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DESCRIPTION

Description

FIELD OF THE INVENTION

[0001] The present invention relates to bispecific antigen-binding molecules (*e.g.*, bispecific antibodies) that bind B-cell maturation antigen (BCMA) and CD3, and methods of use thereof.

BACKGROUND

[0002] B-cell maturation antigen (BCMA), also known as TNFRSF17, or CD269, is a type III transmembrane protein lacking a signal peptide and containing a cysteine-rich extracellular domain. BCMA, along with closely related proteins, promotes B-cell survival at distinct stages of development. BCMA is expressed exclusively in B-cell lineage cells, particularly in the interfollicular region of the germinal center as well as on plasmablasts and differentiated plasma cells. BCMA is selectively induced during plasma cell differentiation, and is required for optimal survival of longlived plasma cells in the bone marrow. In multiple myeloma, BCMA is widely expressed on malignant plasma cells at elevated levels, and BCMA expression is increased with progression from normal cells to active multiple myeloma. BCMA is also expressed in other B-cell malignancies, including Waldenström's macroglobulinemia, Burkitt lymphoma, and Diffuse Large B-Cell Lymphoma. Tai et al., Immunotherapy, 7(11):1187-1199, 2015.

[0003] CD3 is a homodimeric or heterodimeric antigen expressed on T cells in association with the T cell receptor complex (TCR) and is required for T cell activation. Functional CD3 is formed from the dimeric association of two of four different chains: epsilon, zeta, delta and gamma. The CD3 dimeric arrangements include gamma/epsilon, delta/epsilon and zeta/zeta. Antibodies against CD3 have been shown to cluster CD3 on T cells, thereby causing T cell activation in a manner similar to the engagement of the TCR by peptide-loaded MHC molecules. Thus, anti-CD3 antibodies have been proposed for therapeutic purposes involving the activation of T cells. In addition, bispecific antibodies that are capable of binding CD3 and a target antigen have been proposed for therapeutic uses involving targeting T cell immune responses to tissues and cells expressing the target antigen.

[0004] WO 2017/031104 discloses anti-BCMA antibodies, bispecific antigen binding molecules that bind BCMA and CD3, and uses thereof. WO 2014/047231 discloses anti-CD3 antibodies, bispecific antigen-binding molecules that bind CD3 and CD20, and uses thereof. WO 2018/067331 discloses bispecific anti-MUC16-CD3 antibodies and NTI-MUC16 drug conjugates.

[0005] Antigen-binding molecules that target BCMA, including bispecific antigen-binding molecules that bind both BCMA and CD3 would be useful in therapeutic settings in which specific targeting and T cell-mediated killing of cells that express BCMA is desired.

BRIEF SUMMARY OF THE INVENTION

[0006] The present invention provides an isolated bispecific antigen-binding molecule, comprising: (a) a first antigen-binding domain that specifically binds a human B cell maturation antigen (BCMA) comprises HCDR1,HCDR2,HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 68, 70, 72, and LCDR1,LCDR2,LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 84, 86,

88; and (b) a second antigen binding domain that specifically binds human CD3 that comprises HCDR1,HCDR2,HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 92, 94, 96, and LCDR1,LCDR2,LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 84, 86, 88. In some cases, the isolated bispecific antigen-binding molecule comprises: (a) a first antigen binding domain that comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 66, and a LCVR comprising the amino acid sequence of SEQ ID NO: 82; and (b) a second antigen binding domain that comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 90, and a LCVR comprising the amino acid sequence of SEQ ID NO: 82.

[0007] In another aspect, the present invention provides an isolated bispecific antigen-binding molecule, comprising: (a) a first antigen-binding domain that specifically binds a human B cell maturation antigen (BCMA) that comprises HCDR1,HCDR2,HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 68, 70, 72, and LCDR1,LCDR2,LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 84, 86, 88; and (b) a second antigen binding domain that specifically binds human CD3 that comprises HCDR1,HCDR2,HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 100, 102, 104, and LCDR1,LCDR2,LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 84, 86, 88. In some cases, the isolated bispecific antigen-binding molecule comprises: (a) a first antigen binding domain that comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 66, and a LCVR comprising the amino acid sequence of SEQ ID NO: 82; and (b) a second antigen binding domain that comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 98, and a LCVR comprising the amino acid sequence of SEQ ID NO: 82.

[0008] Any of the bispecific antigen-binding molecules discussed above or herein may be a bispecific antibody. In some cases, the bispecific antibody comprises a human IgG heavy chain constant region. In some cases, the human IgG heavy chain constant region is isotype IgG1. In some cases, the human IgG heavy chain constant region is isotype IgG4. In various embodiments, the bispecific antibody comprises a chimeric hinge that reduces Fcγ receptor binding relative to a wild-type hinge of the same isotype.

[0009] In another aspect, the present invention provides a pharmaceutical composition comprising the bispecific antigen-binding molecule (*e.g.*, bispecific antibody) of the invention, and a pharmaceutically acceptable carrier or diluent.

[0010] In another aspect, the present invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a bispecific antigen-binding molecule (*e.g.*, bispecific antibody) of the invention.

[0011] In another aspect, the present invention provides an expression vector comprising the nucleic acid molecule of the invention.

[0012] In another aspect, the present invention provides a host cell comprising the expression vector of the invention.

[0013] In another aspect, the present invention provides an isolated bispecific antigen-binding molecule of the invention, or a pharmaceutical composition of the invention, for use in a method of inhibiting growth of a plasma cell tumor in a subject, comprising administering the isolated bispecific antigen-binding molecule, or the pharmaceutical composition comprising the bispecific antigen-binding molecule to the subject. In some cases, the plasma cell tumor is multiple myeloma. In some cases, the method further comprises administering a second therapeutic agent, or therapeutic regimen. In some embodiments, the second therapeutic agent comprises an anti-tumor agent (*e.g.* chemotherapeutic agents including melphalan, vincristine (Oncovin), cyclophosphamide (Cytoxan), etoposide (VP-16), doxorubicin (Adriamycin), liposomal doxorubicin (Doxil), obendamustine (Treanda), or any others known to be effective in treating a plasma cell tumor in a subject.). In some embodiments, the second therapeutic agent comprises steroids. In some embodiments, the second therapeutic agent comprises targeted therapies including thalidomide, lenalidomide, and bortezomib, which are therapies approved to treat newly diagnosed patients. Lenalidomide, pomalidomide, bortezomib, carfilzomib, panobinostat, ixazomib, elotuzumab, and daratumumab are examples of a second therapeutic agent effective for treating recurrent

myeloma. In certain embodiments the second therapeutic agent is a regimen comprising radiotherapy or a stem cell transplant. In certain embodiments, the second therapeutic agent may be an immunomodulatory agent. In certain embodiments, the second therapeutic agent may be a proteasome inhibitor, including bortezomib (Velcade), carfilzomib (Kyprolis), ixazomib (Ninlaro). In certain embodiments the second therapeutic agent may be a histone deacetylase inhibitor such as panobinostat (Farydak). In certain embodiments, the second therapeutic agent may be a monoclonal antibody, an antibody drug conjugate, a bispecific antibody conjugated to an anti-tumor agent, a checkpoint inhibitor, or combinations thereof.

[0014] In another aspect, the present invention provides an isolated bispecific antigen-binding molecule of the invention, or a pharmaceutical composition of the invention, for use in a method of treating a patient suffering from multiple myeloma, or from another BCMA-expressing B cell malignancy, where the method comprises administering the isolated bispecific antigen-binding molecule or the pharmaceutical composition comprising the bispecific antigen-binding molecule to the subject. In some cases, the BCMA-expressing B cell malignancy is selected from the group consisting of Waldenström's macroglobulinemia, Burkitt's lymphoma and Diffuse Large B-Cell lymphoma, Non-Hodgkin's lymphoma, chronic lymphocytic leukemia, follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, lymphoplasmacytic lymphoma, and Hodgkin's lymphoma. In some cases, the method further comprises administering a second therapeutic agent. In some embodiments, the second therapeutic agent comprises an anti-tumor agent (a chemotherapeutic agent), DNA alkylators, immunomodulators, proteasome inhibitors, histone deacetylase inhibitors radiotherapy, a stem cell transplant, an immunomodulator, a monoclonal antibody that interacts with an antigen expressed on the tumor cell surface, a monoclonal antibody other than those described herein, which may interact with a different antigen on the plasma cell surface, a bispecific antibody, which has one arm that binds to an antigen on the tumor cell surface and the other arm binds to an antigen on a T cell, an antibody drug conjugate, a bispecific antibody conjugated with an anti-tumor agent, a checkpoint inhibitor, for example, one that targets, PD-1 or CTLA-4, or combinations thereof. In certain embodiments, the checkpoint inhibitors may be selected from PD-1 inhibitors, such as pembrolizumab (Keytruda), nivolumab (Opdivo), or cemiplimab (REGN2810). In certain embodiments, the checkpoint inhibitors may be selected from PD-L1 inhibitors, such as atezolizumab (Tecentriq), avelumab (Bavencio), or Durvalumab (Imfinzi)). In certain embodiments, the checkpoint inhibitors may be selected from CTLA-4 inhibitors, such as ipilimumab (Yervoy). Other combinations that may be used in conjunction with an antibody of the invention are described above.

[0015] In another aspect, the present invention provides an isolated bispecific antigen-binding molecule of the invention, or a pharmaceutical composition of the invention, for use in a method of treating a patient suffering from a BCMA-expressing tumor, wherein the method comprises administering the isolated bispecific antigen-binding molecule, or the pharmaceutical composition, to the subject in combination with an anti-PD-1 antibody or antigen-binding fragment thereof. In some cases, the anti-PD-1 antibody or antigen-binding fragment is an anti-PD-1 antibody. In some embodiments, the anti-PD-1 antibody is cemiplimab (REGN2810). In various embodiments, the combination of anti-BCMA x anti-CD3 bispecific antigen-binding molecule (*e.g.*, a bispecific antibody) and an anti-PD-1 antibody or antigen-binding fragment (*e.g.*, an anti-PD-1 antibody) produces a synergistic therapeutic effect in the treatment of BCMA-expressing tumors.

[0016] Other aspects will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017]

Figures 1 and 2 illustrate prophylactic dose-dependent tumor inhibition of BCMA-expressing NCI-H929 human multiple myeloma tumor cells *in vivo* by anti-BCMA x anti-CD3 bispecific antibodies REGN5458 and REGN5459, respectively. NCI-H929 cells express high levels of BCMA.

Figures 3 and 4 illustrate therapeutic dose-dependent tumor inhibition of established BCMA-expressing NCI-H929 human multiple myeloma tumor cells *in vivo* by anti-BCMA x anti-CD3 bispecific antibodies REGN5458 and

REGN5459, respectively. NCI-H929 cells express high levels of BCMA.

Figures 5 and 6 illustrate prophylactic dose-dependent tumor inhibition of BCMA-expressing MOLP-8 human multiple myeloma tumor cells *in vivo* by anti-BCMA x anti-CD3 bispecific antibodies REGN5458 and REGN5459, respectively. MOLP-8 cells express moderate levels of BCMA.

Figure 7 illustrates a therapeutic reduction in established tumor burden of BCMA-expressing OPM-2 human multiple myeloma tumor cells *in vivo* by anti-BCMA x anti-CD3 bispecific antibodies REGN5458 and REGN5459, relative to controls. OPM-2 cells express low levels of BCMA.

DETAILED DESCRIPTION

[0018] The technical disclosure set out below may in some respects go beyond the scope of the claims. Elements of the disclosure which do not fall within the scope of the claims are provided for information. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

Definitions

[0019] The expression "CD3," as used herein, refers to an antigen which is expressed on T cells as part of the multimolecular T cell receptor (TCR) and which consists of a homodimer or heterodimer formed from the association of two of four receptor chains: CD3-epsilon, CD3-delta, CD3-zeta, and CD3-gamma. Human CD3-epsilon comprises the amino acid sequence as set forth in SEQ ID NO:116; human CD3-delta comprises the amino acid sequence as set forth in SEQ ID NO:117; human CD3-zeta comprises the amino acid sequence as set forth in SEQ ID NO: 118; and CD3-gamma comprises the amino acid sequence as set forth in SEQ ID NO 119. All references to proteins, polypeptides and protein fragments herein are intended to refer to the human version of the respective protein, polypeptide or protein fragment unless explicitly specified as being from a non-human species. Thus, the expression "CD3" means human CD3 unless specified as being from a non-human species, *e.g.,* "mouse CD3," "monkey CD3," etc.

[0020] As used herein, "an antibody that binds CD3" or an "anti-CD3 antibody" includes antibodies and antigen-binding fragments thereof that specifically recognize a single CD3 subunit (*e.g.,* epsilon, delta, gamma or zeta), as well as antibodies and antigen-binding fragments thereof that specifically recognize a dimeric complex of two CD3 subunits (*e.g.,* gamma/epsilon, delta/epsilon, and zeta/zeta CD3 dimers). The antibodies and antigen-binding fragments of the present invention may bind soluble CD3 and/or cell surface expressed CD3. Soluble CD3 includes natural CD3 proteins as well as recombinant CD3 protein variants such as, *e.g.,* monomeric and dimeric CD3 constructs, that lack a transmembrane domain or are otherwise unassociated with a cell membrane.

[0021] As used herein, the expression "cell surface-expressed CD3" means one or more CD3 protein(s) that is/are expressed on the surface of a cell *in vitro* or *in vivo,* such that at least a portion of a CD3 protein is exposed to the extracellular side of the cell membrane and is accessible to an antigen-binding portion of an antibody. "Cell surface-expressed CD3" includes CD3 proteins contained within the context of a functional T cell receptor in the membrane of a cell. The expression "cell surface-expressed CD3" includes CD3 protein expressed as part of a homodimer or heterodimer on the surface of a cell (*e.g.,* gamma/epsilon, delta/epsilon, and zeta/zeta CD3 dimers). The expression, "cell surface-expressed CD3" also includes a CD3 chain (*e.g.,* CD3-epsilon, CD3-delta or CD3-gamma) that is expressed by itself, without other CD3 chain types, on the surface of a cell. A "cell surface-

expressed CD3" can comprise or consist of a CD3 protein expressed on the surface of a cell which normally expresses CD3 protein. Alternatively, "cell surface-expressed CD3" can comprise or consist of CD3 protein expressed on the surface of a cell that normally does not express human CD3 on its surface but has been artificially engineered to express CD3 on its surface.

[0022] The expression "BCMA," as used herein, refers to B-cell maturation antigen. BCMA (also known as TNFRSF17 and CD269) is a cell surface protein expressed on malignant plasma cells, and plays a central role in regulating B cell maturation and differentiation into immunoglobulinproducing plasma cells. The amino acid sequence of human BCMA is shown in SEQ ID NO: 115, and can also be found in GenBank accession number NP_001183.2.

[0023] As used herein, "an antibody that binds BCMA" or an "anti-BCMA antibody" includes antibodies and antigen-binding fragments thereof that specifically recognize BCMA.

[0024] The term "antigen-binding molecule" includes antibodies and antigen-binding fragments of antibodies, including, *e.g.,* bispecific antibodies.

[0025] The term "antibody", as used herein, means any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen (*e.g.,* BCMA or CD3). The term "antibody" includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.,* IgM). The term "antibody" also includes immunoglobulin molecules consisting of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from aminoterminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti-BCMA antibody or anti-CD3 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0026] The term "antibody", as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.,* from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.,* commercial sources, DNA libraries (including, *e.g.,* phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0027] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.,* an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-

deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0028] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0029] In certain aspects, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.,* 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.,* by disulfide bond(s)).

[0030] As with full antibody molecules, antigen-binding fragments may be monospecific or multispecific (*e.g.,* bispecific). A multispecific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format, including the exemplary bispecific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

[0031] The antibodies of the present invention may function through complement-dependent cytotoxicity (CDC) or antibody-dependent cell-mediated cytotoxicity (ADCC). "Complement-dependent cytotoxicity" (CDC) refers to lysis of antigen-expressing cells by an antibody of the invention in the presence of complement. "Antibody-dependent cell-mediated cytotoxicity" (ADCC) refers to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (*e.g.*, Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and thereby lead to lysis of the target cell. CDC and ADCC can be measured using assays that are well known and available in the art. (*See, e.g.,* U.S. Patent Nos 5,500,362 and 5,821,337, and Clynes et al. (1998) Proc. Natl. Acad. Sci. (USA) 95:652-656). The constant region of an antibody is important in the ability of an antibody to fix complement and mediate celldependent cytotoxicity. Thus, the isotype of an antibody may be selected on the basis of whether it is desirable for the antibody to mediate cytotoxicity.

[0032] In certain embodiments of the invention, the anti-BCMA x anti-CD3 bispecific antibodies of the invention are human antibodies. The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. Human antibodies may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0033] The antibodies of the invention may, in some embodiments, be recombinant human antibodies. The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo.*

[0034] Human antibodies can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification.

[0035] The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. A single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. The instant invention encompasses antibodies having one or more mutations in the hinge, C_H2 or C_H3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[0036] The antibodies of the invention are isolated antibodies. An "isolated antibody," as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an "isolated antibody" for purposes of the present invention. An isolated antibody also includes an antibody *in situ* within a recombinant cell. Isolated antibodies are antibodies that have been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0037] The present disclosure also includes one-arm antibodies that bind BCMA. As used herein, a "one-arm antibody" means an antigen-binding molecule comprising a single antibody heavy chain and a single antibody light chain. The one-arm antibodies may comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 1.

[0038] The anti-BCMA or anti-BCMA x anti-CD3 antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the antibodies were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present disclosure includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences

disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain aspects, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other aspects, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other aspects, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.,* a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present disclosure may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present disclosure.

[0039] The present disclosure also includes anti-BCMA or anti-BCMA x anti-CD3 antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present disclosure includes anti-BCMA or anti-BCMA x anti-CD3 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences set forth in Tables 1 and 3 herein, or the anti-CD3 antibodies disclosed in WO 2014/047231 or WO 2017/053856.

