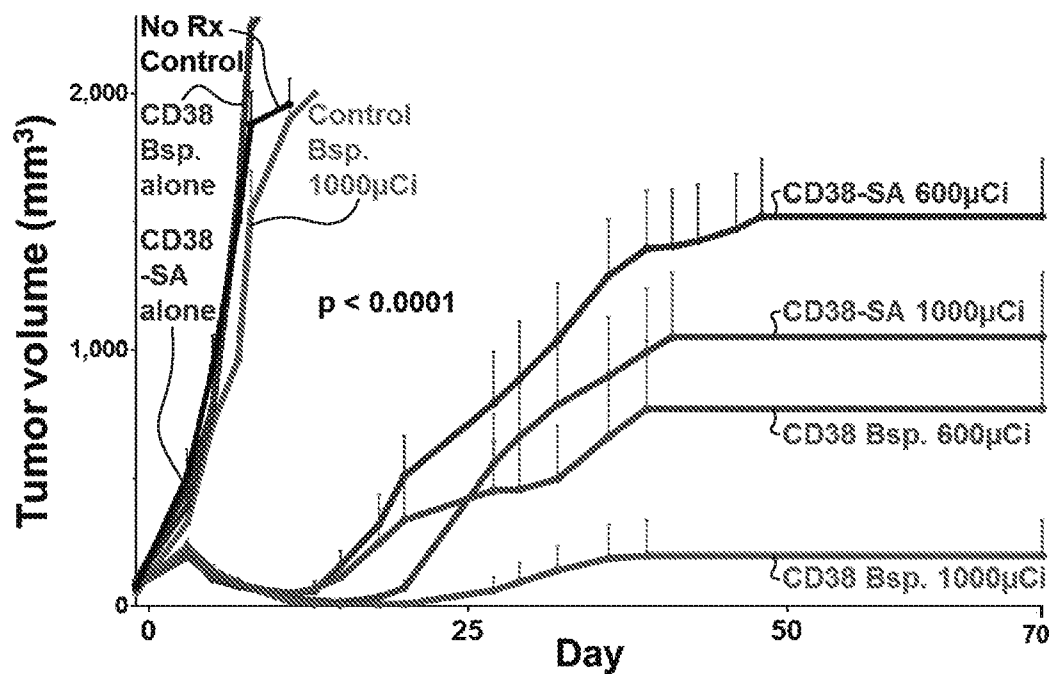
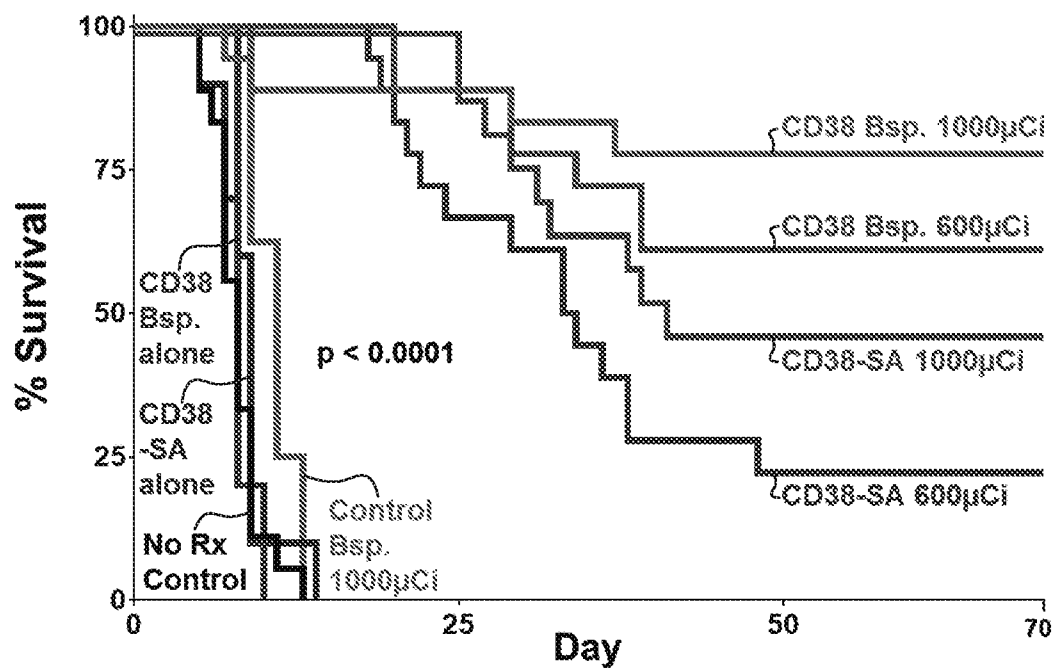
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**FIG. 5A****FIG. 5B**

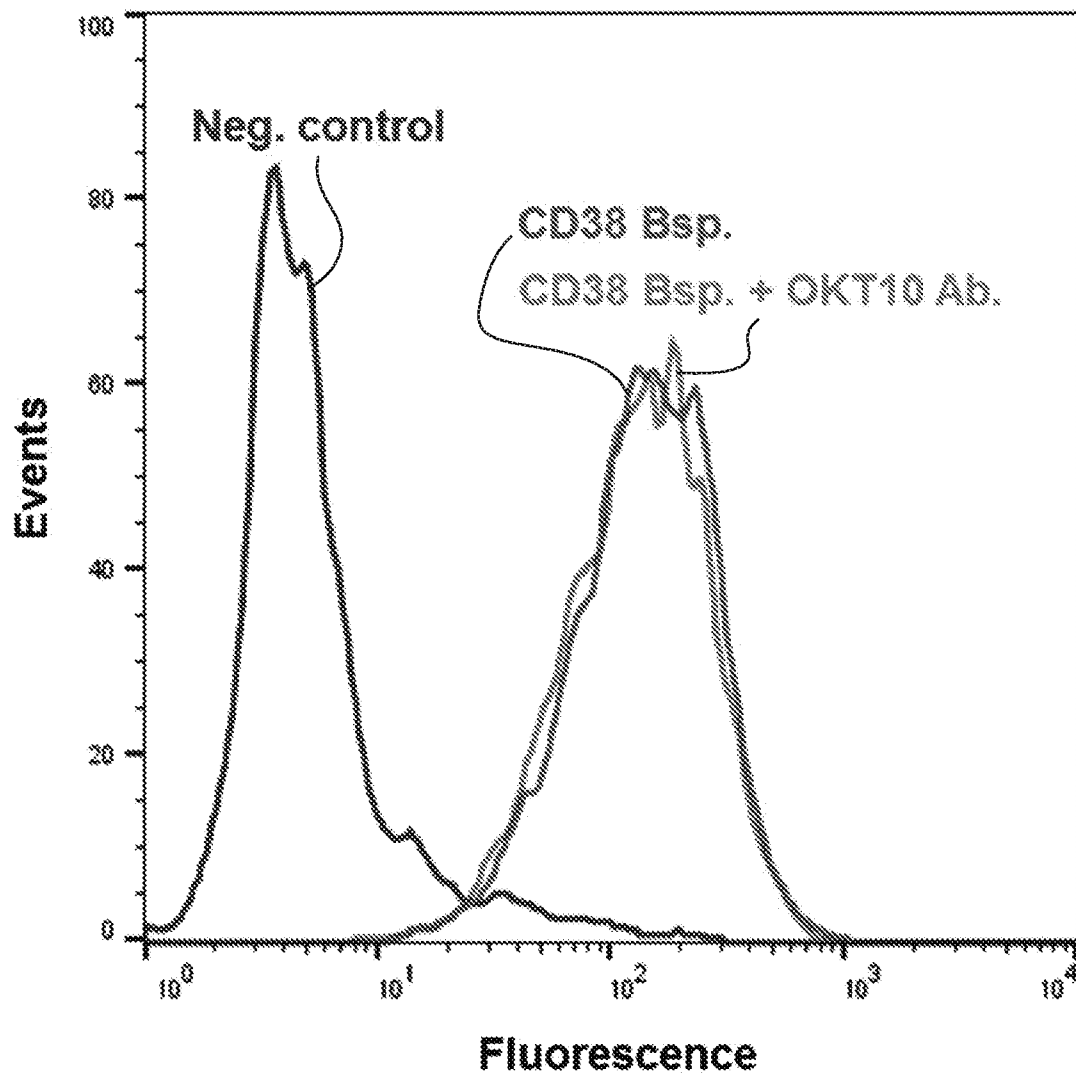
6/18

**FIG. 6A****FIG. 6B**

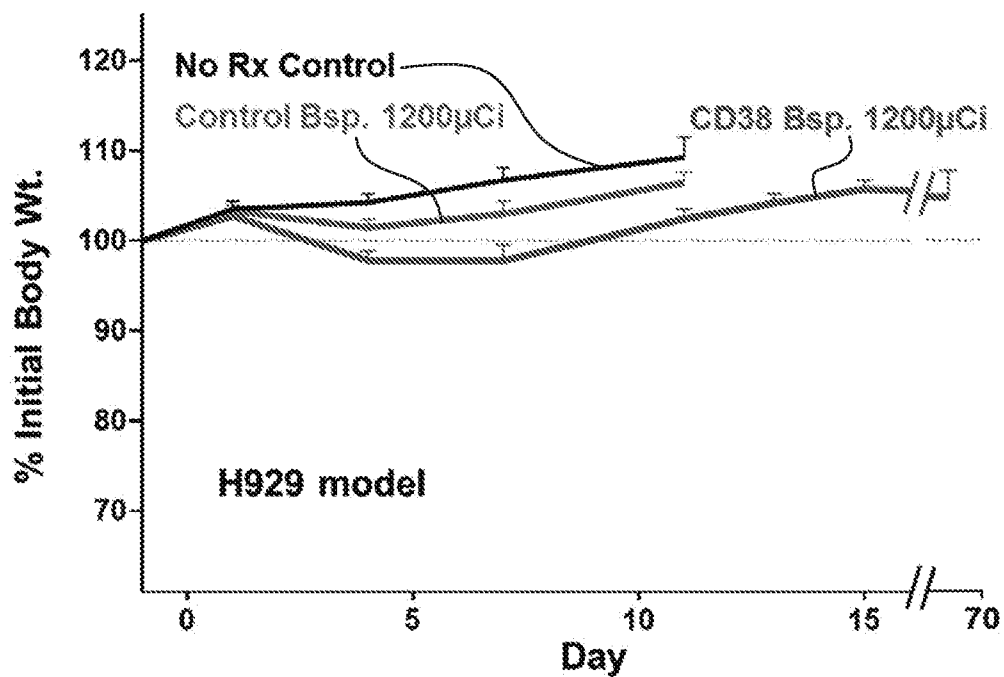
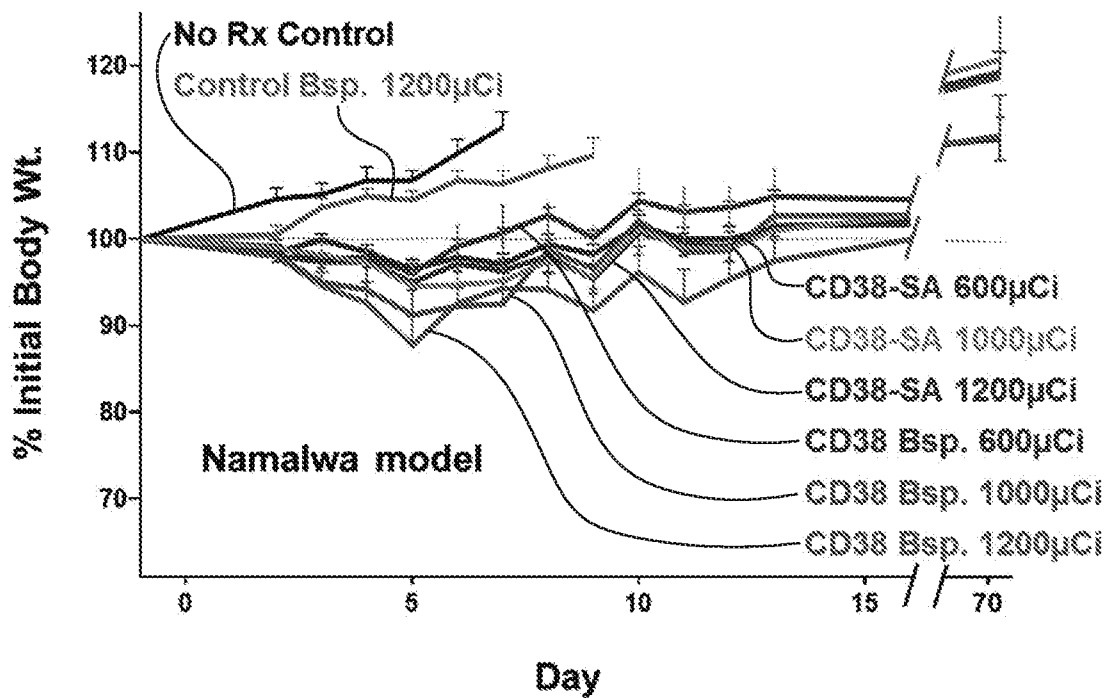
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**FIG. 7A****FIG. 7B**