[0040] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

[0041] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95%, and more preferably at least about 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[0042] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. *See, e.g.,* Pearson (1994) Methods Mol. Biol. 24: 307-331. Examples of groups of amino acids that have side chains with similar chemical properties include (1)

aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagineglutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al. (1992) Science 256: 1443-1445. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0043] Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. *See, e.g.,* GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) supra). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. *See, e.g.,* Altschul et al. (1990) J. Mol. Biol. 215:403-410 and Altschul et al. (1997) Nucleic Acids Res. 25:3389-402.

Germline Mutations

[0044] The anti-CD3 antibodies disclosed herein comprise one or more amino acid substitutions, insertions and/or deletions in the framework regions of the heavy chain variable domains as compared to the corresponding germline sequences from which the antibodies were derived.

[0045] The present disclosure also includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"), and having weak or no detectable binding to a CD3 antigen.

[0046] Furthermore, the antibodies of the present disclosure may contain any combination of two or more germline mutations within the framework regions, *e.g.,* wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be tested for one or more desired properties such as, improved binding specificity, weak or reduced binding affinity, improved or enhanced pharmacokinetic properties, reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner given the guidance of the present disclosure are encompassed within the present disclosure.

[0047] The present also includes antigen-binding molecules comprising an antigen-binding domain with an HCVR amino acid sequence that is substantially identical to any of the HCVR amino acid sequences disclosed herein, while maintaining or improving the desired weak affinity to CD3 antigen. The term "substantial identity" or "substantially identical," when referring to an amino acid sequence means that two amino acid sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95%

sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. *See, e.g.,* Pearson (1994) Methods Mol. Biol. 24: 307-331.

[0048] Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. *See, e.g.,* GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) supra). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. *See, e.g.,* Altschul et al. (1990) J. Mol. Biol. 215:403-410 and Altschul et al. (1997) Nucleic Acids Res. 25:3389-402.

Binding Properties of the Antibodies

[0049] As used herein, the term "binding" in the context of the binding of an antibody, immunoglobulin, antibody-binding fragment, or Fc-containing protein to either, *e.g.,* a predetermined antigen, such as a cell surface protein or fragment thereof, typically refers to an interaction or association between a minimum of two entities or molecular structures, such as an antibody-antigen interaction.

[0050] For instance, binding affinity typically corresponds to a K_D value of about 10^{-7} M or less, such as about 10^{-8} M or less, such as about 10^{-9} M or less when determined by, for instance, surface plasmon resonance (SPR) technology in a BIAcore 3000 instrument using the antigen as the ligand and the antibody, Ig, antibody-binding fragment, or Fc-containing protein as the analyte (or antiligand). Cell-based binding strategies, such as fluorescent-activated cell sorting (FACS) binding assays, are also routinely used, and FACS data correlates well with other methods such as radioligand competition binding and SPR (Benedict, CA, J Immunol Methods. 1997, 201(2):223-31; Geuijen, CA, et al. J Immunol Methods. 2005, 302(1-2):68-77).

[0051] Accordingly, the antibody or antigen-binding protein of the invention binds to the predetermined antigen or cell surface molecule (receptor) having an affinity corresponding to a K_D value that is at least ten-fold lower than its affinity for binding to a non-specific antigen (e.g., BSA, casein). According to the present invention, the affinity of an antibody corresponding to a K_D value that is equal to or less than ten-fold lower than a non-specific antigen may be considered nondetectable binding, however such an antibody may be paired with a second antigen binding arm for the production of a bispecific antibody of the invention.

[0052] The term "K_D" (M) refers to the dissociation equilibrium constant of a particular antibody-antigen interaction, or the dissociation equilibrium constant of an antibody or antibody-binding fragment binding to an antigen. There is an inverse relationship between K_D and binding affinity, therefore the smaller the K_D value, the higher, i.e. stronger, the affinity. Thus, the terms "higher affinity" or "stronger affinity" relate to a higher ability to form an interaction and therefore a smaller K_D value, and conversely the terms "lower affinity" or "weaker affinity" relate to a lower ability to form an interaction and therefore a larger K_D value. In some circumstances, a higher binding affinity (or K_D) of a particular molecule (*e.g.* antibody) to its interactive partner molecule (*e.g.* antigen X) compared to the binding affinity of the molecule (*e.g.* antibody) to another interactive partner molecule (*e.g.*

antigen Y) may be expressed as a binding ratio determined by dividing the larger K_D value (lower, or weaker, affinity) by the smaller K_D (higher, or stronger, affinity), for example expressed as 5-fold or 10-fold greater binding affinity, as the case may be.

[0053] The term "k_d" (sec -1 or 1/s) refers to the dissociation rate constant of a particular antibody-antigen interaction, or the dissociation rate constant of an antibody or antibody-binding fragment. Said value is also referred to as the k_{off} value.

[0054] The term "k_a" (M-1 x sec-1 or 1/M) refers to the association rate constant of a particular antibody-antigen interaction, or the association rate constant of an antibody or antibody-binding fragment.

[0055] The term "K_a" (M-1 or 1/M) refers to the association equilibrium constant of a particular antibody-antigen interaction, or the association equilibrium constant of an antibody or antibody-binding fragment. The association equilibrium constant is obtained by dividing the k_a by the k_d.

[0056] The term "EC50" or "EC_{50}" refers to the half maximal effective concentration, which includes the concentration of an antibody which induces a response halfway between the baseline and maximum after a specified exposure time. The EC_{50} essentially represents the concentration of an antibody where 50% of its maximal effect is observed. In certain embodiments, the EC_{50} value equals the concentration of an antibody of the invention that gives half-maximal binding to cells expressing CD3 or tumor-associated antigen (*e.g.,* BCMA), as determined by *e.g.* a FACS binding assay. Thus, reduced or weaker binding is observed with an increased EC_{50}, or half maximal effective concentration value.

[0057] In one embodiment, decreased binding can be defined as an increased EC_{50} antibody concentration which enables binding to the half-maximal amount of target cells.

[0058] In another embodiment, the EC_{50} value represents the concentration of an antibody of the invention that elicits half-maximal depletion of target cells by T cell cytotoxic activity. Thus, increased cytotoxic activity (e.g. T cell-mediated tumor cell killing) is observed with a decreased EC_{50}, or half maximal effective concentration value.

Bispecific Antigen-Binding Molecules

[0059] The antibodies of the present disclosure may be monospecific, bi-specific, or multispecific. Multispecific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, e.g., Tutt et al., 1991, J. Immunol. 147:60-69; Kufer et al., 2004, Trends Biotechnol. 22:238-244. The anti-BCMA x anti-CD3 bispecific antibodies of the present invention can be linked to or co-expressed with another functional molecule, e.g., another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment to produce a bi-specific or a multispecific antibody with a second or additional binding specificity.

[0060] Use of the expression "anti-CD3 antibody" or "anti-BCMA antibody" herein is intended to include both monospecific anti-CD3 or anti-BCMA antibodies as well as bispecific antibodies comprising a CD3-binding arm and a BCMA-binding arm. The present invention provides bispecific antibodies as defined in the claims wherein one arm of an immunoglobulin binds human CD3, and the other arm of the immunoglobulin is specific for human BCMA.

[0061] In certain instances, the CD3-binding arm binds to human CD3 and induces human T cell activation. In certain instances, the CD3-binding arm binds weakly to human CD3 and induces human T cell activation. In other instances, the CD3-binding arm binds weakly to human CD3 and induces tumor-associated antigen-expressing

cell killing in the context of a bispecific or multispecific antibody. In other instances, the CD3-binding arm binds or associates weakly with human and cynomolgus (monkey) CD3, yet the binding interaction is not detectable by *in vitro* assays known in the art. The BCMA-binding arm can comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 1 herein.

[0062] The present invention provides bispecific antigen-binding molecules as defined in the claims that specifically bind CD3 and BCMA. Such molecules may be referred to herein as, *e.g.,* "anti-BCMA x anti-CD3" or "anti-CD3/anti-BCMA," or "anti-CD3xBCMA" or "CD3xBCMA" bispecific molecules, or other similar terminology (*e.g.,* anti-BCMA/anti-CD3).

[0063] The term "BCMA," as used herein, refers to the human BCMA protein unless specified as being from a non-human species (*e.g.,* "mouse BCMA," "monkey BCMA," etc.). The human BCMA protein has the amino acid sequence shown in SEQ ID NO: 115.

[0064] The aforementioned bispecific antigen-binding molecules that specifically bind CD3 and BCMA may comprise an anti-CD3 antigen-binding molecule which binds to CD3 with a weak binding affinity such as exhibiting a K_D of greater than about 40 nM, as measured by an *in vitro* affinity binding assay.

[0065] As used herein, the expression "antigen-binding molecule" means a protein, polypeptide or molecular complex comprising or consisting of at least one complementarity determining region (CDR) that alone, or in combination with one or more additional CDRs and/or framework regions (FRs), specifically binds to a particular antigen. In certain aspects, an antigen-binding molecule is an antibody or a fragment of an antibody, as those terms are defined elsewhere herein.

[0066] As used herein, the expression "bispecific antigen-binding molecule" means a protein, polypeptide or molecular complex comprising at least a first antigen-binding domain and a second antigen-binding domain. Each antigen-binding domain within the bispecific antigen-binding molecule comprises at least one CDR that alone, or in combination with one or more additional CDRs and/or FRs, specifically binds to a particular antigen. In the context of the present invention, the first antigen-binding domain specifically binds a first antigen (BCMA), and the second antigen-binding domain specifically binds a second, distinct antigen (CD3).

[0067] In certain exemplary embodiments of the present invention, the bispecific antigen-binding molecule is a bispecific antibody. Each antigen-binding domain of a bispecific antibody comprises a heavy chain variable domain (HCVR) and a light chain variable domain (LCVR). In the context of a bispecific antigen-binding molecule comprising a first and a second antigen-binding domain (*e.g.,* a bispecific antibody), the CDRs of the first antigen-binding domain may be designated with the prefix "D1" and the CDRs of the second antigen-binding domain may be designated with the prefix "D2". Thus, the CDRs of the first antigen-binding domain may be referred to herein as D1-HCDR1, D1-HCDR2, and D1-HCDR3; and the CDRs of the second antigen-binding domain may be referred to herein as D2-HCDR1, D2-HCDR2, and D2-HCDR3.

[0068] In certain exemplary aspects of the disclosure, the isolated bispecific antigen binding molecule comprises a first antigen-binding domain that comprises: (a) three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 66; and (b) three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO:82. In the invention, the isolated bispecific antigen binding molecule comprises a HCDR1 comprising the amino acid sequence of SEQ ID NO:68, a HCDR2 comprising the amino acid sequence of SEQ ID NO:70, and a HCDR3 comprising the amino acid sequence of SEQ ID NO:72. In the invention, the isolated bispecific antigen-binding molecule comprises a LCDR1 comprising the amino acid sequence of SEQ ID NO:84, a LCDR2 comprising the amino acid sequence of SEQ ID NO:86, and a LCDR3 comprising the amino acid sequence of SEQ ID NO:88. In some cases, the first antigen-binding domain comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 66, and a LCVR comprising the amino acid sequence of SEQ ID NO: 82.

[0069] In certain exemplary aspects of the disclosure, the isolated bispecific antigen-binding molecule comprises a second antigen-binding domain that comprises: (a) three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 90 or SEQ ID NO: 98; and (b) three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO:82. In the invention, the second antigen-binding domain comprises a LCDR1 comprising the amino acid sequence of SEQ ID NO:84, a LCDR2 comprising the amino acid sequence of SEQ ID NO:86, and a LCDR3 comprising the amino acid sequence of SEQ ID NO:88. In the invention, the second antigen-binding domain comprises: (a) HCDR1,HCDR2,HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 92, 94, 96; and LCDR1,LCDR2,LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 84, 86, 88; or (b) HCDR1, HCDR2, HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 100, 102, 104; and LCDR1, LCDR2, LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 84, 86, 88. In some cases, the second antigen-binding domain comprises: (a) a HCVR comprising the amino acid sequence of SEQ ID NO: 90, and a LCVR comprising the amino acid sequence of SEQ ID NO: 82; or (b) a HCVR comprising the amino acid sequence of SEQ ID NO: 98, and a LCVR comprising the amino acid sequence of SEQ ID NO: 82.

[0070] In one embodiment, the isolated bispecific antigen-binding molecule comprises: (a) a first antigen-binding domain that comprises HCDR1,HCDR2,HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 68, 70, 72, and LCDR1,LCDR2,LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 84, 86, 88; and (b) a second antigen binding domain that comprises HCDR1,HCDR2,HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 92, 94, 96, and LCDR1,LCDR2,LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 84, 86, 88. In some cases, the isolated bispecific antigen-binding molecule comprises: (a) a first antigen binding domain that comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 66, and a LCVR comprising the amino acid sequence of SEQ ID NO: 82; and (b) a second antigen binding domain that comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 90, and a LCVR comprising the amino acid sequence of SEQ ID NO: 82.

[0071] In another embodiment, the isolated bispecific antigen-binding molecule comprises: (a) a first antigen-binding domain that comprises HCDR1,HCDR2,HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 68, 70, 72, and LCDR1,LCDR2,LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 84, 86, 88; and (b) a second antigen binding domain that comprises HCDR1,HCDR2,HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 100, 102, 104, and LCDR1,LCDR2,LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 84, 86, 88. In some cases, the isolated bispecific antigen-binding molecule comprises: (a) a first antigen binding domain that comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 66, and a LCVR comprising the amino acid sequence of SEQ ID NO: 82; and (b) a second antigen binding domain that comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 98, and a LCVR comprising the amino acid sequence of SEQ ID NO: 82.

[0072] The bispecific antigen-binding molecules discussed above or herein may be bispecific antibodies. In some cases, the bispecific antibody comprises a human IgG heavy chain constant region. In some cases, the human IgG heavy chain constant region is isotype IgG1. In some cases, the human IgG heavy chain constant region is isotype IgG4. In various embodiments, the bispecific antibody comprises a chimeric hinge that reduces Fcγ receptor binding relative to a wild-type hinge of the same isotype.

[0073] The first antigen-binding domain and the second antigen-binding domain may be directly or indirectly connected to one another to form a bispecific antigen-binding molecule of the present invention. Alternatively, the first antigen-binding domain and the second antigen-binding domain may each be connected to a separate multimerizing domain. The association of one multimerizing domain with another multimerizing domain facilitates the association between the two antigen-binding domains, thereby forming a bispecific antigen-binding molecule. As used herein, a "multimerizing domain" is any macromolecule, protein, polypeptide, peptide, or amino acid that has the ability to associate with a second multimerizing domain of the same or similar structure or constitution. For

example, a multimerizing domain may be a polypeptide comprising an immunoglobulin C_H3 domain. A non-limiting example of a multimerizing component is an Fc portion of an immunoglobulin (comprising a C_H2-C_H3 domain), *e.g.,* an Fc domain of an IgG selected from the isotypes IgG1, IgG2, IgG3, and IgG4, as well as any allotype within each isotype group.

[0074] Bispecific antigen-binding molecules of the present invention will typically comprise two multimerizing domains, e.g., two Fc domains that are each individually part of a separate antibody heavy chain. The first and second multimerizing domains may be of the same IgG isotype such as, *e.g.,* IgG1/IgG1, IgG2/IgG2, IgG4/IgG4. Alternatively, the first and second multimerizing domains may be of different IgG isotypes such as, *e.g.,* IgG1/IgG2, IgG1/IgG4, IgG2/IgG4, etc.

[0075] In certain embodiments, the multimerizing domain is an Fc fragment or an amino acid sequence of from 1 to about 200 amino acids in length containing at least one cysteine residue. In other embodiments, the multimerizing domain is a cysteine residue, or a short cysteine-containing peptide. Other multimerizing domains include peptides or polypeptides comprising or consisting of a leucine zipper, a helix-loop motif, or a coiled-coil motif.

[0076] Any bispecific antibody format or technology may be used to make the bispecific antigen-binding molecules of the present invention. For example, an antibody or fragment thereof having a first antigen binding specificity can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment having a second antigen-binding specificity to produce a bispecific antigen-binding molecule. Specific exemplary bispecific formats that can be used in the context of the present invention include, without limitation, *e.g.,* scFv-based or diabody bispecific formats, IgGscFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.,* common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab^2 bispecific formats (*see, e.g.,* Klein et al. 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats).

[0077] In the context of bispecific antigen-binding molecules of the present invention, the multimerizing domains, *e.g.,* Fc domains, may comprise one or more amino acid changes *(e.g.,* insertions, deletions or substitutions) as compared to the wild-type, naturally occurring version of the Fc domain. For example, the invention includes bispecific antigen-binding molecules comprising one or more modifications in the Fc domain that results in a modified Fc domain having a modified binding interaction (*e.g.,* enhanced or diminished) between Fc and FcRn. In one embodiment, the bispecific antigen-binding molecule comprises a modification in a C_H2 or a C_H3 region, wherein the modification increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0). Non-limiting examples of such Fc modifications include, *e.g.,* a modification at position 250 *(e.g.,* E or Q); 250 and 428 *(e.g.,* L or F); 252 *(e.g.,* L/Y/F/W or T), 254 *(e.g.,* S or T), and 256 *(e.g.,* S/R/Q/E/D or T); or a modification at position 428 and/or 433 *(e.g.,* L/R/S/P/Q or K) and/or 434 *(e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (e.g., 308F, V308F), and 434. In one embodiment, the modification comprises a 428L *(e.g.,* M428L) and 434S *(e.g.,* N434S) modification; a 428L, 259I *(e.g.,* V259I), and 308F *(e.g.,* V308F) modification; a 433K *(e.g.,* H433K) and a 434 *(e.g.,* 434Y) modification; a 252, 254, and 256 *(e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (e.g., T250Q and M428L); and a 307 and/or 308 modification (e.g., 308F or 308P).

[0078] The present invention also includes bispecific antigen-binding molecules comprising a first C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bispecific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise a Y96F modification (by IMGT; Y436F by EU). See, for example, US Patent No. 8,586,713. Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and

V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies.

[0079] In certain embodiments, the Fc domain may be chimeric, combining Fc sequences derived from more than one immunoglobulin isotype. For example, a chimeric Fc domain can comprise part or all of a C_H2 sequence derived from a human IgG1, human IgG2 or human IgG4 C_H2 region, and part or all of a C_H3 sequence derived from a human IgG1, human IgG2 or human IgG4. A chimeric Fc domain can also contain a chimeric hinge region. For example, a chimeric hinge may comprise an "upper hinge" sequence, derived from a human IgG1, a human IgG2 or a human IgG4 hinge region, combined with a "lower hinge" sequence, derived from a human IgG1, a human IgG2 or a human IgG4 hinge region. A particular example of a chimeric Fc domain that can be included in any of the antigen-binding molecules set forth herein comprises, from N- to C-terminus: [IgG4 C_H1] - [IgG4 upper hinge] - [IgG2 lower hinge] - [IgG4 CH2] - [IgG4 CH3]. Another example of a chimeric Fc domain that can be included in any of the antigen-binding molecules set forth herein comprises, from N- to C-terminus: [IgG1 C_H1] - [IgG1 upper hinge] - [IgG2 lower hinge] - [IgG4 CH2] - [IgG1 CH3]. These and other examples of chimeric Fc domains that can be included in any of the antigen-binding molecules of the present invention are described in US Publication 2014/0243504, published August 28, 2014. Chimeric Fc domains having these general structural arrangements, and variants thereof, can have altered Fc receptor binding, which in turn affects Fc effector function.

Sequence Variants

[0080] The antibodies and bispecific antigen-binding molecules of the present disclosure may comprise one or more amino acid substitutions, insertions and/or deletions in the framework regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the individual antigen-binding domains were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The antigen-binding molecules of the present disclosure may comprise antigen-binding domains which are derived from any of the exemplary amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain aspects, all of the framework residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antigen-binding domain was originally derived. In other aspects, only certain residues are mutated back to the original germline sequence, *e.g.,* only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4. In other aspects, one or more of the framework residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.,* a germline sequence that is different from the germline sequence from which the antigen-binding domain was originally derived). Furthermore, the antigen-binding domains may contain any combination of two or more germline mutations within the framework regions, *e.g.,* wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antigen-binding domains that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Bispecific antigen-binding molecules comprising one or more antigen-binding domains obtained in this general manner are encompassed within the present disclosure.

Antibodies Comprising Fc Variants

[0081] According to certain embodiments of the present invention anti-BCMA x anti-CD3 bispecific antigen-binding molecules are provided comprising an Fc domain comprising one or more mutations which enhance or diminish antibody binding to the FcRn receptor, *e.g.,* at acidic pH as compared to neutral pH. For example, the present invention includes antibodies comprising a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.,* a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., H/L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L *(e.g.,* M428L) and 434S *(e.g.,* N434S) modification; a 428L, 259I *(e.g.,* V259I), and 308F *(e.g.,* V308F) modification; a 433K *(e.g.,* H433K) and a 434 *(e.g.,* 434Y) modification; a 252, 254, and 256 (e.g., 252Y, 254T, and 256E) modification; a 250Q and 428L modification *(e.g.,* T250Q and M428L); and a 307 and/or 308 modification *(e.g.,* 308F or 308P).

[0082] For example, the present invention includes anti-BCMA x anti-CD3 bispecific antigen-binding molecules, comprising an Fc domain comprising one or more pairs or groups of mutations selected from the group consisting of: 250Q and 248L (*e.g.,* T250Q and M248L); 252Y, 254T and 256E *(e.g.,* M252Y, S254T and T256E); 428L and 434S *(e.g.,* M428L and N434S); and 433K and 434F (*e.g.,* H433K and N434F). All possible combinations of the foregoing Fc domain mutations, and other mutations within the antibody variable domains disclosed herein, are contemplated .

Biological Characteristics of the Antibodies and Bispecific Antigen-Binding Molecules

[0083] The present disclosure includes antibodies and antigen-binding fragments thereof that bind human BCMA with high affinity (*e.g.,* nanomolar or sub-nanomolar K_D values).

[0084] According to certain aspects, the present disclosure includes antibodies and antigen-binding fragments of antibodies that bind human BCMA (*e.g.,* at 25°C) with a K_D of less than about 5 nM as measured by surface plasmon resonance, *e.g.,* using an assay format as defined in Example 4 herein. In certain aspects, the antibodies or antigen-binding fragments bind BCMA with a K_D of less than about 20 nM, less than about 10 nM, less than about 8 nM, less than about 7 nM, less than about 6 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, less than about 800 pM, less than about 700 pM, less than about 500 pM, less than about 400 pM, less than about 300 pM, less than about 200 pM, less than about 100 pM, less than about 50 pM, or less than about 25 pM as measured by surface plasmon resonance, *e.g.,* using an assay format as defined in Example 4 herein, or a substantially similar assay. The present invention includes bispecific antigen-binding molecules (e.g., bispecific antibodies which bind human BCMA with a K_D of less than about 25 pM, and which bind monkey BCMA with a K_D of less than about 170 pM, as measured by surface plasmon resonance, *e.g.,* using an assay format as defined in Example 4 herein, or a substantially similar assay.

[0085] The present disclosure also includes antibodies and antigen-binding fragments thereof that bind BCMA with a dissociative half-life (t½) of greater than about 10 minutes or greater than about 125 minutes as measured by surface plasmon resonance at 25°C, *e.g.,* using an assay format as defined in Example 4 herein, or a substantially similar assay. In certain aspects, the antibodies or antigen-binding fragments of the present invention bind BCMA with a t½ of greater than about 3 minutes, greater than about 4 minutes, greater than about 10 minutes, greater than about 20 minutes, greater than about 30 minutes, greater than about 40 minutes, greater than about 50 minutes, greater than about 60 minutes, greater than about 70 minutes, greater than about 80 minutes, greater

than about 90 minutes, greater than about 100 minutes, greater than about 110 minutes, or greater than about 120 minutes, as measured by surface plasmon resonance at 25°C, *e.g.,* using an assay format as defined in Example 4 herein, or a substantially similar assay. The present invention includes bispecific antigen-binding molecules (e.g., bispecific antibodies which bind BCMA with a of greater than about 10 minutes as measured by surface plasmon resonance at 25°C, *e.g.,* using an assay format as defined in Example 4 herein, or a substantially similar assay.

[0086] The present disclosure also includes antibodies and antigen-binding fragments thereof which bind specifically to human cell lines which express endogenous BCMA (e.g., NCI-H929, MOLP-8 or OMP-2), as determined by a FACS binding assay as set forth in Example 6 or a substantially similar assay.

[0087] The present invention also includes anti-BCMA x anti-CD3 bispecific antigen-binding molecules which exhibit one or more characteristics selected from the group consisting of: (a) inhibiting tumor growth in immunocompromised mice bearing human multiple myeloma xenografts; (b) suppressing tumor growth of established tumors in immunocompromised mice bearing human multiple myeloma xenografts (*see, e.g.,* Examples 10-15), and (c) suppressing tumor growth of syngenic melanoma and colon carcinoma cells engineered to express human BCMA in immunocompetent mice expressing human CD3.

[0088] The present disclosure includes antibodies and antigen-binding fragments thereof that bind human CD3 with high affinity. The present disclosure also includes antibodies and antigen-binding fragments thereof that bind human CD3 with medium or low affinity, depending on the therapeutic context and particular targeting properties that are desired. In some cases, the low affinity includes antibodies that bind CD3 with a K_D or EC_{50} (*e.g.,* as measured in a surface plasmon resonance assay) of greater than 300 nM, greater than 500 nM or greater than 1 µM. The present disclosure also includes antibodies and antigen-binding fragments thereof that bind human CD3 with no measureable affinity. For example, in the context of a bispecific antigen-binding molecule, wherein one arm binds CD3 and another arm binds a target antigen (*e.g.,BCMA),* it may be desirable for the target antigen-binding arm to bind the target antigen with high affinity while the anti-CD3 arm binds CD3 with only moderate or low affinity or no affinity. In this manner, preferential targeting of the antigen-binding molecule to cells expressing the target antigen may be achieved while avoiding general/untargeted CD3 binding and the consequent adverse side effects associated therewith.

[0089] The present disclosure includes bispecific antigen-binding molecules (*e.g.,* bispecific antibodies) which are capable of simultaneously binding to human CD3 and a human BCMA. The binding arm that interacts with cells that express CD3 may have weak to no detectable binding as measured in a suitable *in vitro* binding assay. The extent to which a bispecific antigen-binding molecule binds cells that express CD3 and/or BCMA can be assessed by fluorescence activated cell sorting (FACS), as illustrated in Examples 5 and 6 herein.

[0090] The present disclosure includes antibodies, antigen-binding fragments, and bispecific antibodies thereof that bind human CD3 and induce T cell activation.

[0091] The present disclosure includes anti-BCMA x anti-CD3 bispecific antigen-binding molecules which are capable of depleting or reducing tumor antigen-expressing cells in a subject (*see, e.g.,* Examples 8-16, or a substantially similar assay). For example, according to certain aspects, anti-BCMA x anti-CD3 bispecific antigen-binding molecules are provided, wherein a single administration, or multiple administrations, of 0.04 mg/kg, 0.4 mg/kg or 4 mg/kg of the bispecific antigen-binding molecule to a subject causes a reduction in the number of BCMA-expressing cells in the subject (*e.g.,* tumor growth in the subject is suppressed or inhibited).

Preparation of Antigen-Binding Domains and Construction of Bispecific Molecules

[0092] Antigen-binding domains specific for particular antigens can be prepared by any antibody generating technology known in the art. Once obtained, two different antigen-binding domains, specific for two different antigens (*e.g.,* CD3 and BCMA), can be appropriately arranged relative to one another to produce a bispecific

antigen-binding molecule of the present invention using routine methods. (A discussion of exemplary bispecific antibody formats that can be used to construct the bispecific antigen-binding molecules of the present invention is provided elsewhere herein). In certain embodiments, one or more of the individual components (*e.g.,* heavy and light chains) of the multispecific antigen-binding molecules of the invention are derived from chimeric, humanized or fully human antibodies. Methods for making such antibodies are well known in the art. For example, one or more of the heavy and/or light chains of the bispecific antigen-binding molecules can be prepared using VELOCIMMUNE™ technology. Using VELOCIMMUNE™ technology (or any other human antibody generating technology), high affinity chimeric antibodies to a particular antigen (*e.g.,* CD3 or BCMA) are initially isolated having a human variable region and a mouse constant region. The antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate fully human heavy and/or light chains that can be incorporated into the bispecific antigen-binding molecules.

[0093] Genetically engineered animals may be used to make human bispecific antigen-binding molecules. For example, a genetically modified mouse can be used which is incapable of rearranging and expressing an endogenous mouse immunoglobulin light chain variable sequence, wherein the mouse expresses only one or two human light chain variable domains encoded by human immunoglobulin sequences operably linked to the mouse kappa constant gene at the endogenous mouse kappa locus. Such genetically modified mice can be used to produce fully human bispecific antigen-binding molecules comprising two different heavy chains that associate with an identical light chain that comprises a variable domain derived from one of two different human light chain variable region gene segments. (*See, e.g.,* US 2011/0195454). Fully human refers to an antibody, or antigen-binding fragment or immunoglobulin domain thereof, comprising an amino acid sequence encoded by a DNA derived from a human sequence over the entire length of each polypeptide of the antibody or antigen-binding fragment or immunoglobulin domain thereof. In some instances, the fully human sequence is derived from a protein endogenous to a human. In other instances, the fully human protein or protein sequence comprises a chimeric sequence wherein each component sequence is derived from human sequence. While not being bound by any one theory, chimeric proteins or chimeric sequences are generally designed to minimize the creation of immunogenic epitopes in the junctions of component sequences, e.g. compared to any wild-type human immunoglobulin regions or domains.

Therapeutic Formulation and Administration

[0094] The present invention provides pharmaceutical compositions comprising the antigen-binding molecules of the present invention. The pharmaceutical compositions of the invention are formulated with suitable carriers, excipients, and other agents that provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™, Life Technologies, Carlsbad, CA), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0095] The dose of antigen-binding molecule administered to a patient may vary depending upon the age and the size of the patient, target disease, conditions, route of administration, and the like. The preferred dose is typically calculated according to body weight or body surface area. When a bispecific antigen-binding molecule of the present invention is used for therapeutic purposes in an adult patient, it may be advantageous to intravenously administer the bispecific antigen-binding molecule of the present invention normally at a single dose of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. Effective dosages and schedules for administering a bispecific antigen-binding

molecule may be determined empirically; for example, patient progress can be monitored by periodic assessment, and the dose adjusted accordingly. Moreover, interspecies scaling of dosages can be performed using well-known methods in the art (*e.g.,* Mordenti et al., 1991, Pharmaceut. Res. 8:1351).

[0096] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.,* encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, e.g., Wu et al., 1987, J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0097] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0098] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park IL), to name only a few.

[0099] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201). In another embodiment, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.,* Goodson, 1984, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

[0100] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.,* by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate

solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0101] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Therapeutic Uses of the Antigen-Binding Molecules

[0102] In the present invention, a therapeutic composition comprising a bispecific antigen-binding molecule of the invention that specifically binds CD3 and BCMA is provided for use in a method of treating multiple myeloma, another BCMA-expressing B cell malignancy, or a BCMA-expressing tumor. The therapeutic composition comprises a pharmaceutically acceptable carrier or diluent. As used herein, the expression "a subject in need thereof" means a human or non-human animal that exhibits one or more symptoms or indicia of cancer (*e.g.,* a subject expressing a tumor or suffering from any of the cancers mentioned herein below), or who otherwise would benefit from an inhibition or reduction in BCMA activity or a depletion of BCMA+ cells (*e.g.,* multiple myeloma cells).

[0103] The bispecific antigen-binding molecules of the invention (and therapeutic compositions comprising the same) are useful, *inter alia,* for treating any disease or disorder in which stimulation, activation and/or targeting of an immune response would be beneficial. In particular, the anti-BCMA x anti-CD3 bispecific antigen-binding molecules of the present invention may be used for the treatment, prevention and/or amelioration of any disease or disorder associated with or mediated by BCMA expression or activity or the proliferation of BCMA+ cells. The mechanism of action by which the therapeutic methods are achieved include killing of the cells expressing BCMA in the presence of effector cells, for example, by CDC, apoptosis, ADCC, phagocytosis, or by a combination of two or more of these mechanisms. Cells expressing BCMA which can be inhibited or killed using the bispecific antigen-binding molecules of the invention include, for example, multiple myeloma cells.

[0104] The antigen-binding molecules of the present invention may be used to treat a disease or disorder associates with BCMA expression including, *e.g.,* a cancer including multiple myeloma or other B-cell or plasma cell cancers, such as Waldenström's macroglobulinemia, Burkitt lymphoma, and diffuse large B-Cell lymphoma, Non-Hodgkin's lymphoma, chronic lymphocytic leukemia, follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, lymphoplasmacytic lymphoma, and Hodgkin's lymphoma. According to certain embodiments of the present invention, the anti-BCMA x anti-CD3 bispecific antibodies are useful for treating a patient afflicted with multiple myeloma. According to other related embodiments of the invention, an anti-BCMA x anti-CD3 bispecific antigen-binding molecule of the invention is administered to a patient who is afflicted with multiple myeloma. Analytic/diagnostic methods known in the art, such as tumor scanning, etc., may be used to ascertain whether a patient harbors multiple myeloma or another B-cell lineage cancer.

[0105] The present invention also includes the bispecific antigen-binding molecules for treating residual cancer in a subject. As used herein, the term "residual cancer" means the existence or persistence of one or more cancerous cells in a subject following treatment with an anti-cancer therapy.

[0106] According to certain aspects, the present invention provides bispecific antigen-binding molecules of the invention for use in a method of treating multiple myeloma comprising administering one or more of bispecific antigen-binding molecules to the subject after the subject has been determined to have multiple myeloma. For

example, the present invention includes an anti-BCMA x anti-CD3 bispecific antigen-binding molecule of the invention for use in treating multiple myeloma, wherein the antigen-binding molecule is administered 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks or 4 weeks, 2 months, 4 months, 6 months, 8 months, 1 year, or more after the subject has received other immunotherapy or chemotherapy.

Combination Therapies and Formulations

[0107] A pharmaceutical composition of the invention may be administered to a subject in combination with one or more additional therapeutic agents. Exemplary additional therapeutic agents that may be combined with or administered in combination with an antigen-binding molecule of the present invention include, *e.g.,* an anti-tumor agent *(e.g.* chemotherapeutic agents including melphalan, vincristine (Oncovin), cyclophosphamide (Cytoxan), etoposide (VP-16), doxorubicin (Adriamycin), liposomal doxorubicin (Doxil), obendamustine (Treanda), or any others known to be effective in treating a plasma cell tumor in a subject.). In some embodiments, the second therapeutic agent comprises steroids. In some embodiments, the second therapeutic agent comprises targeted therapies including thalidomide, lenalidomide, and bortezomib, which are therapies approved to treat newly diagnosed patients. Lenalidomide, pomalidomide, bortezomib, carfilzomib, panobinostat, ixazomib, elotuzumab, and daratumumab are examples of a second therapeutic agent effective for treating recurrent myeloma. In certain embodiments the second therapeutic agent is a regimen comprising radiotherapy or a stem cell transplant. In certain embodiments, the second therapeutic agent may be an immunomodulatory agent. In certain embodiments, the second therapeutic agent may be a proteasome inhibitor, including bortezomib (Velcade), carfilzomib (Kyprolis), ixazomib (Ninlaro). In certain embodiments the second therapeutic agent may be a histone deacetylase inhibitor such as panobinostat (Farydak). In certain embodiments, the second therapeutic agent may be a monoclonal antibody, an antibody drug conjugate, a bispecific antibody conjugated to an anti-tumor agent, a checkpoint inhibitor, or combinations thereof. Other agents that may be beneficially administered in combination with the antigen-binding molecules of the invention include cytokine inhibitors, including small-molecule cytokine inhibitors and antibodies that bind to cytokines such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-11, IL-12, IL-13, IL-17, IL-18, or to their respective receptors. The pharmaceutical compositions of the present invention (*e.g.,* pharmaceutical compositions comprising an anti-BCMA x anti-CD3 bispecific antigen-binding molecule) may also be administered as part of a therapeutic regimen comprising one or more therapeutic combinations selected from a monoclonal antibody other than those described herein, which may interact with a different antigen on the plasma cell surface, a bispecific antibody, which has one arm that binds to an antigen on the tumor cell surface and the other arm binds to an antigen on a T cell, an antibody drug conjugate, a bispecific antibody conjugated with an anti-tumor agent, a checkpoint inhibitor, for example, one that targets, PD-1 or CTLA-4, or combinations thereof. In certain embodiments, the checkpoint inhibitors may be selected from PD-1 inhibitors, such as pembrolizumab (Keytruda), nivolumab (Opdivo), or cemiplimab (REGN2810). In certain embodiments, the checkpoint inhibitors may be selected from PD-L1 inhibitors, such as atezolizumab (Tecentriq), avelumab (Bavencio), or Durvalumab (Imfinzi)). In certain embodiments, the checkpoint inhibitors may be selected from CTLA-4 inhibitors, such as ipilimumab (Yervoy). Other combinations that may be used in conjunction with an antibody of the invention are described above.