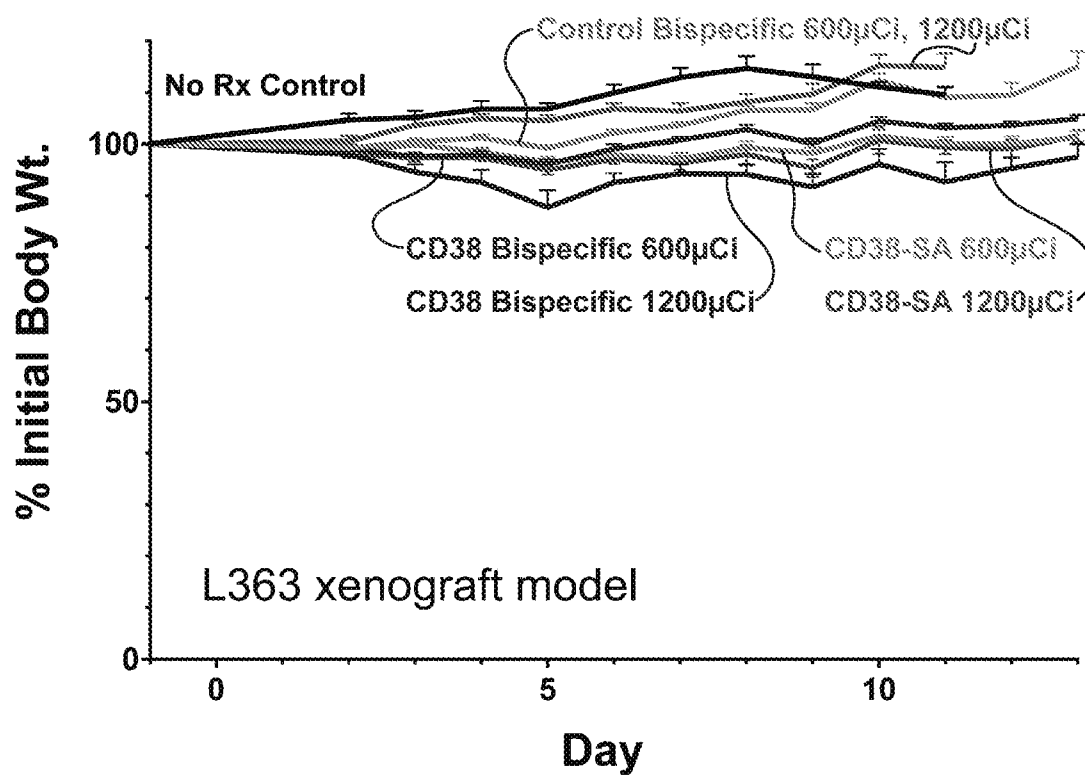
8/18

**FIG. 8**

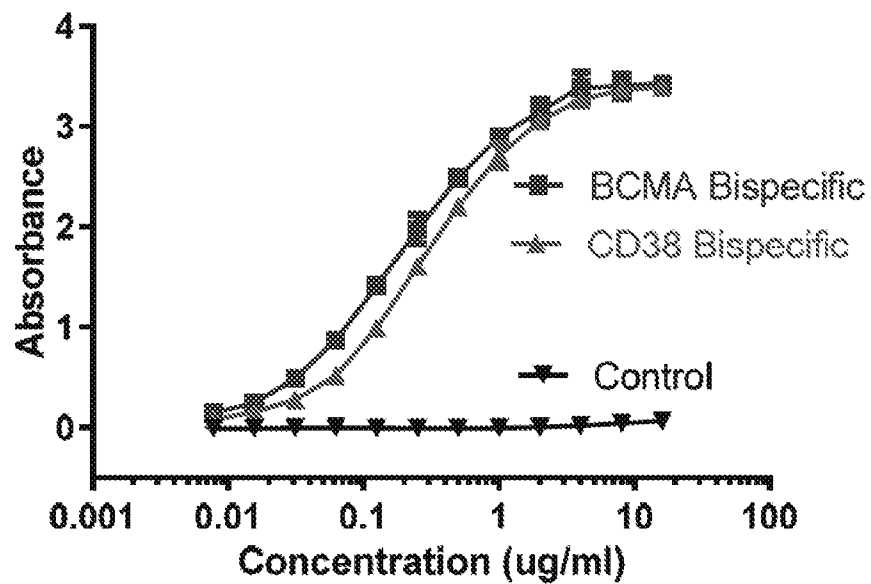
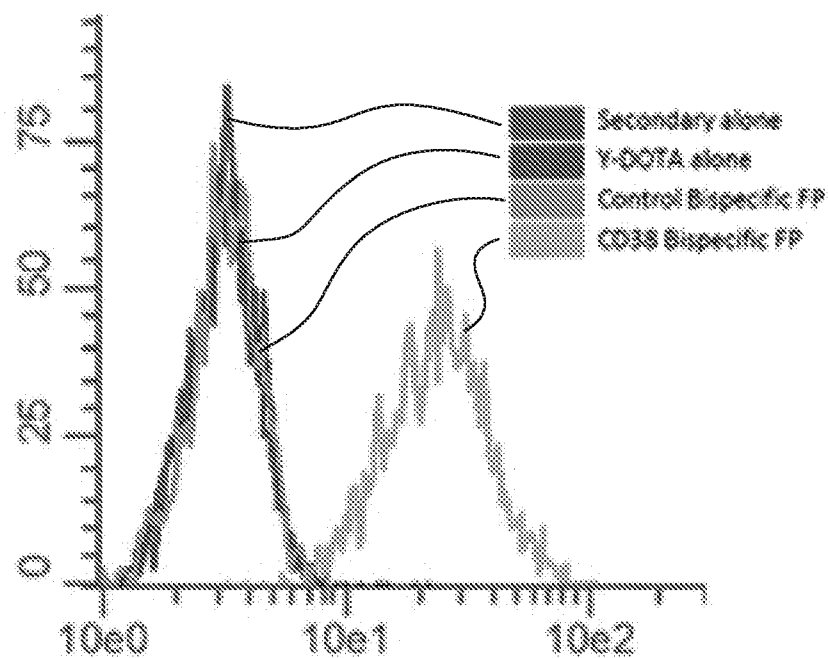
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**FIG. 9A****FIG. 9B**

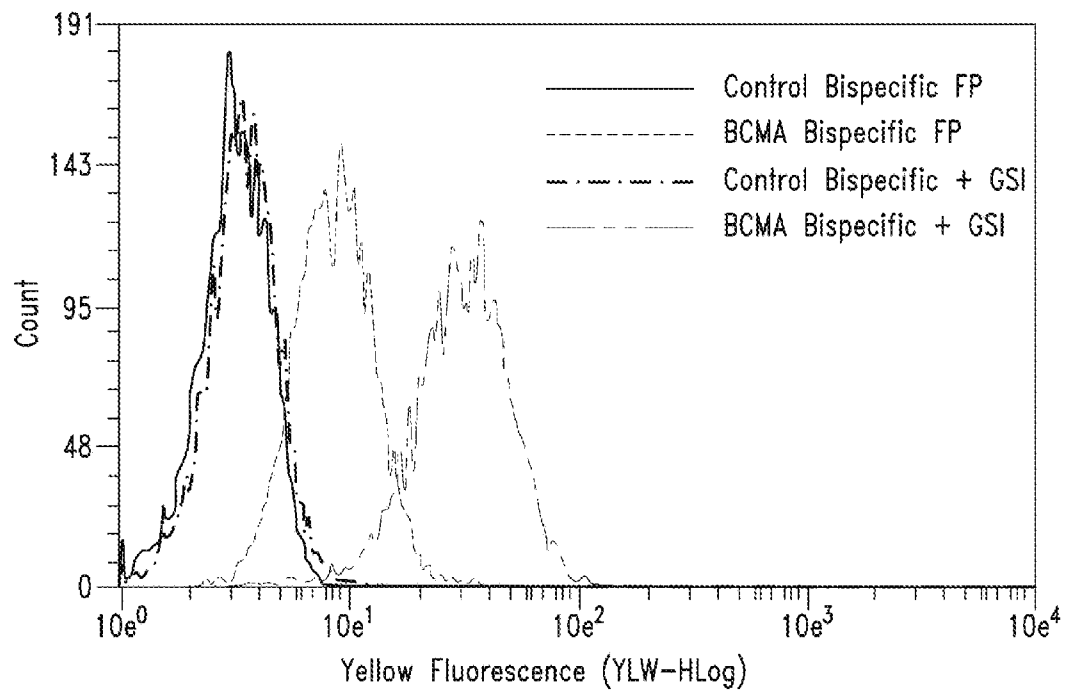
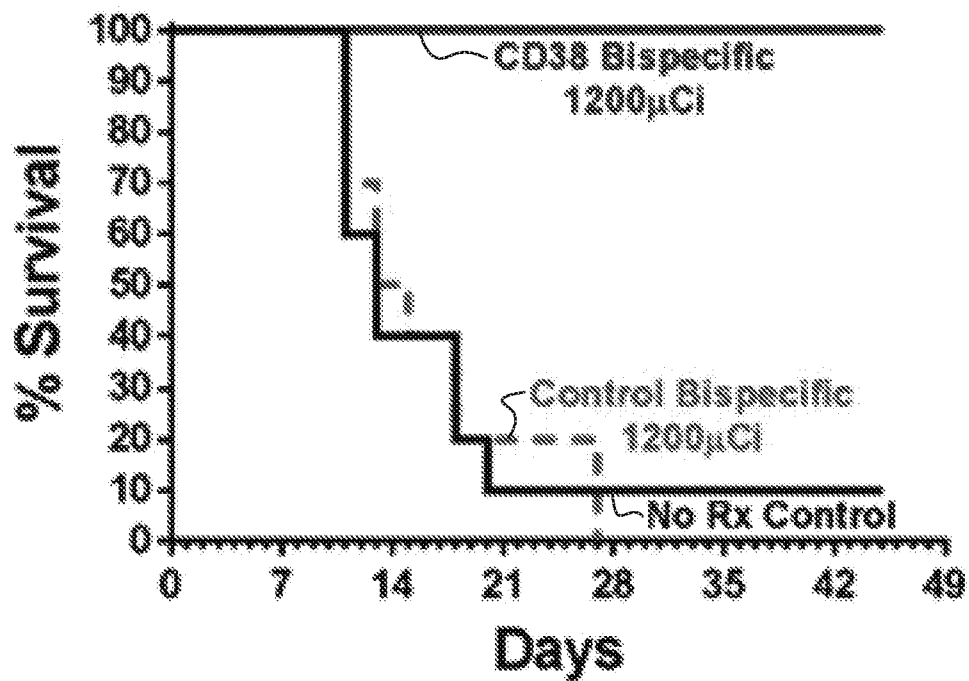
10/18

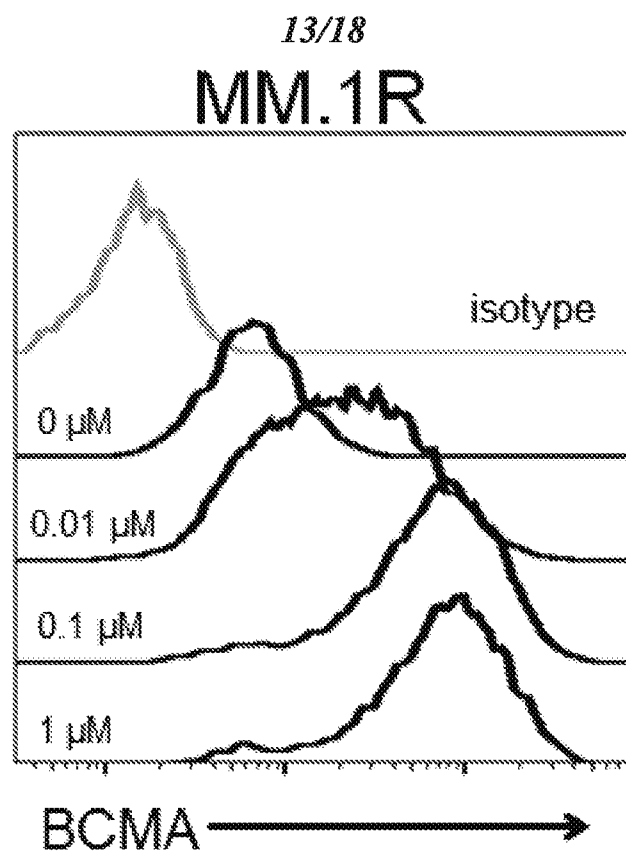
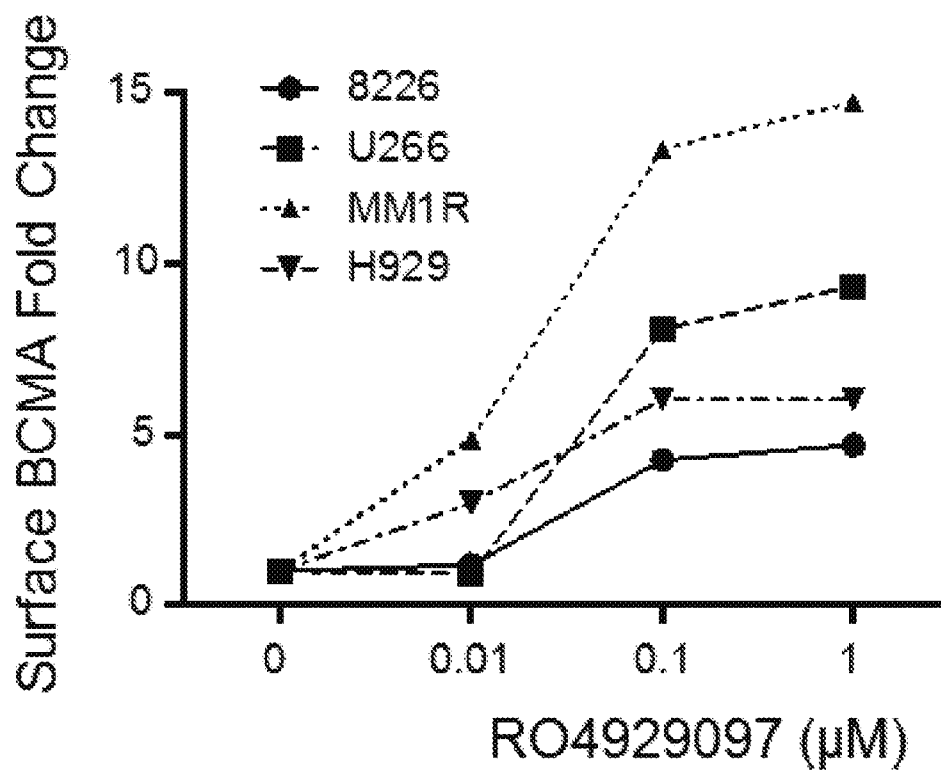
**FIG. 9C**