[0108] The present invention also includes therapeutic combinations comprising any of the antigen-binding molecules of the invention and an inhibitor of one or more of VEGF, Ang2, DLL4, EGFR, ErbB2, ErbB3, ErbB4, EGFRvIII, cMet, IGF1R, B-raf, PDGFR-α, PDGFR-β, FOLH1 (PSMA), PRLR, STEAP1, STEAP2, TMPRSS2, MSLN, CA9, uroplakin, or any of the aforementioned cytokines, wherein the inhibitor is an aptamer, an antisense molecule, a ribozyme, an siRNA, a peptibody, a nanobody or an antibody fragment (*e.g.,* Fab fragment; $F(ab')_2$ fragment; Fd fragment; Fv fragment; scFv; dAb fragment; or other engineered molecules, such as diabodies, triabodies, tetrabodies, minibodies and minimal recognition units). The antigen-binding molecules of the invention may also be administered and/or co-formulated in combination with antivirals, antibiotics, analgesics, corticosteroids and/or NSAIDs. The antigen-binding molecules of the invention may also be administered as part of a treatment regimen that also includes radiation treatment and/or conventional chemotherapy.

[0109] The additional therapeutically active component(s) may be administered just prior to, concurrent with, or

shortly after the administration of an antigen-binding molecule of the present invention; (for purposes of the present disclosure, such administration regimens are considered the administration of an antigen-binding molecule "in combination with" an additional therapeutically active component).

[0110] The present invention includes pharmaceutical compositions in which an antigen-binding molecule of the present invention is co-formulated with one or more of the additional therapeutically active component(s) as described elsewhere herein.

Administration Regimens

[0111] According to certain embodiments of the present invention, multiple doses of an antigen-binding molecule (a bispecific antigen-binding molecule that specifically binds BCMA and CD3 as defined in the claims) may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of an antigen-binding molecule of the invention. As used herein, "sequentially administering" means that each dose of an antigen-binding molecule is administered to the subject at a different point in time, *e.g.,* on different days separated by a predetermined interval (*e.g.,* hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of an antigen-binding molecule, followed by one or more secondary doses of the antigen-binding molecule, and optionally followed by one or more tertiary doses of the antigen-binding molecule.

[0112] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the antigen-binding molecule of the invention. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of the antigen-binding molecule, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of an antigen-binding molecule contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.,* adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (e.g., 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.,* "maintenance doses").

[0113] In one exemplary embodiment of the present invention, each secondary and/or tertiary dose is administered 1 to 26 (*e.g.,* 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of antigen-binding molecule which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0114] The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of an antigen-binding molecule (*e.g.,* an anti-BCMA antibody or a bispecific antigen-binding molecule that specifically binds BCMA and CD3). For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.,* 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.,* 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0115] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 2 weeks after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example,

each tertiary dose may be administered to the patient 2 to 4 weeks after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

Diagnostic Uses of the Antibodies

[0116] The anti-BCMA antibodies of the present disclosure may also be used to detect and/or measure BCMA, or BCMA-expressing cells in a sample, *e.g.,* for diagnostic purposes. For example, an anti-BCMA antibody, or fragment thereof, may be used to diagnose a condition or disease characterized by aberrant expression (*e.g.,* over-expression, under-expression, lack of expression, etc.) of BCMA. Exemplary diagnostic assays for BCMA may comprise, *e.g.,* contacting a sample, obtained from a patient, with an anti-BCMA antibody of the disclosure, wherein the anti-BCMA antibody is labeled with a detectable label or reporter molecule. Alternatively, an unlabeled anti-BCMA antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, betagalactosidase, horseradish peroxidase, or luciferase. Another exemplary diagnostic use of the anti-BCMA antibodies of the invention includes ^{89}Zr-labeled, such as ^{89}Zr-desferrioxamine-labeled, antibody for the purpose of noninvasive identification and tracking of tumor cells in a subject (*e.g.* positron emission tomography (PET) imaging). (*See, e.g.,* Tavare, R. et al. Cancer Res. 2016 Jan 1;76(1):73-82; and Azad, BB. et al. Oncotarget. 2016 Mar 15;7(11):12344-58.) Specific exemplary assays that can be used to detect or measure BCMA in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0117] Samples that can be used in BCMA diagnostic assays include any tissue or fluid sample obtainable from a patient which contains detectable quantities of BCMA protein, or fragments thereof, under normal or pathological conditions. Generally, levels of BCMA in a particular sample obtained from a healthy patient (*e.g.,* a patient not afflicted with a disease or condition associated with abnormal BCMA levels or activity) will be measured to initially establish a baseline, or standard, level of BCMA. This baseline level of BCMA can then be compared against the levels of BCMA measured in samples obtained from individuals suspected of having a BCMA related disease (*e.g.,* a tumor containing BCMA-expressing cells) or condition.

EXAMPLES

[0118] The following examples illustrate but do not limit the scope of the claims. The may encompass details about constructs which do not fall within the scope of the claims; such details are provided for information only. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Generation of Anti-BCMA Antibodies

[0119] Anti-BCMA antibodies were obtained by immunizing a genetically modified mouse with a human BCMA antigen (*e.g.,* hBCMA, SEQ ID NO: 115) or by immunizing an engineered mouse comprising DNA encoding human immunoglobulin heavy and kappa light chain variable regions with a human BCMA antigen.

[0120] Following immunization, splenocytes were harvested from each mouse and either (1) fused with mouse

myeloma cells to preserve their viability and form hybridoma cells and screened for BCMA specificity, or (2) B-cell sorted (as described in US 2007/0280945A1) using a human BCMA fragment as the sorting reagent that binds and identifies reactive antibodies (antigen-positive B cells).

[0121] Chimeric antibodies to BCMA were initially isolated having a human variable region and a mouse constant region. The antibodies were characterized and selected for desirable characteristics, including affinity, selectivity, etc. If necessary, mouse constant regions were replaced with a desired human constant region, for example wild-type or modified IgG1 or IgG4 constant region, to generate a fully human anti-BCMA antibody. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

[0122] ***Heavy and Light Chain Variable Region Amino Acid and Nucleic Acid Sequences of anti-BCMA antibodies*:** Table 1 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs of selected anti-BCMA antibodies of the invention. The corresponding nucleic acid sequence identifiers are set forth in Table 2.

Table 1: Amino Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
mAb16711	2	4	6	8	10	12	14	16
mAb16716	18	20	22	24	26	28	30	32
mAb16732	34	36	38	40	42	44	46	48
mAb16747	50	52	54	56	58	60	62	64
mAb21581	66	68	70	72	74	76	78	80
mAb21587	122				123			
mAb21589	124				125			

Table 2: Nucleic Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
mAb16711	1	3	5	7	9	11	13	15
mAb16716	17	19	21	23	25	27	29	31
mAb16732	33	35	37	39	41	43	45	47
mAb16747	49	51	53	55	57	59	61	63
mAb21581	65	67	69	71	73	75	77	79

Example 2: Generation of Anti-CD3 Antibodies

[0123] Anti-CD3 antibodies were generated as described in WO 2017/053856. Two such anti-CD3 antibodies were selected from the production of bispecific anti-BCMA x anti-CD3 antibodies in accordance with the present invention. Table 3 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs of selected anti-CD3 antibodies. The corresponding nucleic acid sequence identifiers are set forth in Table 4. Other anti-CD3 antibodies for use in preparing bispecific antibodies in accordance with the present invention can be found in, e.g., WO 2014/047231.

Table 3: Amino Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
mAb7221G	90	92	94	96	82	84	86	88
mAb7221G20	98	100	102	104	82	84	86	88

Table 4: Nucleic Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
mAb7221G	89	91	93	95	81	83	85	87
mAb7221G20	97	99	101	103	81	83	85	87

Example 3: Generation of Bispecific Antibodies that Bind BCMA and CD3

[0124] The present invention provides bispecific antigen-binding molecules that bind CD3 and BCMA; such bispecific antigen-binding molecules are also referred to herein as "anti-BCMA x anti-CD3 or anti-CD3xBCMA or anti-BCMA x anti-CD3 bispecific molecules." The anti-BCMA portion of the anti-BCMA x anti-CD3 bispecific molecule is useful for targeting tumor cells that express BCMA (also known as CD269), and the anti-CD3 portion of the bispecific molecule is useful for activating T-cells. The simultaneous binding of BCMA on a tumor cell and CD3 on a T-cell facilitates directed killing (cell lysis) of the targeted tumor cell by the activated T-cell.

[0125] Bispecific antibodies comprising an anti-BCMA-specific binding domain and an anti-CD3-specific binding domain were constructed using standard methodologies, wherein the anti-BCMA antigen binding domain and the anti-CD3 antigen binding domain each comprise different, distinct HCVRs paired with a common LCVR. In exemplified bispecific antibodies, the molecules were constructed utilizing a heavy chain from an anti-CD3 antibody, a heavy chain from an anti-BCMA antibody and a common light chain from the anti-CD3 antibody (10082). In other instances, the bispecific antibodies may be constructed utilizing a heavy chain from an anti-CD3 antibody, a heavy chain from an anti-BCMA antibody and an antibody light chain known to be promiscuous or pair effectively with a variety of heavy chain arms.

Table 5: Summary of Component Parts of Anti-BCMA x Anti-CD3 Bispecific Antibodies

	Anti-BCMA	**Anti-CD3**	
Bispecific Antibody Identifier	**Antigen-Binding Domain**	**Antigen-Binding Domain**	**Common Light Chain Variable Region**
	Heavy Chain Variable Region	***Heavy Chain Variable Region***	
bsAb25441D9 (also referred to as REGN5458)	mAb21581	mAb7221G	mAb7221G
bsAb25442D (also referred to as REGN5459)	mAb21581	mAb7221G20	mAb7221G20

[0126] Table 6 shows the amino acid sequence identifiers for the bispecific anti-BCMA x anti-CD3 antibodies exemplified herein.

Table 6: Amino Acid Sequences of Anti-BCMA x Anti-CD3 Bispecific Antibodies

Bispecific Antibody Identifier	Anti-BCMA First Antigen-Binding Domain				Anti-CD3 Second Antigen-Binding Domain				Common Light Chain Variable Region			
	HC VR	HCDR 1	HCDR 2	HCDR 3	HC VR	HCDR 1	HCDR 2	HCDR 3	LC VR	LCDR 1	LCDR 2	LCDR 3
bsAb25441D (REGN5458)	66	68	70	72	90	92	94	96	82	84	86	88
bsAb25442D (REGN5459)	66	68	70	72	98	100	102	104	82	84	86	88

Example 4: Surface Plasmon Resonance Derived Binding Affinities and Kinetic Constants of Anti-BCMA Antibodies and Anti-BCMA x Anti-CD3 Bispecific Antibodies

[0127] Equilibrium dissociation constants (K_D values) for hBCMA.mmh (SEQ ID NO: 106) binding to purified anti-BCMA mAbs and anti-BCMA x anti-CD3 bispecific mAbs were determined using a real-time surface plasmon resonance biosensor using a Biacore 4000 instrument. The CM5 Biacore sensor surface was derivatized by amine coupling with a monoclonal mouse anti-human Fc antibody (GE, # BR-1008-39) to capture purified anti-BCMA mAbs and anti-BCMA x anti-CD3 bispecific mAbs. All Biacore binding studies were performed in a buffer composed of 0.01M HEPES pH 7.4, 0.15M NaCl, 3mM EDTA, 0.05% v/v Surfactant P20 (HBS-ET running buffer). For monomeric affinities, different concentrations of the extracellular domain of human BCMA expressed with C-terminal myc-myc-hexahistidine tag (human BCMA-MMH; SEQ ID NO: 106) or monkey BCMA expressed with C-terminal myc-myc-hexahistidine tag (monkey BCMA-MMH; SEQ ID NO: 110) were prepared in HBS-ET running buffer (ranging from 90 to 1.11 nM, 3-fold dilutions). For dimeric affinities, different concentrations of the extracellular domain of human BCMA expressed with C-terminal mFc tag (human BCMA-MFC; SEQ ID NO: 108) monkey BCMA expressed with C-terminal mFc tag (monkey BCMA-MFC; SEQ ID NO: 112) prepared in HBS-ET running buffer (ranging from 30 to 0.37 nM, 3-fold dilutions) or 30nM BCMA expressed with C-terminal mFc tag (mouse BCMA-MFC; SEQ ID NO: 114) were prepared. Antigen samples were then injected over the anti-BCMA and anti-BCMA x anti-CD3 bispecific mAbs captured surfaces at a flow rate of 30uL/minute. Antibody-reagent association was monitored for 5 minutes while dissociation in HBS-ET running buffer was monitored for 10 minutes. All of the binding kinetics experiments were performed at 25°C. Kinetic association (k_a) and dissociation (k_d) rate constants were determined by fitting the real-time sensorgrams to a 1:1 binding model using Scrubber 2.0c curve fitting software. Binding dissociation equilibrium constants (K_D) and dissociative half-lives (t½) were calculated from the kinetic rate constants as:

$K_D\,(M) = \frac{kd}{ka}$, and $t½\,(min) = \frac{\ln(2)}{60 \times kd}$

[0128] As shown in Table 7, at 25°C, all of the anti-BCMA antibodies of the invention bound to human BCMA-MMH with K_D values ranging from 1.06nM to 3.56nM. As shown in Table 8, at 25°C, all of the anti-BCMA antibodies of the invention bound to human BCMA-MFC with K_D values ranging from 22.3pM to 103pM. As shown in Table 9, at 25°C, two of the anti-BCMA antibodies of the invention bound to monkey BCMA-MMH with K_D values ranging from 38.8nM to 49.92nM. As shown in Table 10, at 25°C, four of the anti-BCMA antibodies of the invention bound to monkey BCMA-MFC with K_D values ranging from 148pM to 14.7nM. As shown in Table 11, at 25°C, four of the anti-BCMA antibodies of the invention bound to mouse BCMA-MFC with K_D values ranging from 677pM to 18.8nM.

Table 7: Binding Kinetics parameters of anti-BCMA monoclonal antibodies binding to human BCMA-MMH at 25°C

REGN #	Ab PID #	mAb Capture (RU)	90nM hBCMA.mmh Bind (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t1/2 (min)
REGN5458	bsAb25441D	437.5 ± 1.1	19.9	8.27E+05	8.74E-04	1.06E-09	13.2
REGN5459	bsAb25442D	384.8 ± **1.4**	17.0	7.30E+05	1.01E-03	1.38E-09	11.5

REGN #	Ab PID #	mAb Capture (RU)	90nM hBCMA.mmh Bind (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t1/2 (min)
	mAb16711	275.0 ± 2.8	22.2	2.01E+06	3.47E-03	1.73E-09	3.3
	mAb16716	310.3 ± 2.2	26.4	8.41E+05	2.99E-03	3.56E-09	3.9
REGN4514	mAb16732	284.1 ± 0.9	25.3	1.06E+06	2.85E-03	2.69E-09	4.1
REGN4515	mAb16747	332.5 ± 0.9	31.4	8.69E+05	2.47E-03	2.84E-09	4.7

Table 8: Binding Kinetics parameters of anti-BCMA monoclonal antibodies binding to human BCMA-MFC at 25°C

REGN #	Ab PID #	mAb Capture (RU)	30nM hBCMA.mFc Bind (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t1/2 (min)
REGN5458	bsAb25441D	437.9 ± 0.1	106.8	4.48E+05	≤ 1E-5	2.23E-11	≤1155
REGN5459	bsAb25442D	385.2 ± 0.1	96.8	4.49E+05	≤ 1E-5	2.23E-11	≤1155
	mAb16711	268.9 ± 1.4	113.5	1.85E+06	1.90E-04	1.03E-10	60.8
	mAb16716	303.4 ± 1.2	120.3	8.62E+05	8.35E-05	9.68E-11	138.4
REGN4514	mAb16732	282.3 ± 1.0	124.1	1.07E+06	4.53E-05	4.22E-11	255.2
REGN4515	mAb16747	327.3 ± 1.5	146.0	1.41E+06	8.95E-05	6.33E-11	129.0

Table 9: Binding Kinetics parameters of anti-BCMA monoclonal antibodies binding to monkey BCMA-MMH at 25°C

REGN #	Ab PID #	mAb Capture (RU)	90 nM mfBCMA.mmh Bind (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t1/2 (min)
REGN5458	bsAb25441D	438.2 ± 0.9	14.8	1.82E+05	9.09E-03	4.99E-08	1.3
REGN5459	bsAb25442D	384.6 ± 1.4	12.7	2.23E+05	8.64E-03	3.88E-08	1.3
	mAb16711	263.5 ± 1.7	-0.5	NB	NB	NB	NB
	mAb16716	301.8 ± 0.5	0.8	NB	NB	NB	NB
REGN4514	mAb16732	279.1 ± 0.8	1.1	NB	NB	NB	NB
REGN4515	mAb16747	326.2 ± 0.5	1.9	NB	NB	NB	NB