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**FIG. 10A****FIG. 10B**

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**FIG. 10C****FIG. 10D**

**FIG. 11A****FIG. 11B**

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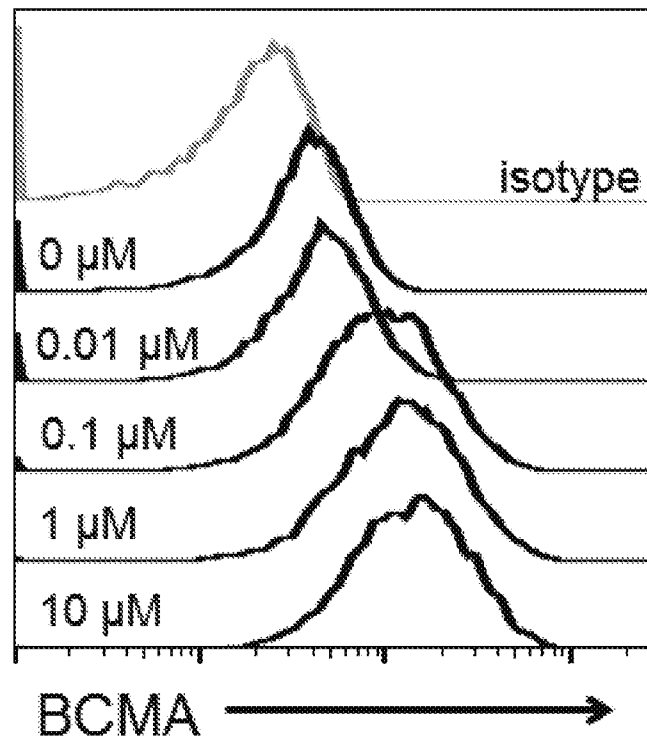


FIG. 11C

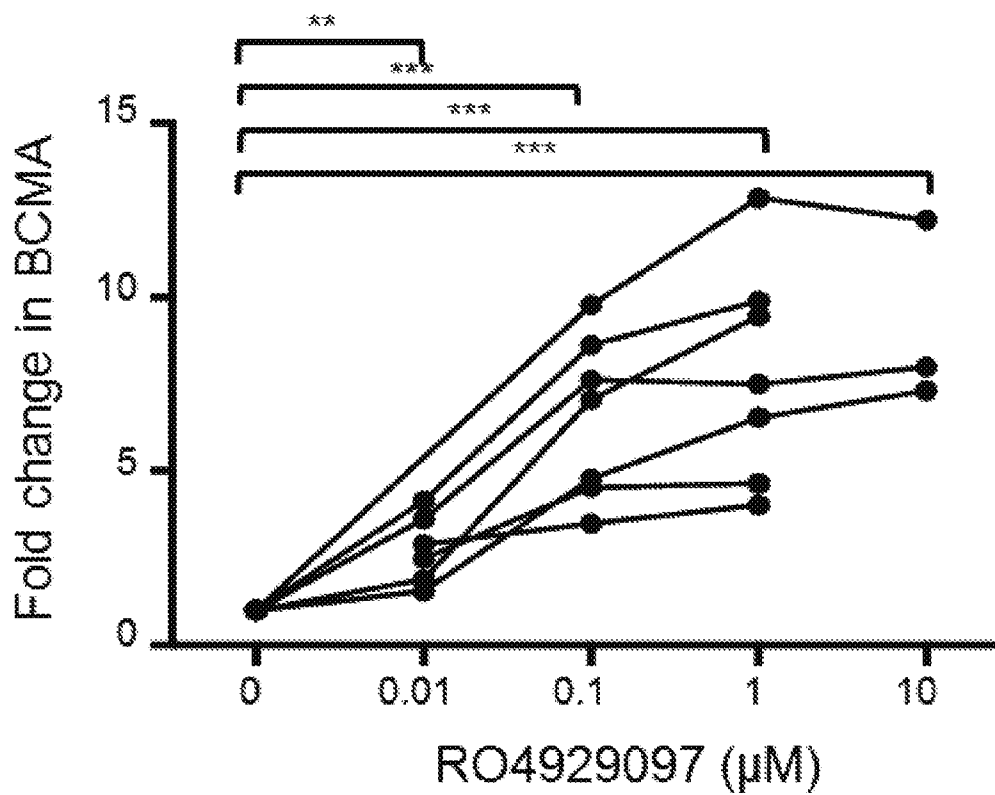
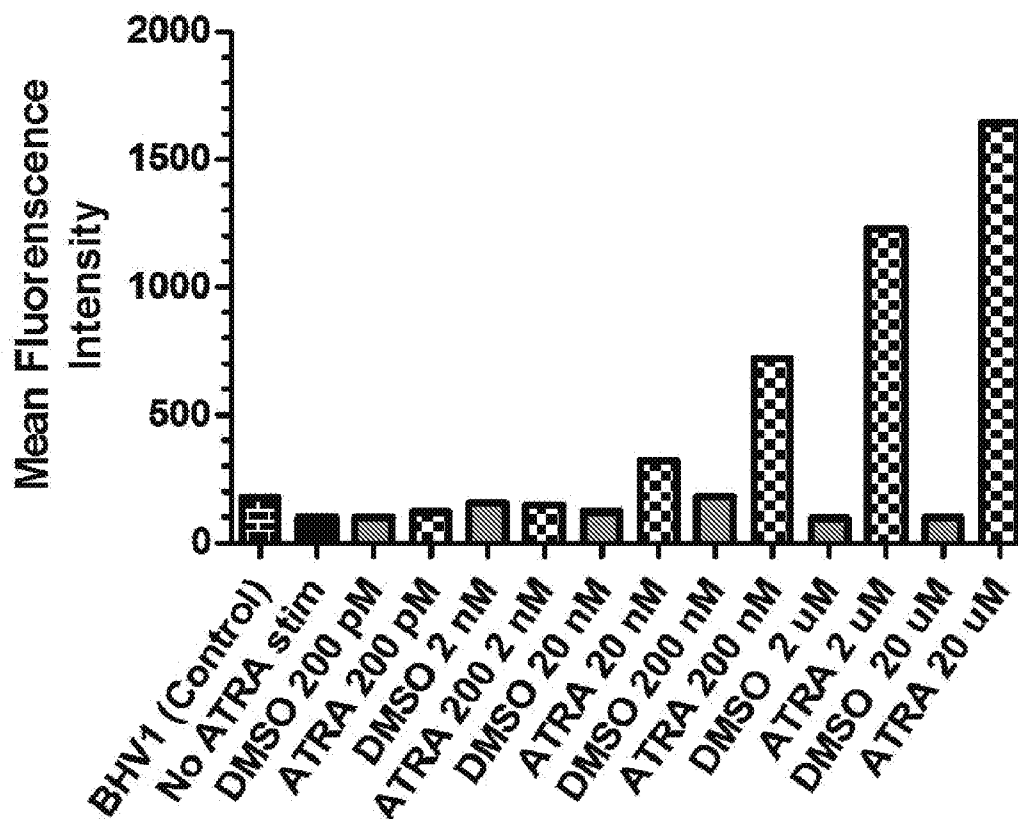


FIG. 11D

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**FIG. 12**

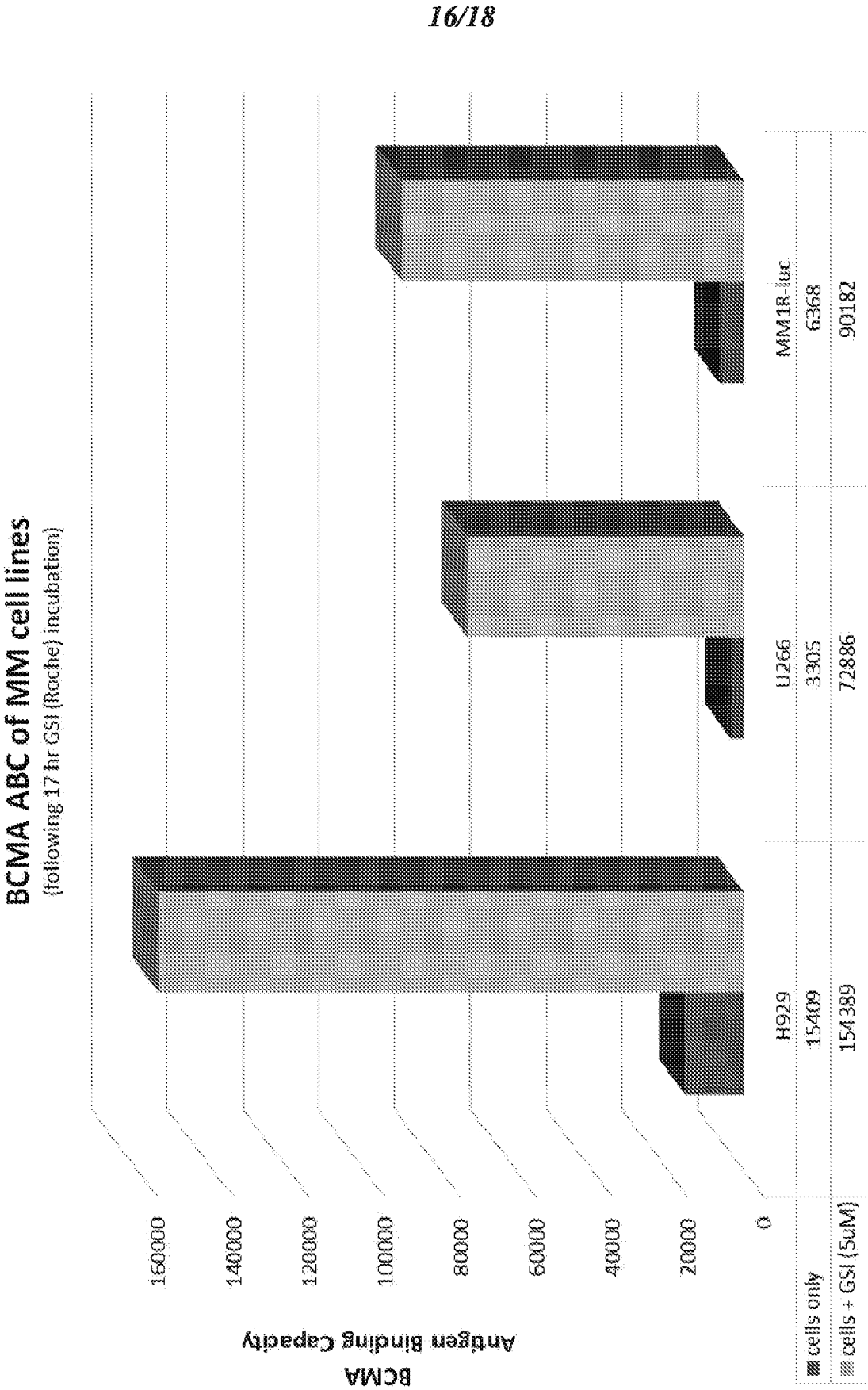


FIG. 13

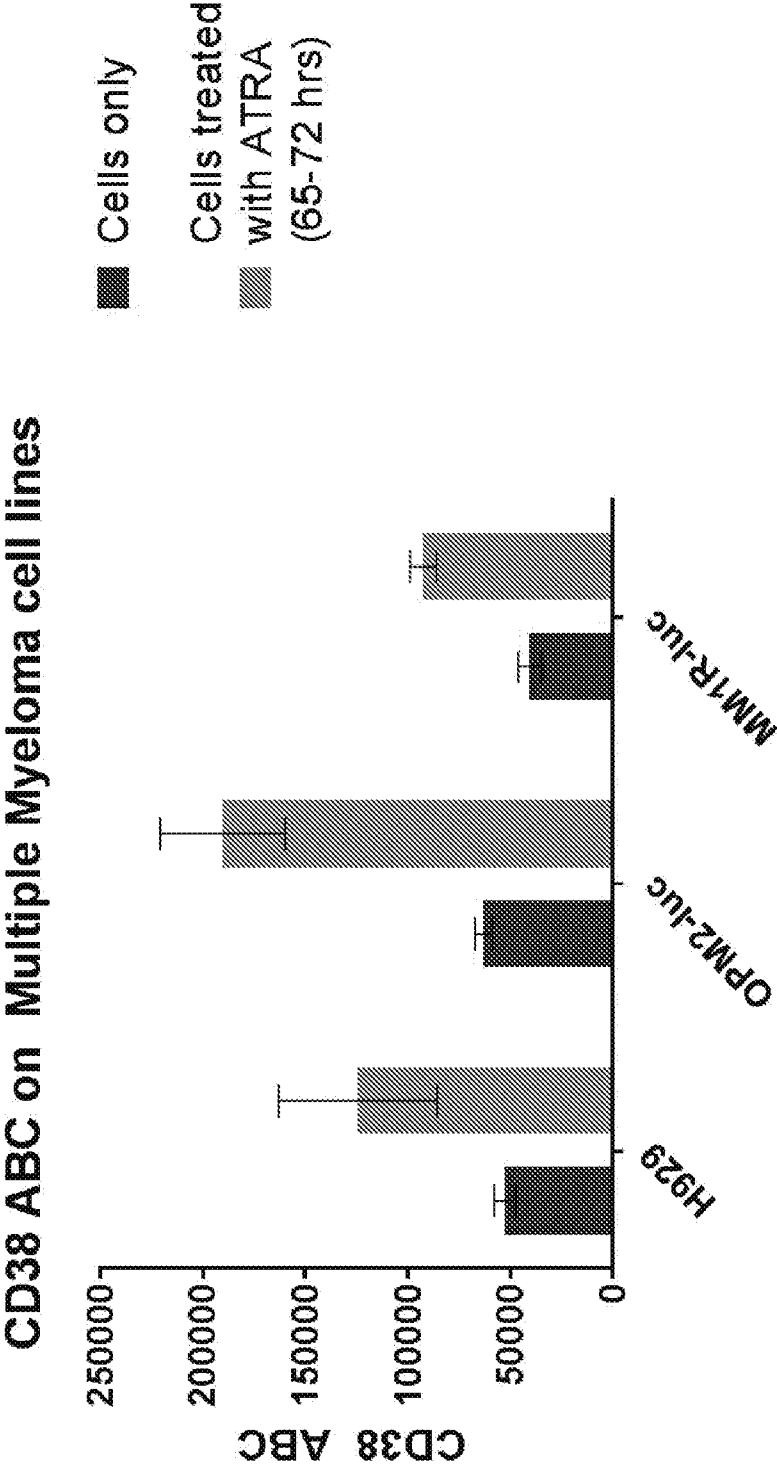
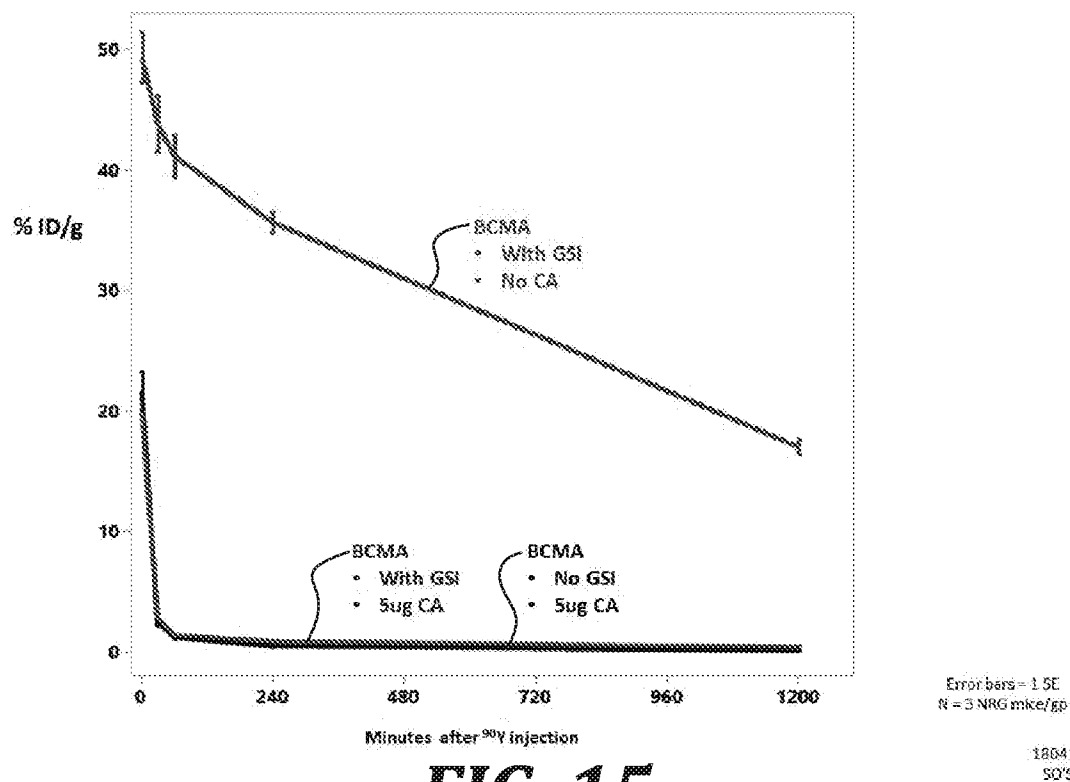
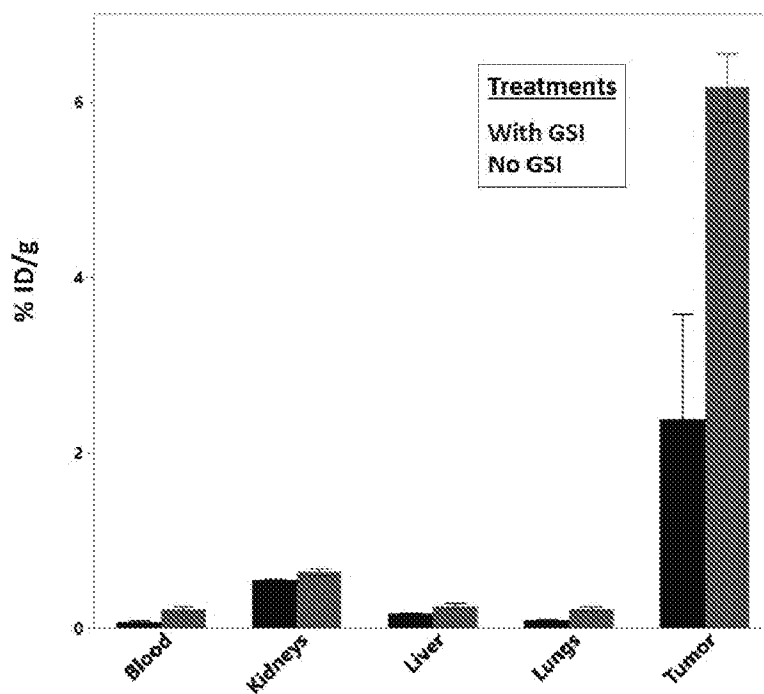