Table 10: Binding Kinetics parameters of anti-BCMA monoclonal antibodies binding to monkey BCMA-MFC at 25°C

REGN #	Ab PID #	mAb Capture (RU)	30 nM mfBCMA.mFc Bind (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t1/2 (min)
REGN5458	bsAb25441D	437.9 ± 1.1	107.7	5.28E+05	8.80E-05	1.67E-10	131.2
REGN5459	bsAb25442D	386.2 ± 0.22	97.0	4.82E+05	7.15E-05	1.48E-10	161.6
	mAb16711	259.4 ± 1.4	0.9	NB	NB	NB	NB
	mAb16716	300.8 ± 0.6	3.2	IC	IC	IC	IC
REGN4514	mAb16732	276.9 ± 1.1	40.3	4.92E+05	7.24E-03	1.47E-08	1.6
REGN4515	mAb16747	324.4 ± 0.7	101.3	2.13E+06	7.16E-03	3.37E-09	1.6

Table 11: Binding Kinetics parameters of anti-BCMA monoclonal antibodies binding to mouse BCMA-MFC at 25°C

REGN #	Ab PID #	mAb Capture (RU)	30 nM mBCMA.mFc Bind (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t1/2 (min)
REGN5458	bsAb25441D	438.8	2.7	NB	NB	NB	NB
REGN5459	bsAb25442D	383.9	2.4	NB	NB	NB	NB

REGN #	Ab PID #	mAb Capture (RU)	30 nM mBCMA.mFc Bind (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t1/2 (min)
	mAb16711	257.0	90.0	1.07E+06	1.10E-03	1.02E-09	10.5
	mAb16716	300.0	33.4	2.05E+05	3.85E-03	1.88E-08	3.0
REGN4514	mAb16732	276.1	109.6	3.97E+05	2.69E-04	6.77E-10	43.0
REGN4515	mAb16747	323.1	107.6	9.47E+05	4.18E-03	4.42E-09	2.8

Example 5: FACS Binding of Anti-BCMA x Anti-CD3 Bispecific Antibodies to Human and Cynomolgous CD3 Expressing Cells

[0129] Flow cytometric analysis was utilized to determine binding of BCMAxCD3 bispecific antibodies to human and cynomolgus CD3 (Jurkat cells, mfCD3 engineered Jurkat cells, primary human CD8+ and cynomolgus CD8+ T cells). Briefly, 1e05 cells/well were incubated in the presence of FACS wash with block (PBS+1% filtered FBS+5% mouse serum) with a serial dilution of BCMAxCD3 and control antibodies for 30 minutes on ice. After incubation, the cells were washed twice with cold FACS wash (PBS + 1% filtered FBS) and bound antibody was detected by incubating with Alexa647-conjugated anti-human secondary antibody on ice for an additional 30 minutes. Wells containing no antibody or secondary only were used as a control. For the detection of monkey and human T cells, a cocktail of human and cynomolgus cross-reactive antibodies to CD4, CD8 and CD16 was added to the anti-human secondary. After incubation, cells were washed, re-suspended in 200 µL cold PBS containing 1% filtered FBS and analyzed by flow cytometry on a BD FACS Canto II. Cells were gated by FSC-H by FSC-A to select singlet events, followed by side and forward scatters to select for live events. For monkey T cells, additional gating on CD8+/CD16- cells was performed.

[0130] EC50 values for FACS binding were calculated using 4-parameter non-linear regression analysis in Prism software.

[0131] Jurkat cells are a human CD3 expressing T cell lymphoblastic cell line. REGN5458 bound to human CD3 on Jurkat cells and primary human CD8+ T cells with median EC50s 1.50×10^{-8}M and 3.20×10^{-8}M respectively. Binding of REGN5459 was to human CD3 was weaker, with median EC50 of 5.58×10^{-7}M to Jurkat cells and 4.71×10^{-6} to primary human CD8+ T cells. Utilizing CRISPR/Cas9 technology, a Jurkat cell line was engineered to express cynomolgus CD3ε and CD3δ chains in place of the human versions. Median EC50 of binding of REGN5458 to the mfCD3 engineered Jurkat cell line was 1.51×10^{-8}M and to primary cynomolgus CD8+ T cells was 4.66×10^{-8}M. REGN5459 did not bind to mfCD3 expressing cells.

[0132] No binding was observed on any cell line for the negative isotype control antibody, designated mAb15260.

Table 12: Binding to CD3 expressing cells: Median EC50

	Jurkat-hCD3		Jurkat-mfCD3		Human CD8+ T cells		Mf (Cyno) CD8+ T cells	
REGN	EC50 [M]	n	EC50 [M]	n	EC50 [M]	n	EC50 [M]	n
REGN5458	1.50E-08	5	1.51E-08	2	3.20E-08	1	4.66E-08	1
REGN5459	5.58E-07	5	No Binding	2	4.71E-06	1	No binding	1

Example 6: FACS Binding Assay to Assess Cell Surface Antigen Binding Capacity

[0133] The ability of the anti-BCMA x CD3 antibody, mAb25442D, to bind the surface of BCMA positive multiple myeloma (NCI-H929, MM.1S, OPM-2, and RPMI-8226), BCMA positive lymphoma (Raji and Daudi), and BCMA negative (HEK293) cells was determined via flow cytometry. Cells were harvested from the flasks using cell dissociation buffer (Millipore, Cat# S-004-C) and plated in staining buffer (PBS, without Calcium and Magnesium (Irving 9240) + 2% FBS (ATCC 30-2020) at a density of 500,000 cells per well in a 96 well V-Bottom plate. Cells were stained for 30 mins at 4°C with two-fold serial dilutions of an Alexa647 conjugated anti-BCMA x CD3 antibody (mAb25442D-A647) or an Alexa 647 conjugated isotype control with the same CD3 binding arm paired with an irrelevant tumor targeting arm (Isotype-A647). Cells were washed twice with staining buffer and labeled with the LIVE/DEAD™ Fixable Green Dead Cell Stain Kit (Invitrogen, L34970) according to manufacture instructions to discriminate between live and dead cells. Cells were then washed and fixed for 25 mins at 4°C using a 50% solution of BD Cytofix (BD, Cat # 554655) diluted in PBS. Samples were run on the Accuri C6 flow cytometer (BD Biosciences) and analyzed in Flowjo 10.2 (Tree Star). After gating for live cells and single cells, the mean fluorescent intensity (MFI) was determined, and MFI values were plotted in Graphpad Prism using a four-parameter logistic equation over a 10-point response curve to calculate EC_{50}s. The zero condition for each dose-response curve is also included in the analysis as a continuation of the two-fold serial dilution and is represented as the lowest dose. The signal to noise (S/N) is determined by taking the ratio of the mAb25442D-A647 MFI to the Isotype-A647 MFI. (Table 13). The mAb25442D-A647 S/N ranged from 2 to 470 and the EC_{50} values ranged from 27 to 83 nM. No detectable binding was observed on HEK293 cells.

Table 13: Binding to Cells

Cell Line	mAb25442D-A647 S/N	mAb25442D-A647 EC_{50} (nM)
NCI-H929	470	79
MM.1S	43	83
OPM-2	19	57
RPMI-8226	9	27
Daudi	3	ND
Raji	2	ND
HEK293	1	ND
ND = not determined due to non-sigmoidal curves		

Example 7: T-Cell Activation via Bispecific Anti-BCMA x Anti-CD3 Antibodies in the Presence of BCMA-Expressing Cells

[0134] Activity of the anti-BCMA x anti-CD3 bispecific antibodies were assessed in a Jurkat/NFATLuc reporter bioassay utilizing several cell lines with varying levels of BCMA surface expression. The Jurkat cells were engineered to express an NFAT-luciferase reporter (Jurkat/NFATLuc.3C7), and 50,000 Jurkat reporter cells were combined with 50,000 BCMA positive (Daudi, MM1-S, NCI-H929, OPM-2, RPMI-8226, MOLP-8, or Raji) or BCMA negative (HEK293) cells in Thermo Nunclon delta 96 well white microwell plates (Thermo Scientific, Cat # 136102) in 50 ul of assay media (RPMI media with 10% FBS and 1% P/S/G). Three-fold serial dilutions of the BCMA x CD3 bispecific antibodies (mAb25441D or mAb25442D), or a bivalent anti-BCMA antibody (mAb21581) were immediately added in 50uL of assay buffer. The plates were gently agitated and incubated in a 37°C, 5% CO_2 incubator for 4-6 hours. NFAT-Luciferase activity was determined using Promega One-Glo (Cat # E6130) and a Perkin Elmer Envision plate reader. RLU were plotted in GraphPad Prism using a four-parameter logistic equation over a 12-point response curve to calculate EC_{50} values. The no antibody treatment condition for each dose-response curve is also included in the analysis as a continuation of the three-fold serial dilution and is represented as the lowest dose. The signal to noise (S:N) is determined by taking the ratio of the highest RLU on the curve to the lowest.

[0135] mAb25441D activated Jurkat/NFATLuc cells in the presence of BCMA expressing cells with EC50s ranging from 0.61 nM to 2.1 nM and S:N ranging from 8 to 123. mAb25442D activated Jurkat/NFATLuc cells in the presence of BCMA expressing cells with EC50s ranging from 2.6 nM to 11 nM and S:N ranging from 7 to 120. The BCMA x CD3 bispec mAb25441D with the higher affinity CD3 binding arm was consistently more potent than mAb25442D with a lower affinity CD3 binding arm; whereas, the S:N was similar for the two bispecifics. Neither antibody activated Jurkat/NFATLuc cells in the presence of HEK293 cells, and control bispecific antibodies did not significantly increase Jurkat reporter activity with any of the tested cell lines. The results are shown in Tables 14A and 14B, below.

Table 14A: Activation of T-Cells

	Daudi		MM1-S		NCI-H929		OPM-2	
Antibodies	**EC50**	**S:N**	**EC50**	**S:N**	**EC50**	**S:N**	**EC50**	**S:N**
bsAb25441D	2.1E-9	43	1.2E-9	165	6.8E-10	39	6.6E-10	8
bsAb25442D	7.9E-9	25	4.4E-9	120	2.7E-9	32	2.6E-9	7
mAb21581	ND	1	ND	1	ND	1	ND	1

Table 14B: Activation of T-Cells

	RPMI-8226		MOLP-8		Raji		HEK293	
Antibodies	**EC50**	**S:N**	**EC50**	**S:N**	**EC50**	**S:N**	**EC50**	**S:N**
bsAb25441D	6.1E-10	55	1.4E-9	32	1.6E-9	123	ND	1
bsAb25442D	2.6E-9	42	1.1E-8	31	7.4E-9	78	ND	1
mAb21581	ND	1	ND	1	ND	1	ND	1

Example 8: FACS Based Cytotoxicity Assay to Assess T Cell-Mediated Killing of BCMA-Expressing Multiple Myeloma Cells in the Presence of Anti-BCMA x Anti-CD3 Bispecific Antibodies

[0136] Antibody binding capacity (ABC) of a commercially available anti-human BCMA antibody (clone 19F2) was determined on a panel of multiple myeloma cell lines using a Quantum Simply Cellular anti-human IgG kit and following the manufacturer's instructions (Bangs Laboratories).

[0137] Briefly, multiple myeloma (MM) cell lines (H929, MM1S, U266, MOLP8 and RPMI8226) and Quantum Simply Cellular beads were incubated for 30 minutes at 4°C with a titration of APC conjugated anti-hBCMA-19F2 antibody. After incubation, cells and beads were washed three times, re-suspended in 200 µL cold PBS containing 1% filtered FBS and analyzed by flow cytometry. Using the QuickCal® template (Bangs Labs), the ABC of a saturating level of anti-BCMA 19F2 for each cell line was interpolated from the standard curve generated by the channel intensity of the bead populations at saturation.

[0138] Killing of BCMA expressing target cells by resting human or cynomolgus monkey T cells was determined by flow cytometry. Briefly, human or cynomolgus monkey peripheral blood mononuclear cells (PBMC) were plated in supplemented RPMI (human) or X-Vivo (cyno) media at 1×10^6 cells/mL and incubated overnight at 37°C in order to enrich for lymphocytes by depleting adherent macrophages, dendritic cells, and some monocytes. The next day, BCMA expressing target cells were labeled with 1uM of Violet CellTrace and co-incubated with adherent cell-depleted PBMC (effector/target cell 4:1 ratio) and a serial dilution of BCMAxCD3 bispecifics, or control antibodies at 37°C. After 48-72 hrs, cells were removed from cell culture plates, stained with a cocktail phenotyping antibodies and live/dead cell viability dye, and analyzed by FACS. In order to quantify the number of live target cells present in the wells, 20µl CountBright absolute counting beads were added to the wells just prior to acquisition. For the assessment of specificity of killing, cells were gated on Violet cell tracker labeled populations. Percent survival of target cells was calculated as followed: Target survival=(R_1/R_2)*100, where R_1= absolute number of live target cells in the presence of effector cells and antibody, and R_2 = number of live target cells only (cultured without effector cells or test antibody).

[0139] Human CD8+ T cells were gated as CD45+/CD14-/CD4-/CD8+. Cynomolgus CD8+ T cells were gated as CD45+/CD20-/CD14-/CD4-/CD8+ T cell activation was reported as the percent of CD25+ or CD69+ T cells out of total CD8+ T cells.

[0140] EC50 values for target cell survival and T cell activation were calculated using 4-parameter non-linear regression analysis in Prism software.

[0141] Anti-BCMA x anti-CD3 bispecific antibodies were tested for their ability to activate resting human and cynomolgus T cells to kill a panel of BCMA expressing cells with differing surface BCMA levels. With resting human T cells as effector cells, REGN5458 mediated killing of 5 different BCMA cell lines with EC_{50} values ranging from 7.07×10^{-10} M to 3.45×10^{-11} M. REGN5459 showed killing of the same 5 cell lines with EC50s values ranging from 1.66×10^{-9} M to 1.06×10^{-10} M. EC_{50}s for T cell activation, as measured by CD25 upregulation on CD8+ T cells were similar to killing EC_{50}s. Modest T cell activation was observed in the presence of 1-arm CD3 isotype control mAb17664D, but only for the U266 cell line. No cytotoxicity was observed for the isotype controls tested.

[0142] BCMAxCD3 mediated killing by cynomolgus T cells was tested only on the MM cell line H929. The EC_{50} for cytotoxicity mediated by REGN5458 and REGN5459 was 2.34×10^{-11} and 6.92×10^{-11} respectively. No cytotoxicity or T cell activation was observed for the isotype control antibody mAb15260 with either human or cynomolgus effector cells. The results are shown in Tables 15A, 15B and 16, below.

Table 15A: Median EC_{50}, Human Effector Cells

	H929 (40000 ABC)			MM1S (18000 ABC)			U266 (13000 ABC)		
REGN#	n	% Survival	% T activation	n	% Survival	% T activation	n	% Survival	% T activation
REGN5458	3	1.03E-10	2.11E-10	2	6.46E-11	7.06E-11	1	3.28E-10	1.07E-10
REGN5459	4	3.01E-10	3.00E-10	2	2.88E-10	4.58E-10	1	1.66E-09	4.69E-10

Table 15B: Median EC_{50}, Human Effector Cells

	RPMI8226 (10000 ABC)			Molp8 (2000 ABC)		
REGN#	n	% Survival	% T activation	n	% Survival	% T activation
REGN5458	1	3.45E-11	6.49E-11	2	7.07E-10	1.10E-9
REGN5459	1	1.06E-10	7.50E-10	3	1.36E-09	6.47E-9

Table 16: Median EC_{50}, Cynomolgus effector cells

	H929		
REGN#	n	% Survival	% T activation
REGN5458	4	2.34E-11	6.83E-11
REGN5459	4	6.92E-11	1.58E-10

Example 9: FACS Cytotoxicity Assay to Autologous T Cell-Mediated Killing of Primary Multiple Myeloma Blast Cells in the Presence of Anti-BCMA x Anti-CD3 Bispecific Antibodies

[0143] In order to monitor the specific killing of multiple myeloma cells by flow cytometry, bone marrow mononuclear cells (BMMC) from multiple myeloma patients were plated on human stromal cells (HS5) and rested overnight at 37C. Separately, matching patient peripheral blood mononuclear cells (PBMC) were thawed and cultured in supplemented RPMI media at 1×10^6 cells/mL overnight at 37°C in order to enrich for lymphocytes by depleting adherent cells. The next day, BMMC were co-incubated with adherent cell-depleted naïve PBMC on stromal cells (HS5) and a serial 10x dilution of BCMAxCD3 bispecific or 1-arm CD3 isotype control (starting

concentration 66.7nM) at 37°C. Cells were removed from cell culture plates at day 3, 4 or 7 and analyzed by FACS. For the assessment of specificity of killing, multiple myeloma cells were gated as single, live, CD90 negative (to exclude stromal cells), CD2 negative, CD56 positive. CD45 was low on multiple myeloma cells in most samples except MM455. Percent of live target cells was reported for the calculation of adjusted survival as follows: Adjusted survival=(R1/R2)*100, where R1= % live target cells in the presence of antibody, and R2= % live target cells in the absence of test antibody.

[0144] T cells were gated as CD2 positive, CD56 negative and either CD4 or CD8 positive. T cell activation was reported as the percent of CD25+ CD4 or CD8 T cells out of total CD4 or CD8 T cells.

[0145] BCMAxCD3 bispecific antibodies were tested for their ability to redirect killing of primary multiple myeloma blast cells by autologous donor PBMC. Maximal BCMAxCD3 mediated cytotoxicity of primary MM blast ranged from 52-96%, with EC50s ranging from 9.89×10^{-11} M to 3.67×10^{-9} M for REGN5458 and 4.96×10^{-10} M to 7.94×10^{-8} M for REGN5459. T cell activation was measured by assessing the upregulation of CD25 on CD8+ T cells. EC50s of T cell activation ranged from 3.23×10^{-9} to 1.69×10^{-1}°. Modest cytotoxicity and T cell activation was observed for the 1-arm CD3 (no target binding) isotype control. Results are shown in Tables 17A and 17B, below.