FIG. 14

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Blood clearance (pharmacokinetics) of BCMA bispecific ^{90}Y -PRIT in a xenograft model of MM (H929)**FIG. 15**Biodistribution of BCMA bispecific ^{90}Y -PRIT after 24hrs in a xenograft model of MM
(H929 cell line, NRG mice, n = 5/gp)**FIG. 16**180410
SQ'S

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/59872

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 49/14, G01N 33/50, C07K 16/28 (2019.01)

CPC - A61K 49/14, G01N 33/50, C07K 16/2896, C07K 16/2827

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y ----- A	GREEN et al., Comparative analysis of bispecific antibody and streptavidin-targeted radioimmunotherapy for B cell cancers. Cancer Res. 15 November 2016, Vol. 76, No. 22, pp 6669-6679. Abstract; p6670, col 2, para 2; p6670, Figure 1 legend; p6672, col 1, last para; p6678, col 1, para 4	1-10, 23, (24-29, 34, 35)/(1-10, 23), 63 ----- 11, 12, (24-29, 34, 35)/(11-12)
Y ----- A	US 2017/0107295 A1 (JANSSEN BIOTECH INC.) 20 April 2017 (20.04.2017) Abstract; Claim 1; Claim 5; para [0009]; para [0012]; para [0043]; para [0131]	1-10, 23, (24-29, 34, 35)/(1-10, 23), 63 ----- 11, 12, (24-29, 34, 35)/(11-12)
Y	WO 2010/099536 A2 (MASSACHUSETTS INST TECHNOLOGY et al.) 2 September 2010 (2.09.2010) Claim 1	23, (24-29, 34-35)/23
Y	WO 2001/074384 A1 (ARONEX PHARMACEUTICALS INC.) 11 October 2001 (11.10.2001) Abstract; Claim 1; Claim 3; p8, ln 21-p9, ln 1	35/(1-10,23)
A	WO 2009/082624 A2 (ZYMOGENETICS INC et al.) 2 July 2009 (2.07.2009) para [0351]	11, 12, (24-29, 34, 35)/(11-12)

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

10 February 2019

Date of mailing of the international search report

01 APR 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application no.

PCT/US 18/59872

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 68-76
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
---Please see continuation in first extra sheet -----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-12, 23, (24-29, 34-35)(in part), 63 limited to SEQ ID NOs: 2 and 20

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application no.

PCT/US 18/59872

Continuation of Box No. III. Observations where unity of invention is lacking.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-67, drawn to a bispecific affinity reagent and a method of treating a hematological malignancy in a subject. The bispecific affinity reagent will be searched to the extent that the first binding domain targets CD38 (SEQ ID NO: 2) and the second binding domain targets DOTA (SEQ ID NO: 20). It is believed that claims 1-12, 23, (24-29, 34-35)(in part), 63, encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 2 and 20. Additional bispecific affinity reagent(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected bispecific affinity reagent(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a bispecific affinity agent comprising the first binding domain targets CD20 (SEQ ID NO: 12) and the second binding domain targets DOTA (SEQ ID NO: 21) [note: this is in addition to the first named invention] (claims 1-12, 23, (24-35)(in part), 41-53, 61-63).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

No technical features are shared between the amino acid sequences of binding domains of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of including: a bispecific affinity reagent and a method of treating a hematological malignancy in a subject, these shared technical features are previously disclosed by the article "Comparative analysis of bispecific antibody and streptavidin-targeted radioimmunotherapy for B cell cancers" by Green et al. (Cancer Res., 15 November 2016, Vol 76, No 22, pages 6669-6679) (hereinafter 'Green') and US 2017/0107295 A1 to Janssen Biotech, Inc. (hereinafter 'Janssen').