Table 17A: MM % lysis

Sample ID	Disease Stage	E:T ratio	length of treatment	% MM lysis at 66nM REGN5458	% MM lysis at 66nM REGN5459	% MM lysis at 66nM Isotype
MM2	newly diagnosed	1.4	7 days	88	85	27.5
MM369	newly diagnosed	0.3	3 days	96	94	0
MM453	newly diagnosed	2.4	3 days	82	80	40
MM455	progression, treated	0.4	3 days	63	52	24

Table 17B: MM lysis EC50 and T cell activation

Sample ID	Disease Stage	E:T ratio	length of treatment	MM Lysis EC50 REGN5458	MM lysis EC50 REGN5459	CD25 upreg EC50 REGN5456	CD25 upreg EC50 REGN5456
MM2	newly diagnosed	1.4	7 days	7.47E-10	7.24E-09	Not done	Not done
MM369	newly diagnosed	0.3	3 days	1.07E-10	4.96E-10	1.69E-10	2.03E-10
MM453	newly diagnosed	2.4	3 days	9.89E-11	1.19E-09	1.71E-10	3.23E-9
MM455	progression, treated	0.4	3 days	3.67E-09	7.94E-08	2.06E-10	1.16E-9

Example 10: Anti-BCMA x Anti-CD3 Bispecific Antibodies Prevent Growth of BCMA-Expressing Tumors (NCI-H929) In Vivo in a Xenogenic Tumor Model

[0146] To determine the in vivo efficacy of BCMAxCD3 bispecific antibodies (Abs), a xenogenic tumor study was performed. Immunodeficient NOD.Cg-Prkdc^{scid}Il2rg^{tm1wjl}/SzJ (NSG) mice were subcutaneously implanted with a mixture of 10×10^{6} BCMA-expressing NCI-H929 multiple myeloma cells and 0.5×10^{6} human peripheral blood mononuclear cells (PBMC) isolated from a normal donor. The mice (n=7 per group) were immediately administered a PBS vehicle control, an irrelevant anti-FelD1 bivalent isotype control Ab (REGN2759), a CD3-binding control bispecific Ab (mAb17664D), a BCMAxCD3 (G; REGN5458) bispecific Ab, or a BCMAxCD3 (G20; REGN5459) bispecific Ab at a dose of 4 mg/kg. The mice were administered Abs twice per week for a total of three

weeks, and tumor growth was assessed over 40 days. While $BCMA^+$ tumors grew similarly in the vehicle-, isotype control-, and CD3-binding control-treated mice, both BCMAxCD3 Abs that were tested prevented the growth of tumors in vivo.

[0147] *Implantation and measurement of syngeneic tumors:* NSG mice were subcutaneously implanted with a mixture of 10×10^6 BCMA-expressing NCI-H929 multiple myeloma cells and 0.5×10^6 PBMC derived from a normal donor. The mice (n=7 per group) were immediately administered a PBS vehicle control, an irrelevant anti-FelD1 bivalent isotype control Ab (REGN2759), a CD3-binding control bispecific Ab (mAb17664D), a BCMAxCD3 (G; REGN5458) bispecific Ab, or a BCMAxCD3 (G20; REGN5459) bispecific Ab at a dose of 4 mg/kg. The mice were administered Abs twice per week for a total of three weeks. Tumor growth was measured with calipers twice per week for the duration of the experiment. Mice were sacrificed 40 days after tumor implantation.

[0148] *Calculation of syngeneic tumor growth and inhibition:* In order to determine tumor volume by external caliper, the greatest longitudinal diameter (length in mm) and the greatest transverse diameter (width in mm) were determined. Tumor volumes based on caliper measurements were calculated by the formula: Volume (mm^3) = (length x $width^2$)/2.

[0149] BCMAxCD3 bispecific Abs prevented the growth of $BCMA^+$ NCI-H929 tumors in vivo in a xenogenic tumor model. Results are shown in Table 18, below.

Table 18: Average Tumor Size at Various Time Points

Antibody (4 mg/kg)	**Average Tumor Size (mm3) ± SEM on Day 4**
PBS (Vehicle Control)	67.1 ± 5.9
REGN2759 (Isotype Control)	62.6 ± 3.7
mAb17664D (CD3 Binding Control)	76.1 ± 7.6
REGN5458 (BCMAxCD3-G)	39.5 ± 9.1
REGN5459 (BCMAxCD3-G20)	26.5 ± 6.2
Antibody (4 mg/kg)	**Average Tumor Size (mm3) ± SEM on Day 7**
PBS (Vehicle Control)	123.0 ± 25.2
REGN2759 (Isotype Control)	109.7 ± 20.3
mAb17664D (CD3 Binding Control)	182.0 ± 19.4
REGN5458 (BCMAxCD3-G)	0 ± 0
REGN5459 (BCMAxCD3-G20)	0 ± 0
Antibody (4 mg/kg)	**Average Tumor Size (mm3) ± SEM on Day 11**
PBS (Vehicle Control)	361.5 ± 35.7
REGN2759 (Isotype Control)	415.3 ± 11.4
mAb17664D (CD3 Binding Control)	449.6 ±46.6
REGN5458 (BCMAxCD3-G)	0 ± 0
REGN5459 (BCMAxCD3-G20)	0 ± 0
Antibody (4 mg/kg)	**Average Tumor Size (mm3) ± SEM on Day 14**
PBS (Vehicle Control)	581.4 ± 57.9
REGN2759 (Isotype Control)	734.3 ± 41.8
mAb17664D (CD3 Binding Control)	741.2 ± 56.0
REGN5458 (BCMAxCD3-G)	0 ± 0
REGN5459 (BCMAxCD3-G20)	0 ± 0

Antibody (4 mg/kg)	**Average Tumor Size (mm3) ± SEM on Day 18**
PBS (Vehicle Control)	1033.4 ± 143.7
REGN2759 (Isotype Control)	1586.1 ± 101.4
mAb17664D (CD3 Binding Control)	1511.4 ± 80.7
REGN5458 (BCMAxCD3-G)	0 ± 0
REGN5459 (BCMAxCD3-G20)	0 ± 0
Antibody (4 mg/kg)	**Average Tumor Size (mm3) ± SEM on Day 21**
PBS (Vehicle Control)	1730.9 ± 244.8
REGN2759 (Isotype Control)	2554.7 ± 148.8
mAb17664D (CD3 Binding Control)	2474.0 ± 132.6
REGN5458 (BCMAxCD3-G)	0 ± 0
REGN5459 (BCMAxCD3-G20)	0 ± 0
Antibody (4 mg/kg)	**Average Tumor Size (mm3) ± SEM on Day 28**
PBS (Vehicle Control)	Euthanized - Not measured
REGN2759 (Isotype Control)	Euthanized - Not measured
mAb17664D (CD3 Binding Control)	Euthanized - Not measured
REGN5458 (BCMAxCD3-G)	0 ± 0
REGN5459 (BCMAxCD3-G20)	0 ± 0
Antibody (4 mg/kg)	**Average Tumor Size (mm3) ± SEM on Day 40**
PBS (Vehicle Control)	Euthanized - Not measured
REGN2759 (Isotype Control)	Euthanized - Not measured
mAb17664D (CD3 Binding Control)	Euthanized - Not measured
REGN5458 (BCMAxCD3-G)	0 ± 0
REGN5459 (BCMAxCD3-G20)	0 ± 0

Example 11: Anti-BCMA x Anti-CD3 Bispecific Antibodies Prevent Growth of BCMA-Expressing Tumors (NCI-H929) in a Dose-Dependent Manner in a Xenogenic In Vivo Tumor Model

[0150] To determine the in vivo efficacy of anti-BCMA x anti-CD3 bispecific antibodies (Abs), a xenogenic tumor study was performed. Immunodeficient NOD.Cg-Prkdc^{scid}Il2rg^{tm1wjl}/SzJ (NSG) mice were subcutaneously implanted with a mixture of 10×10^6 BCMA-expressing NCI-H929 human multiple myeloma tumor cells and 0.5×10^6 human peripheral blood mononuclear cells (PBMC) isolated from a normal, healthy donor. The mice (n=7 per group) were then immediately administered a PBS vehicle control, a CD3-binding control bispecific Ab (G; mAb17664D) at a dose of 4 mg/kg, a CD3-binding control bispecific Ab (G20; REGN4460) at a dose of 4 mg/kg, a BCMAxCD3 (G; REGN5458) bispecific Ab at doses of either 4 mg/kg, 0.4 mg/kg, or 0.04 mg/kg, or a BCMAxCD3 (G20; REGN5459) bispecific Ab at doses of either 4 mg/kg, 0.4 mg/kg, or 0.04 mg/kg. The mice were administered these Abs twice per week for a total of seven doses, and tumor growth was assessed over 60 days. While BCMA$^+$ NCI-H929 tumors grew similarly in the vehicleand CD3-binding control-treated mice, both anti-BCMA x anti-CD3 Abs that were tested prevented the growth of tumors in a dose-dependent manner in vivo.

[0151] *Implantation and measurement of xenogenic tumors:* NSG mice were subcutaneously implanted with a mixture of 10×10^6 BCMA-expressing NCI-H929 multiple myeloma cells and 0.5×10^6 PBMC derived from a normal, healthy donor. The mice (n=7 per group) were immediately administered a PBS vehicle control, a CD3-binding control bispecific Ab (G; mAb17664D), a CD3-binding control bispecific Ab (G20; REGN4460), a BCMAxCD3 (G;

REGN5458) bispecific Ab, or a BCMAxCD3 (G20; REGN5459) bispecific Ab. mAb17664D and REGN4460 were dosed at 4 mg/kg, while REGN5458 and REGN5459 were administered at either 4 mg/kg, 0.4 mg/kg, or 0.04 mg/kg. The mice were administered Abs twice per week for a total of seven doses. Tumor growth was measured with calipers twice per week for the duration of the experiment.

[0152] *Calculation of xenogenic tumor growth and inhibition:* In order to determine tumor volume by external caliper, the greatest longitudinal diameter (length in mm) and the greatest transverse diameter (width in mm) were determined. Tumor volumes based on caliper measurements were calculated by the formula: Volume (mm^3) = (length x $width^2$)/2.

[0153] BCMAxCD3 bispecific Abs prevented the growth of $BCMA^+$ NCI-H929 tumors in a dose-dependent manner in this xenogenic in vivo tumor model. Results are shown in Table 19, below, and illustrated in Figures 1 and 2.

Table 19: Average Tumor Size at Various Time Points

Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 4**
PBS (Vehicle Control)	60.1 ± 7.9
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	42.5 ± 4.7
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	52.0 ± 5.9
REGN5458 (BCMAxCD3-G) - 4mg/kg	18.0 ± 1.2
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	31.9 ± 2.0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	32.0 ± 2.9
REGN5459 (BCMAxCD3-G20) - 4mg/kg	21.8 ± 3.4
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	19.6 ± 4.4
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	33.0 ± 4.4
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 7**
PBS (Vehicle Control)	138.2 ± 25.1
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	108.6 ± 17.8
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	132.4 ± 21.1
REGN5458 (BCMAxCD3-G) - 4mg/kg	1.3 ± 1.3
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	11.3 ± 3.0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	30.8 ± 5.5
REGN5459 (BCMAxCD3-G20) - 4mg/kg	8.0 ± 4.3
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	7.3 ± 3.6
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	8.4 ± 4.0
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 12**
PBS (Vehicle Control)	545.4 ± 88.7
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	493.4 ± 67.5
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	616.2 ± 84.4
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	1.6 ± 1.6
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	71.5 ± 22.4
REGN5459 (BCMAxCD3-G20) - 4mg/kg	1.7 ± 1.7
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	0 ± 0

Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 15**
PBS (Vehicle Control)	921.4 ± 147.5
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	874.8 ± 86.6
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	1190.7 ± 91.2
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	133.4 ± 50.9
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	7.9 ± 7.9
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 19**
PBS (Vehicle Control)	1785.3 ± 282.2
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	1833.4 ± 186.6
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	2336.5 ± 188.3
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	413.7 ± 162.7
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	23.1 ± 23.1
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 22**
PBS (Vehicle Control)	2601.5 ± 414.5
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	2878.5 ± 257.6
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	3374.3 ± 267.2
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	669.4 ± 248.5
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	69.5 ± 69.5
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 26**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	1167.0 ± 431.7
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	156.7 ± 156.7
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 29**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized

Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 29**
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	1781.8 ± 620.7
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	226.6 ± 226.6
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 34**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	Animals Euthanized
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	Animals Euthanized
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 39**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	Animals Euthanized
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	Animals Euthanized
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 42**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	Animals Euthanized
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	Animals Euthanized
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 46**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized

Antibody Treatment	Average Tumor Size (mm3) ± SEM on Day 46
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	Animals Euthanized
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	Animals Euthanized
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 55**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	Animals Euthanized
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	Animals Euthanized
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 60**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	Animals Euthanized
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	Animals Euthanized

Example 12: Anti-BCMA × Anti-CD3 Bispecific Antibodies Reduce the Size of and Prevent Growth of Established BCMA-Expressing Tumors (NCI-H929) in a Dose-Dependent Manner in a Xenogenic In Vivo Tumor Model

[0154] To determine the in vivo efficacy of anti-BCMA × anti-CD3 bispecific antibodies (Abs), a xenogenic tumor study was performed. Immunodeficient NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice were subcutaneously implanted with a mixture of 10×10^6 BCMA-expressing NCI-H929 human multiple myeloma tumor cells and 0.5×10^6 human peripheral blood mononuclear cells (PBMC) isolated from a normal, healthy donor. The tumors were allowed to grow and establish for 5 days until they were approximately 70 mm^3 in size. On day 5, the mice (n=7-8 per group) were then administered a PBS vehicle control, a CD3-binding control bispecific Ab (G; mAb17664D) at a dose of 4 mg/kg, a CD3-binding control bispecific Ab (G20; REGN4460) at a dose of 4 mg/kg, a BCMAxCD3 (G; REGN5458) bispecific Ab at doses of either 4 mg/kg, 0.4 mg/kg, or 0.04 mg/kg, or a BCMAxCD3 (G20; REGN5459) bispecific Ab at doses of either 4 mg/kg, 0.4 mg/kg, or 0.04 mg/kg. The mice were administered these Abs twice per week for a total of seven doses, and tumor growth was assessed over 55 days. While BCMA$^+$ NCI-H929 tumors grew similarly in the vehicle- and CD3-binding control-treated mice, both BCMAxCD3 Abs that were

tested shrank established tumors and prevented the growth of tumors in a dose-dependent manner in vivo.

[0155] *Implantation and measurement of xenogenic tumors:* NSG mice were subcutaneously implanted with a mixture of 10×10^6 BCMA-expressing NCI-H929 multiple myeloma cells and 0.5×10^6 PBMC derived from a normal, healthy donor. The tumors were allowed to grow and establish for 5 days until they were approximately 70 mm^3 in size. On day 5, the mice (n=7-8 per group) were then administered a PBS vehicle control, a CD3-binding control bispecific Ab (G; mAb17664D), a CD3-binding control bispecific Ab (G20; REGN4460), a BCMAxCD3 (G; REGN5458) bispecific Ab, or a BCMAxCD3 (G20; REGN5459) bispecific Ab. mAb17664D and REGN4460 were dosed at 4 mg/kg, while REGN5458 and REGN5459 were administered at either 4 mg/kg, 0.4 mg/kg, or 0.04 mg/kg. The mice were administered Abs twice per week for a total of seven doses. Tumor growth was measured with calipers twice per week for the duration of the experiment.

[0156] *Calculation of xenogenic tumor growth and inhibition:* In order to determine tumor volume by external caliper, the greatest longitudinal diameter (length in mm) and the greatest transverse diameter (width in mm) were determined. Tumor volumes based on caliper measurements were calculated by the formula: Volume (mm^3) = (length × $width^2$)/2.

[0157] Anti-BCMA × anti-CD3 bispecific antibodies reduced the size of and prevented the growth of established $BCMA^+$ NCI-H929 tumors in a dose-dependent manner in this xenogenic in vivo tumor model. Results are shown in Table 20, below, and illustrated in Figures 3 and 4.

Table 20: Average Tumor Size at Various Time Points

Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 5**
PBS (Vehicle Control)	61.5 ± 6.4
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	63.7 ± 5.4
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	62.6 ± 3.6
REGN5458 (BCMAxCD3-G) - 4mg/kg	71.9 ± 10.3
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	69.3 ± 7.3
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	58.1 ± 5.6
REGN5459 (BCMAxCD3-G20) - 4mg/kg	61.8 ± 5.2
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	69.5 ± 4.1
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	74.9 ± 6.4
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 8**
PBS (Vehicle Control)	124.3 ± 17.3
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	145.3 ± 22.0
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	170.7 ± 15.5
REGN5458 (BCMAxCD3-G) - 4mg/kg	64.7 ± 16.4
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	120.3 ± 16.3
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	130.3 ± 16.7
REGN5459 (BCMAxCD3-G20) - 4mg/kg	45.8 ± 9.8
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	171.9 ± 23.2
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	152.3 ± 20.0
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 12**
PBS (Vehicle Control)	565.7 ± 64.7
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	585.0 ± 64.4
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	706.8 ± 46.3
REGN5458 (BCMAxCD3-G) - 4mg/kg	19.5 ± 10.9

Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 12**
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	262.7 ± 61.6
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	525.9 ± 71.5
REGN5459 (BCMAxCD3-G20) - 4mg/kg	11.5 ± 8.9
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	233.8 ± 63.5
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	462.5 ± 57.7
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 15**
PBS (Vehicle Control)	1150.4 ± 105.7
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	1041.4 ± 101.3
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	1298.4 ± 71.0
REGN5458 (BCMAxCD3-G) - 4mg/kg	25.6 ± 19.2
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	476.2 ± 133.5
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	1031.2 ± 164.3
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	327.2 ± 135.6
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	1094.2 ± 78.9
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 19**
PBS (Vehicle Control)	2621.3 ± 190.9
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	2557.5 ± 241.1
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	3383.3 ± 183.1
REGN5458 (BCMAxCD3-G) - 4mg/kg	40.6 ± 32.8
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	1347.5 ± 334.7
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	2467.5 ± 370.0
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	606.2 ± 288.8
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	2412.5 ± 184.6
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 22**
PBS (Vehicle Control)	3717.9 ± 214.5
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	3688.9 ± 272.0
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	4492.2 ± 344.0
REGN5458 (BCMAxCD3-G) - 4mg/kg	78.3 ± 60.8
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	2068.5 ± 465.0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	3745.7 ± 541.2
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	815.4 ± 387.1
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	3285.9 ± 227.3
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 27**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	252.3 ± 185.1
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	3463.9 ± 1025.0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	1589.1 ± 0

Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 27**
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	1849.9 ± 903.1
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	Animals Euthanized
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 30**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	411.3 ± 307.2
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	2144.2 ± 2144.2
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	2886.5 ± 0
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	661.8 ± 490.1
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	Animals Euthanized
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 35**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	633.5 ± 473.5
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	Animals Euthanized
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	996.8 ± 771.0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	Animals Euthanized
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 40**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	369.5 ± 369.5
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	Animals Euthanized
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	375.6 ± 375.6
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	Animals Euthanized
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 55**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	Animals Euthanized
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0

Antibody Treatment	Average Tumor Size (mm3) ± SEM on Day 55
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	Animals Euthanized

Example 13: Anti-BCMA × Anti-CD3 Bispecific Antibodies Prevent Growth of BCMA-Expressing Tumors (MOLP-8) in a Dose-Dependent Manner in a Xenogenic In Vivo Tumor Model

[0158] To determine the in vivo efficacy of anti-BCMA × anti-CD3 bispecific antibodies (Abs), a xenogenic tumor study was performed. Immunodeficient NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice were subcutaneously implanted with a mixture of 5×10^6 BCMA-expressing MOLP-8 human multiple myeloma tumor cells and 1×10^6 human peripheral blood mononuclear cells (PBMC) isolated from a normal, healthy donor. The mice (n=7 per group) were then immediately administered a PBS vehicle control, a CD3-binding control bispecific Ab (G; mAb17664D) at a dose of 4 mg/kg, a CD3-binding control bispecific Ab (G20; REGN4460) at a dose of 4 mg/kg, a BCMAxCD3 (G; REGN5458) bispecific Ab at doses of either 4 mg/kg, 0.4 mg/kg, or 0.04 mg/kg, or a BCMAxCD3 (G20; REGN5459) bispecific Ab at doses of either 4 mg/kg, 0.4 mg/kg, or 0.04 mg/kg. The mice were administered these Abs twice per week for a total of seven doses, and tumor growth was assessed over 56 days. While the BCMA$^+$ MOLP-8 tumors grew similarly in the vehicle- and CD3-binding control-treated mice, both BCMAxCD3 Abs that were tested prevented the growth of tumors in a dose-dependent manner in vivo.

[0159] *Implantation and measurement of xenogenic tumors:* NSG mice were subcutaneously implanted with a mixture of 5×10^6 BCMA-expressing MOLP-8 multiple myeloma cells and 1×10^6 PBMC derived from a normal, healthy donor. The mice (n=7 per group) were immediately administered a PBS vehicle control, a CD3-binding control bispecific Ab (G; mAb17664D), a CD3-binding control bispecific Ab (G20; REGN4460), a BCMAxCD3 (G; REGN5458) bispecific Ab, or a BCMAxCD3 (G20; REGN5459) bispecific Ab. mAb17664D and REGN4460 were dosed at 4 mg/kg, while REGN5458 and REGN5459 were administered at either 4 mg/kg, 0.4 mg/kg, or 0.04 mg/kg. The mice were administered Abs twice per week for a total of seven doses. Tumor growth was measured by caliper twice per week for the duration of the experiment.

[0160] *Calculation of xenogenic tumor growth and inhibition:* In order to determine tumor volume by external caliper, the greatest longitudinal diameter (length in mm) and the greatest transverse diameter (width in mm) were determined. Tumor volumes based on caliper measurements were calculated by the formula: Volume (mm^3) = (length × width^2)/2.

[0161] Anti-BCMA × anti-CD3 bispecific antibodies prevented the growth of BCMA$^+$ MOLP-8 tumors in a dose-dependent manner in this xenogenic in vivo tumor model. Results are shown in Table 21, below, and illustrated in Figures 5 and 6.

Table 21: Average Tumor Size at Various Time Points

Antibody Treatment	Average Tumor Size (mm3) ± SEM on Day 3
PBS (Vehicle Control)	10.3 ± 3.0
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	11.6 ± 2.0
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	14.1 ± 3.9
REGN5458 (BCMAxCD3-G) - 4mg/kg	12.5 ± 1.3
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	13.5 ± 1.5
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	9.3 ± 2.4
REGN5459 (BCMAxCD3-G20) - 4mg/kg	12.9 ± 1.3
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	14.0 ± 1.6

Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 3**
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	11.7 ± 2.1
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 7**
PBS (Vehicle Control)	73.4 ± 13.5
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	50.0 ± 6.6
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	45.7 ± 6.1
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	1.0 1.0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	18.3 ± 5.0
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0.6 ± 0.6
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	37.0 ± 5.7
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 10**
PBS (Vehicle Control)	249.9 ± 47.6
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	125.0 ± 6.8
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	173.9 ± 99
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	73.9 ± 25.7
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	104 ± 23.0
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 14**
PBS (Vehicle Control)	677.0 ± 62.7
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	530.0 ± 44.6
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	549.1 ± 59.2
REGN5458 (BCMAxCD3-G) - 4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0 ± 0
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	255.4 ± 79.7
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	356.7 ± 84.6
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 17**
PBS (Vehicle Control)	1349.5 ± 149.7
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	935.3 ± 71.3
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	1027.1 ± 86.6
REGN5458 (BCMAxCD3-G) - 4mg/kg	14.5 ± 7.3
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	1.7 ± 1.7
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	494.3 ± 144.3
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	645.6 ± 140.9

Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 21**
PBS (Vehicle Control)	2990.9 ± 291.7
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	2249.6 ± 113.5
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	2473.4 ± 170.3
REGN5458 (BCMAxCD3-G) - 4mg/kg	102.7 ± 66.2
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	5.3 ± 5.3
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	1373.0 ± 366.6
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	1442.4 ± 310.7
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 23**
PBS (Vehicle Control)	4155.1 ± 401.8
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	3288.4 ± 204.6
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	3592.7 ± 224.2
REGN5458 (BCMAxCD3-G) - 4mg/kg	193.3 ± 117.7
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	9.7 ± 9.7
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	1882.3 ± 551.5
REGN5459 (BCMAxCD3-G20) - 4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	2124.4 ± 444.1
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 28**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	627.4 ± 318.1
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	47.4 ± 47.4
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	2542.5 ± 613.3
REGN5459 (BCMAxCD3-G20) - 4mg/kg	1.9 ± 1.9
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	1939.3 ± 840.6
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 31**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	1018.5 ± 498.3
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	104.7 ± 92.6
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	2906.1 ± 532.6
REGN5459 (BCMAxCD3-G20) - 4mg/kg	3.8 ± 3.0
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	2688.7 ± 1176.6
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 35**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized

Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 35**
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	1342.9 ± 629.6
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	375.1 ± 307.5
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	3538.0 ± 0.0
REGN5459 (BCMAxCD3-G20) - 4mg/kg	9.3 ± 7.5
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	612.1 ± 0
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 42**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	2363.0 ± 890.2
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	968.8 ± 689.2
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	Animals Euthanized
REGN5459 (BCMAxCD3-G20) - 4mg/kg	12.8 ± 12.8
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	Animals Euthanized
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 49**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	1683.5 ± 1683.5
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	No Recording
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	Animals Euthanized
REGN5459 (BCMAxCD3-G20) - 4mg/kg	No Recording
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	No Recording
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	Animals Euthanized
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 56**
PBS (Vehicle Control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN4460 (CD3 Binding Control-G20) - 4mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	3108.1 ± 3108.1
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	1742.4 ± 635.2
REGN5458 (BCMAxCD3-G) - 0.04mg/kg	Animals Euthanized
REGN5459 (BCMAxCD3-G20) - 4mg/kg	17.2 ± 17.2
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	0 ± 0
REGN5459 (BCMAxCD3-G20) - 0.04mg/kg	Animals Euthanized

Example 14: Anti-BCMA × Anti-CD3 Bispecific Antibodies Delay Growth of BCMA-Expressing Tumors (MOLP-8) in a Xenographic In Vivo Tumor Model

[0162] To determine the in vivo efficacy of anti-BCMA × anti-CD3 bispecific antibodies (Abs), a xenogenic tumor study was performed. On day -11, immunodeficient NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice were intraperitoneally injected with 4×10^6 human peripheral blood mononuclear cells (PBMC) from a normal, healthy donor. On day 0, the mice were intravenously administered 2×10^6 BCMA$^+$ MOLP-8 human multiple myeloma tumor cells that were engineered to also express firefly luciferase (MOLP-8-luciferase cells). The mice (n=5 per group) were then immediately administered a CD3-binding control bispecific Ab (G; mAb17664D) at a dose of 4 mg/kg or a BCMAxCD3 (G; REGN5458) bispecific Ab at a dose of 4 mg/kg. The mice were administered these Abs twice more on days 3 and 7, for a total of three doses. Tumor growth was assessed over 48 days by measuring tumor bioluminescence (BLI) in anesthetized animals. As a positive control, a group of mice (n=5) was given only MOLP-8-luciferase cells, but not PBMC or antibody. In order to measure background BLI levels, a group of mice (n=5) were untreated and did not receive tumors, PBMC, or antibody. While the BCMA$^+$ MOLP-8-luciferase tumors grew progressively in the CD3-binding control-treated mice, BCMAxCD3 Ab treatment with REGN5458 delayed the growth of tumors in vivo.

[0163] *Implantation and measurement of xenogenic tumors:* On day -11, immunodeficient NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice were intraperitoneally injected with 5×10^6 human PBMC from a normal, healthy donor. On day 0, the mice were intravenously administered 2×10^6 BCMA$^+$ MOLP-8-luciferase cells. The mice (n=5 per group) were then immediately administered a CD3-binding control bispecific Ab (G; mAb17664D) at a dose of 4 mg/kg or a BCMAxCD3 (G; REGN5458) bispecific Ab at a dose of 4 mg/kg. The mice were administered these Abs twice more on days 3 and 7, for a total of three doses. Tumor growth was assessed over 48 days by measuring tumor BLI in anesthetized animals. As a positive control, a group of mice (n=5) was given only MOLP-8-luciferase cells, but not PBMC or antibody. In order to measure background BLI levels, a group of mice (n=5) were untreated and did not receive tumors, PBMC, or antibody.

[0164] *Measurement of xenogenic tumor growth:* BLI imaging was used to measure tumor burden. Mice were injected IP with 150 mg/kg of the luciferase substrate D-luciferin suspended in PBS. Five minutes after this injection, BLI imaging of the mice was performed under isoflurane anesthesia using the Xenogen IVIS system. Image acquisition was carried out with the field of view at D, subject height of 1.5 cm, and medium binning level with automatic exposure time determined by the Living Image Software. BLI signals were extracted using Living Image software: regions of interest were drawn around each tumor mass and photon intensities were recorded as p/s/cm2/sr.

[0165] Anti-BCMA × anti-CD3 bispecific antibody REGN5458 delayed the growth of BCMA$^+$ MOLP-8-luciferase tumors in this xenogenic in vivo tumor model. Results are shown in Table 22, below.

Table 22: Average Tumor Size (by radiance) at Various Time Points

Antibody Treatment	**Radiance [p/s/cm^2/sr] 8 days post-implantation (mean ± SEM)**
No tumor (background BLI)	4.93E+05 ± 1.66E+04
No PBMC/Antibody (positive control)	5.73E+05 ± 5.27E+04
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	6.08E+05 ± 5.16E+04
REGN5458 (BCMAxCD3-G) - 4mg/kg	5.66E+05 ± 1.97E+04
Antibody Treatment	**Radiance [p/s/cm^2/sr] 15 days post-implantation (mean ± SEM)**
No tumor (background BLI)	5.37E+05 ± 1.46E+04
No PBMC/Antibody (positive control)	1.24E+06 ± 9.67E+04
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	1.61E+06 ± 9.64E+04
REGN5458 (BCMAxCD3-G) - 4mg/kg	5.28E+05 ± 4.13E+04

Antibody Treatment	**Radiance [p/s/cm2^2/sr] 15 days post-implantation (mean ± SEM)**
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 22 days post-implantation (mean ± SEM)**
No tumor (background BLI)	7.00E+05 ± 1.03E+04
No PBMC/Antibody (positive control)	1.23E+07 ± 1.02E+06
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	1.98E+07 ± 8.86E+06
REGN5458 (BCMAxCD3-G) - 4mg/kg	1.08E+06 ± 1.71E+05
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 24 days post-implantation (mean ± SEM)**
No tumor (background BLI)	5.24E+05 ± 1.86E+04
No PBMC/Antibody (positive control)	1.56E+07 ± 1.29E+06
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	5.26E+07 ± 1.91E+07
REGN5458 (BCMAxCD3-G) - 4mg/kg	1.02E+06 ± 1.99E+05
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 28 days post-implantation (mean ± SEM)**
No tumor (background BLI)	7.09E+05 ± 2.28E+04
No PBMC/Antibody (positive control)	3.01E+07 ± 4.78E+06
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	5.69E+07 ± 2.77E+07
REGN5458 (BCMAxCD3-G) - 4mg/kg	3.56E+06 ± 6.34E+05
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 30 days post-implantation (mean ± SEM)**
No tumor (background BLI)	6.44E+05 ± 4.56E+04
No PBMC/Antibody (positive control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	6.92E+06 ± 1.40E+06
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 34 days post-implantation (mean ± SEM)**
No tumor (background BLI)	7.78E+05 ± 3.02E+04
No PBMC/Antibody (positive control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg.	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	2.65E+07 ± 1.36E+07
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 37 days post-implantation (mean ± SEM)**
No tumor (background BLI)	7.59E+05 ± 2.96E+04
No PBMC/Antibody (positive control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	4.52E+07 ± 1.40E+07

Antibody Treatment	**Radiance [p/s/cm2^2/sr] 43 days post-implantation (mean ± SEM)**
No tumor (background BLI)	6.26E+05 ± 4.18E+04
No PBMC/Antibody (positive control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mq/kq	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	1.06E+08 ± 3.43E+07
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 48 days post-implantation (mean ± SEM)**
No tumor (background BLI)	8.24E+05 ± 1.73E+04
No PBMC/Antibody (positive control)	Animals Euthanized
mAb17664D (CD3 Binding Control-G) - 4 mg/kg	Animals Euthanized
REGN5458 (BCMAxCD3-G) - 4mg/kg	3.22E+08 ± 1.27E+08

Example 15: Anti-BCMA × Anti-CD3 Bispecific Antibodies Reduce Tumor (OPM-2) Burdens to Background Levels In Vivo

[0166] To determine the in vivo efficacy of anti-BCMA × anti-CD3 bispecific antibodies (Abs), a xenogenic tumor study was performed. On day 0, immunodeficient NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice were intravenously administered 2×10^6 BCMA$^+$ OPM-2 human multiple myeloma tumor cells that were engineered to also express firefly luciferase (OPM-2-luciferase cells). On day 10, the mice were intraperitoneally injected with 4×10^6 human peripheral blood mononuclear cells (PBMC) from a normal, healthy donor. On day 21, the mice (n=5 per group) were administered a CD3-binding control bispecific Ab (G; mAb17664D) at a dose of 0.4 mg/kg, a BCMAxCD3 (G; REGN5458) bispecific Ab at 0.4 mg/kg, or a BCMAxCD3 (G20; REGN5459) bispecific Ab at 0.4 mg/kg. The mice were administered these Abs twice more on days 25 and 28, for a total of three doses. Tumor growth was assessed through day 61 by measuring tumor bioluminescence (BLI) in anesthetized animals. As a positive control, a group of mice (n=5) was given only OPM-2-luciferase cells, but not PBMC or antibody. In order to measure background BLI levels, a group of mice (n=5) were untreated and did not receive tumors, PBMC, or antibody. While the BCMA$^+$ OPM-2-luciferase tumors grew progressively in the CD3-binding control-treated mice, BCMAxCD3 Ab treatment with REGN5458 and REGN5459 reduced tumor burdens to background levels in the majority of animals.

[0167] *Implantation and measurement of xenogenic tumors:* On day 0, immunodeficient NOD.Cg-PrkdC^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice were intravenously administered 2×10^6 BCMA$^+$ OPM-2 human multiple myeloma tumor cells that were engineered to also express firefly luciferase (OPM-2-luciferase cells). On day 10, the mice were intraperitoneally injected with 4×10^6 human peripheral blood mononuclear cells (PBMC) from a normal, healthy donor. On day 21, the mice (n=5 per group) were administered a CD3-binding control bispecific Ab (G; mAb17664D) at a dose of 0.4 mg/kg, a BCMAxCD3 (G; REGN5458) bispecific Ab at 0.4 mg/kg, or a BCMAxCD3 (G20; REGN5459) bispecific Ab at 0.4 mg/kg. The mice were administered these Abs twice more on days 25 and 28, for a total of three doses. Tumor growth was assessed through day 61 by measuring tumor bioluminescence (BLI) in anesthetized animals. As a positive control, a group of mice (n=5) was given only OPM-2-luciferase cells, but not PBMC or antibody. In order to measure background BLI levels, a group of mice (n=5) were untreated and did not receive tumors, PBMC, or antibody.

[0168] *Measurement of xenogenic tumor growth:* BLI imaging was used to measure tumor burden. Mice were injected IP with 150 mg/kg of the luciferase substrate D-luciferin suspended in PBS. Five minutes after this

injection, BLI imaging of the mice was performed under isoflurane anesthesia using the Xenogen IVIS system. Image acquisition was carried out with the field of view at D, subject height of 1.5 cm, and medium binning level with automatic exposure time determined by the Living Image Software. BLI signals were extracted using Living Image software: regions of interest were drawn around each tumor mass and photon intensities were recorded as p/s/cm2/sr.