Green teaches (instant claim 1) a bispecific affinity reagent, comprising: a first binding domain that specifically binds to a target antigen; and a second binding domain that specifically binds to a radioactive ligand (Abstract - 'In this study, we engineered a bispecific fusion protein (FP)...one arm of the FP was an anti-human CD20 antibody (2H7), with the other arm of the FP an anti-chelated radiometal trap for a radiolabeled ligand (yttrium[Y]-DOTA) captured by a very high-affinity anti-Y-DOTA scFv antibody (C825)').

Green teaches (instant claim 41) a method of treating a hematological malignancy in a subject, comprising: administering to the subject a first bispecific affinity reagent, and thereafter administering to the subject a therapeutically effective amount of a radioactive ligand; wherein the first bispecific affinity reagent comprises a first binding domain that specifically binds to a cancer antigen and a second binding domain that specifically binds to the radioactive ligand (Abstract - 'In this study, we engineered a bispecific fusion protein (FP) that evades the limitations imposed by this system. Briefly, one arm of the FP was an anti-human CD20 antibody (2H7), with the other arm of the FP an anti-chelated radiometal trap for a radiolabeled ligand (yttrium[Y]-DOTA) captured by a very high-affinity anti-Y-DOTA scFv antibody (C825) ... Therapy studies in subjects bearing either Ramos or Granta subcutaneous lymphomas demonstrated that 2H7-Fc-C825 PRIT is highly effective and significantly less myelosuppressive than 1F5-SA (P < 0.0001). All animals receiving optimal doses of 2H7-Fc-C825 followed by 90Y-DOTA were cured by 150 days'), wherein the first binding domain of the first bispecific affinity reagent specifically binds to a cancer antigen CD20 (p6670, col 2, para 2 - 'Construction of a bispecific 2H7-Fc-C825 (anti-CD20 # anti-YDOTA) fusion gene'; p6670, Figure 1 legend - B) 'Infusion of the anti-CD20 construct is followed by injection of a synthetic CA (N-acetylgalactosamine or DOTAY-dextran) and then infusion of the radiolabeled small molecule.').

Green does not expressly teach administering a therapeutically effective amount of a second bispecific affinity reagent, wherein the second bispecific affinity reagent comprises a first binding domain against a cancer antigen different from CD38. Janssen teaches a method of treating a subject having a CD38-positive hematological malignancy, with a CD38-binding affinity reagent (Claim 1 - 'A method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 antibody in combination with all-trans retinoic acid (ATRA)'). Since Janssen teaches the anti-CD38 antibody reagent could be bispecific (para [0131] - 'In some embodiments, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is a bispecific antibody.'), and further teaches use of anti-CD38 antibody in a combinatorial treatment (para [0161] - 'The anti-CD38 antibody in the methods of the invention and in some embodiments of each and every one of the numbered embodiments listed below may be administered in combination with all-trans retinoic acid (ATRA) and a third therapeutic agent.'), it would have been obvious to one of ordinary skill in the art that the bispecific anti-CD38 antibody of Janssen could be modified using routine methods to engineer a second binding domain that specifically binds to a radioactive ligand according to Green, and used in combination with the anti-CD20 binding reagent according to Green, to provide a combinatorial immunotherapy against cancer cells that express CD20 and CD38, for a more efficacious therapy due to the combined action of the two antibody reagents.

---continued on next sheet---

INTERNATIONAL SEARCH REPORT

International application no.

PCT/US 18/59872

Continuation of Box No. III. Observations where unity of invention is lacking.

Continuation from prior sheet

Janssen teaches (instant claim 63) a method of treating a malignancy characterized by expression of CD38 (Claim 1 - 'A method of treating a subject having a CD38-positive hematological malignancy'), comprising administering to the subject an amount of all trans retinoic acid (ATRA), or functional derivatives or subunits thereof, sufficient to upregulate expression of CD38 in the malignant cells (para [0009] - 'FIG. 1A shows that all-trans retinoic acid (ATRA) enhances CD38 expression on multiple myeloma (MM) cell lines in a dose dependent manner MM cell lines RPMI8226, UM9 and XG1 were incubated with RPMI-1640 medium alone or with 0-25 nM ATRA for 48 hours and then harvested to determine CD38 expression by flow cytometry.'; para [0012] - 'FIG. 3A shows daratumumab-induced complement-dependent cytotoxicity (CDC) (top panel) and antibody-dependent cell mediated cytotoxicity (ADCC) (bottom panel) in MM XG1 cell line pretreated with or without 10 nM ATRA for 48 hours prior to performing CDC or ADCC in the presence of 10 ?g/ml daratumumab.'),

administering to the subject a therapeutically effective amount of a bispecific affinity reagent comprising a first binding domain that specifically binds to CD38 (Claim 1 - 'A method of treating a subject having a CD38-positive hematological malignancy comprising administering to the subject in need thereof an anti-CD38 antibody in combination with all-trans retinoic acid (ATRA).'; para [0131] - 'In some embodiments, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is a bispecific antibody.').

Janssen does not expressly teach a second binding domain that specifically binds to a radioactive ligand, and thereafter administering to the subject a therapeutically effective amount of a radioactive ligand.

Green teaches use of bispecific antibodies comprising a second binding domain that specifically binds to a radioactive ligand, and thereafter administering to the subject a therapeutically effective amount of a radioactive ligand (Abstract - 'In this study, we engineered a bispecific fusion protein (FP) that evades the limitations imposed by this system. Briefly, one arm of the FP was an anti-human CD20 antibody (2H7), with the other arm of the FP an anti-chelated radiometal trap for a radiolabeled ligand (yttrium[Y]-DOTA) captured by a very high-affinity anti-Y-DOTA scFv antibody (C825)....Therapy studies in subjects bearing either Ramos or Granta subcutaneous lymphomas demonstrated that 2H7-Fc-C825 PRIT is highly effective and significantly less myelosuppressive than 1F5-SA (P < 0.0001). All animals receiving optimal doses of 2H7-Fc-C825 followed by 90Y-DOTA were cured by 150 days'; p6670, Figure 1 legend - 'A and B, schema comparing SA-biotin multistep PRIT (A) and 2H7-Fc-C825 bispecific FP PRIT (B). Infusion of the anti-CD20 construct is followed by injection of a synthetic CA (N-acetylgalactosamine or DOTAY-dextran) and then infusion of the radiolabeled small molecule.').

Since Green teaches radioimmunotherapy with bispecific antibodies is highly effective against cancer, it would have been obvious to one of ordinary skill in the art that the anti-CD38 bispecific antibody of Janssen could be modified using routine methods to engineer a second binding domain that specifically binds to a radioactive ligand according to Green, to provide a highly effective radioimmunotherapy against cancer cells expressing CD38.

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Continuation of Item 4:

Claims 68-76 are improper multiple dependent claims because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).