[0169] While the BCMA$^+$ OPM-2-luciferase tumors grew progressively in the CD3-binding control-treated mice, BCMAxCD3 Ab treatment with REGN5458 and REGN5459 reduced tumor burdens to background levels in the majority of animals. Results are shown in Table 23, below, and illustrated in Figure 7.

Table 23: Average Tumor Size (by radiance) at Various Time Points

Antibody Treatment	**Radiance [p/s/cm2^2/sr] 5 days post-implantation (mean ± SEM)**
No tumor (background BLI)	6.22E+05 ± 2.77E+04
No PBMC/Antibody (positive control)	5.62E+05 ± 2.75E+04
mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg	5.73E+05 ± 3.02E+04
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	5.87E+05 ± 2.40E+04
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	5.09E+05 ± 3.56E+04
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 11 days post-implantation (mean ± SEM)**
No tumor (background BLI)	6.90E+05 ± 3.64E+04
No PBMC/Antibody (positive control)	6.22E+05 ± 3.34E+04
mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg	6.25E+05 ± 3.80E+04
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	6.19E+05 ± 4.39E+04
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	6.45E+05 ± 2.39E+04
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 20 days post-implantation (mean ± SEM)**
No tumor (background BLI)	7.59E+05 ± 5.82E+04
No PBMC/Antibody (positive control)	2.32E+06 ± 2.94E+05
mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg	2.36E+06 ± 5.46E+05
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	1.81E+06 ± 2.37E+05
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	2.13E+06 ± 1.69E+05
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 26 days post-implantation (mean ± SEM)**
No tumor (background BLI)	5.51E+05 ± 2.51E+04
No PBMC/Antibody (positive control)	5.96E+06 ± 8.74E+05
mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg	6.05E+06 ± 1.32E+06
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	1.73E+06 ± 8.69E+05
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	1.28E+06 ± 7.36E+05
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 31 days post-implantation (mean ± SEM)**
No tumor (background BLI)	6.62E+05 ± 3.35E+04
No PBMC/Antibody (positive control)	1.58E+07 ± 4.84E+06

Antibody Treatment	**Radiance [p/s/cm2^2/sr] 31 days post-implantation (mean ± SEM)**
mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg	1.35E+07 ± 2.35E+06
REGN5458 (BCMAxCD3-G) - 0.4mq/kg	3.50E+06 ± 2.42E+06
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	1.98E+06 ± 1.36E+06
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 34 days post-implantation (mean ± SEM)**
No tumor (background BLI)	4.57E+05 ± 1.04E+04
No PBMC/Antibody (positive control)	3.36E+07 ± 1.27E+07
mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg.	2.35E+07 ± 5.72E+06
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	4.85E+06 ± 3.24E+06
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	4.24E+06 ± 3.69E+06
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 38 days post-implantation (mean ± SEM)**
No tumor (background BLI)	6.60E+05 ± 3.13E+04
No PBMC/Antibody (positive control)	3.91E+07 ± 6.87E+06
mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg	4.84E+07 ± 1.65E+07
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	5.30E+06 ± 3.44E+06
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	3.21E+06 ± 2.52E+06
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 40 days post-implantation (mean ± SEM)**
No tumor (background BLI)	5.39E+05 ± 9.67E+03
No PBMC/Antibody (positive control)	Animals euthanized
mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg	Animals euthanized
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	5.06E+06 ± 3.36E+06
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	3.84E+06 ± 3.34E+06
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 47 days post-implantation (mean ± SEM)**
No tumor (background BLI)	7.73E+05 ± 1.91E+04
No PBMC/Antibody (positive control)	Animals euthanized
mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg	Animals euthanized
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	7.76E+05 ± 7.85E+04
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	7.34E+05 ± 2.62E+04
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 54 days post-implantation (mean ± SEM)**
No tumor (background BLI)	7.49E+05 ± 1.95E+04
No PBMC/Antibody (positive control)	Animals euthanized
mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg	Animals euthanized
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	5.78E+05 ± 1.15E+05

Antibody Treatment	**Radiance [p/s/cm2^2/sr] 54 days post-implantation (mean ± SEM)**
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	6.41E+05 ± 5.96E+04
Antibody Treatment	**Radiance [p/s/cm2^2/sr] 61 days post-implantation (mean ± SEM)**
No tumor (background BLI)	6.18E+05 ± 2.77E+04
No PBMC/Antibody (positive control)	Animals euthanized
mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg	Animals euthanized
REGN5458 (BCMAxCD3-G) - 0.4mg/kg	5.23E+05 ± 4.10E+04
REGN5459 (BCMAxCD3-G20) - 0.4mg/kg	6.03E+05 ± 5.29E+04

Example 16: BCMA×CD3 Bispecific Antibodies Suppress Growth of Syngenic Tumors In Vivo in a Dose-Dependent Manner

[0170] To determine the in vivo efficacy of anti-BCMA × anti-CD3 bispecific antibodies (Abs), a syngenic tumor study was performed in mice expressing human CD3. C57BL/6 mice that express human CD3deg in place of murine CD3deg (CD3-humanized mice) were subcutaneously implanted with either 0.5×10^6 B16 melanoma cells that have been engineered to express full-length human BCMA (B16/BCMA cells) or 1×10^6 MC38 colon carcinoma cells that have been engineered to express full-length human BCMA (MC38/BCMA). The mice (n=7 per group) were then immediately administered a CD3-binding control bispecific Ab (G; mAb17664D) at a dose of 0.4 mg/kg or a BCMAxCD3 (G; REGN5458) bispecific Ab at doses of either 0.4 mg/kg or 0.04 mg/kg. The mice were administered these Abs twice more on days 4 and 7 for a total of three doses, and tumor growth was assessed throughout the experiment. While the B16/BCMA tumors and the MC38/BCMA tumors grew in the CD3-binding control-treated mice, BCMAxCD3 REGN5458 was able to suppress the growth of both tumor lines in a dose-dependent manner in vivo.

[0171] *Implantation and measurement of syngenic tumors:* C57BL/6 mice that express human CD3deg in place of murine CD3deg (CD3-humanized mice) were subcutaneously implanted with either 0.5×10^6 B16F10 melanoma cells that have been engineered to express full-length human BCMA (B16/BCMA cells) or 1×10^6 MC38 colon carcinoma cells that have been engineered to express full-length human BCMA (MC38/BCMA). The mice (n=7 per group) were then immediately administered a CD3-binding control bispecific Ab (G; mAb17664D) at a dose of 0.4 mg/kg or a BCMAxCD3 (G; REGN5458) bispecific Ab at doses of either 0.4 mg/kg or 0.04 mg/kg. The mice were administered these Abs twice more on days 4 and 7 for a total of three doses, and tumor growth was assessed throughout the experiment.

[0172] *Calculation of syngenic tumor growth and inhibition:* In order to determine tumor volume by external caliper, the greatest longitudinal diameter (length in mm) and the greatest transverse diameter (width in mm) were determined. Tumor volumes based on caliper measurements were calculated by the formula: Volume (mm^3) = (length × width^2)/2.

[0173] While the B16/BCMA tumors and the MC38/BCMA tumors grew in the CD3-binding control-treated mice, BCMAxCD3 REGN5458 was able to suppress the growth of both tumor lines in a dose-dependent manner in vivo. Results are shown in Table 24, below.

Table 24: Average Tumor Size at Various Time Points

Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 5**
B16/BCMA Tumor mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg	25.6 ± 2.7
B16/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.4mg/kg	0.0 ± 0.0
B16/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.04mg/kg	3.3 ± 2.2
MC38/BCMA Tumor mAb17664D (CD3 Binding Control-G) - 0.4 mq/kq	29.3 ± 4.4
MC38/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.4mg/kg	1.4 ± 1.4
MC38/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.04mq/kg	11.9 ± 2.9
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 10**
B16/BCMA Tumor mAb17664D (CD3 Binding Control-G) - 0.4 mq/kq	179.2 ± 30.6
B16/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.4mq/kq.	0.0 ± 0.0
B16/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.04mq/kg	15.4 ± 12.5
MC38/BCMA Tumor mAb17664D (CD3 Binding Control-G) - 0.4 mq/kq	123.1 ± 14.6
MC38/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.4mq/kq.	0.0 ± 0.0
MC38/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.04mq/kg	66.7 ± 22.5
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 14**
B16/BCMA Tumor mAb17664D (CD3 Binding Control-G) - 0.4 mq/kq	763.1 ± 156.2
B16/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.4mg/kg	8.1 ± 4.4
B16/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.04mq/kg	81.4 ± 49.2
MC38/BCMA Tumor mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg	477.1 ± 77.1
MC38/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.4mq/kq.	2.9 ± 2.9
MC38/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.04mg/kg	273.3 ± 115.3
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 18**
B16/BCMA Tumor mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg	2068.9 ± 357.7
B16/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.4mg/kg	47.1 ± 17.0
B16/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.04mg/kg	127.2 ± 63.9
MC38/BCMA Tumor mAb17664D (CD3 Binding Control-G) - 0.4 mg/kg	1432.5 ± 231.6
MC38/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.4mg/kg.	7.5 ± 7.5
MC38/BCMA Tumor REGN5458 (BCMAxCD3-G) - 0.04mg/kg	641.5 ± 309.8

Example 17: Epitope Mapping of REGN5458 Binding to BCMA by Hydrogen Deuterium Exchange

[0174] H/D exchange epitope mapping with mass spectrometry (HDX-MS) was performed to determine the amino acid residues of BCMA (recombinant human BCMA, amino acid sequence of SEQ ID NO: 115) interacting with

REGN5458 (BCMA × CD3 bispecific antibody). A general description of the H/D exchange method is set forth in e.g., Ehring (1999) Analytical Biochemistry 267(2):252-259; and Engen and Smith (2001) Anal. Chem. 73:256A-265A.

[0175] The HDX-MS experiments were performed on an integrated HDX/MS platform, consisting of a Leaptec HDX PAL system for the deuterium labeling and quenching, a Waters Acquity M-Class (Auxiliary solvent manager) for the sample digestion and loading, a Waters Acquity M-Class (μBinary solvent manager) for the analytical gradient, and Thermo Q Exactive HF mass spectrometer for peptide mass measurement.

[0176] The labeling solution was prepared as PBS buffer in D_2O at pD 7.0 (10 mM phosphate buffer, 140 mM NaCl, and 3 mM KCl, equivalent to pH 7.4 at 25°C). For deuterium labeling, 10 μL of hBCMA.hFc (REGN2746, 54.5 μM; SEQ ID NO: 120 or hBCMA.hFc premixed with REGN5458 in 1:2 molar ratio (Ag-Ab complex) was incubated at 20°C with 90 μL D_2O labeling solution for various time-points in duplicates (e.g., Undeuterated control = 0 second; deuterium-labeled for 5 minutes and 10 minutes). The deuteration reaction was quenched by adding 100 μL of pre-chilled quench buffer (0.5 M TCEP-HCl, 8 M urea and 1% formic acid) to each sample for a 5-minute incubation at 20 °C. The quenched sample was then injected into a Waters HDX Manager for online pepsin/protease XIII digestion. The digested peptides were separated by a C8 column (1.0 mm × 50 mm, NovaBioassays) with a 13-minute gradient from 10%-32% B (mobile phase A: 0.5% formic acid in water, mobile phase B: 0.1% formic acid in acetonitrile). The eluted peptides were analyzed by Q Exactive HF mass spectrometry in LC-MS/MS or LC-MS mode.

[0177] The LC-MS/MS data of undeuterated BCMA sample were searched against a database including BCMA and its randomized sequence using Byonic search engine (Protein Metrics). The search parameters (in ELN) were set as default using non-specific enzymatic digestion and human glycosylation as common variable modification. The list of identified peptides was then imported into the HDX Workbench software (version 3.3) to calculate the deuterium uptake of each peptide detected by LC-MS from all deuterated samples. For a given peptide, the centroid mass (intensity-weighted average mass) at each time point was used to calculate the deuterium uptake (D) and percentage of deuterium uptake (%D):

$$\text{Deuterium Uptake (D-uptake)} = \text{Average Mass (deuterated)} - \text{Average Mass (undeuterated)}$$

$$\text{Percentage of deuterium uptake (\%D)} = \frac{\text{D-uptake for peptide at each time point X 100\%}}{\text{Maximum D-uptake of the peptide (defined in ELN)}}$$

[0178] A total of 8 peptides from hBCMA.hFc were identified from both hBCMA.hFc alone and hBCMA.hFc in complex with REGN5458 samples, representing 100% sequence coverage of hBCMA. The averaged standard deviation (SD) of all peptides was evaluated to be 1.4% (detailed calculations were defined in ELN and Pascal, BD et al (2012) Journal of the American Society for Mass Spectrometry 23(9):1512-1521). Therefore, any peptide which exhibited a differential percent D-uptake values above 4.2% (3-fold of the averaged SD) was defined as significantly protected. For hBCMA.hFc, peptides corresponding to amino acids 1-43 of SEQ ID NO: 106 (MLQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNA; SEQ ID NO: 121) were significantly protected by REGN5458. Protection of these residues by REGN5458 was confirmed using hBCMA.mmH (REGN2744, amino acid sequence of SEQ ID NO: 106).

Table 25: Selected BCMA.hFc peptides with significant protection upon binding to REGN5458

BCMA Residues	5 min			10 min			-hFc
	REGN2746	REGN2746 + REGN5458		REGN2746	REGN2746 + REGN5458		
	Centroid MH^+	Centroid MH^+	ΔD	Centroid MH^+	Centroid MH^+	ΔD	Δ%D
1-28	3217.16	3212.39	-4.77	3218.05	3212.62	-5.43	-25.2

BCMA Residues	5 min			10 min			-hFc
	REGN2746	REGN2746 + REGN5458		REGN2746	REGN2746 + REGN5458		
	Centroid MH^+	Centroid MH^+	ΔD	Centroid MH^+	Centroid MH^+	ΔD	Δ%D
4-26	2582.03	2577.26	-4.77	2582.71	2577.45	-5.26	-31
27-43	1921.75	1920.69	-1.06	1922.1	1920.83	-1.27	-11.1

Example 18: FACS Binding Assay of BCMAxCD3 Bispecific Antibodies and Additional BCMA Antibodies on Multiple Myeloma Cell Lines After Overnight Incubation with Anti-BCMA Antibodies

[0179] Flow cytometric analysis was utilized to determine the impact of overnight incubation of multiple myeloma cell lines with anti-BCMA antibodies on the level of surface BCMA. MM cell lines (H929, Molp8, U266 and MM1.S) were washed two times and cultured at 37°C in R10 media (RPMI+10% FBS+pen/strep/glut) containing 66.7 or 667 nM anti-BCMA antibodies, DAPT (a gamma-secretase inhibitor) or media only. After 18 hours, wells were washed with cold FACS wash (PBS+1% filtered FBS) and resuspended in 667 nM of the same anti-BCMA antibody in cold stain buffer (Miltenyi 130-091-221) for 30 minutes on ice. After incubation, the cells were washed twice with cold FACS wash (PBS + 1% filtered FBS) and bound antibody was detected by incubating with the appropriate anti-human secondary antibody (anti-hIgG or anti-HIS) on ice for an additional 30-45 minutes. After incubation, cells were washed, re-suspended in 200 µL cold PBS containing 1% filtered FBS and analyzed by flow cytometry on a BD FACS Canto II. Fold increase in staining was calculated by dividing the MFI of stained cells previously incubated overnight in BCMA abs or DAPT by the MFI of stained cells that were incubated overnight in media only.

[0180] BCMA is rapidly cleaved from the surface of cells by the enzyme gamma-secretase. Overnight incubation with the gamma-secretase inhibitors, such as DAPT, prevents BCMA cleavage resulting in increased levels of BCMA on the cell surface. Tables 26-29 report the fold increase in the median fluorescence intensity (MFI) of BCMA on cells incubated overnight in anti-BCMA antibodies or DAPT compared to cells incubated in media only. We observed that overnight incubation with DAPT increased BCMA levels detected by anti-BCMA antibodies (BCMAxCD3 bispecific R5458, the parental BCMA antibody mAb15281, and other in house BCMA antibodies) on H929, Molp8, U266 and MM.1S, 2.3-4 fold, 2.4-8.6 fold, 5.3-9.0 fold, and 11.9 fold, respectively.

[0181] Of note, we also observed that overnight incubation of MM cell lines with 66.7 or 667 nM REGN5458 or the parental bivalent anti-BCMA antibody mAb21581 similarly resulted in increased levels of surface BCMA detected by FACS, suggesting that binding of anti-BCMA antibodies prevents cleavage of BCMA by gamma-secretase. Antibody induced increases in surface BCMA differed by cell line, with greater fold increases on Molp8 and MM1S cells compared to H929 or U266. The phenomenon was not limited to REGN5458, as it was also observed with other in house BCMA antibodies.

Table 26: MFI fold change over cells incubated in media only (NCI-H929)

NCI-H929		67 nM		667 nM		DAPT	
		Average	n	Average	n	Average	n
mAb21581	aBCMA (parent to R5458)	1.2	5	1.4	3	3.5	6
REGN5458	BCMAxCD3	2.0	3	3.0	1	4.0	3
mAb16749	aBCMA	1.0	2	0.8	1	2.3	3
mAb16711	aBCMA	2.8	2	2.1	1	3.8	3
mAb16747	aBCMA	1.8	2	2.1	1	3.9	3
REGN960	scFv IsoC	1.0	2	1.1	1	1.1	3
mAb11810	IgG1 IsoC	1.0	2	1.0	1	1.1	3

NCI-H929		67 nM		667 nM		DAPT	
		Average	n	Average	n	Average	n
mAb21581	aBCMA (parent to R5458)	1.2	5	1.4	3	3.5	6
mAb11810	IgG4s IsoC	1.3	2	1.0	1	1.1	3

Table 27: MFI fold change over cells incubated in media only (MoIp8)

MoIp8		67 nM		667 nM		DAPT	
		Average	n	Average	n	Average	n
mAb21581	aBCMA (parent to R5458)	2.3	5	3.7	3	6.3	6
REGN5458	BCMAxCD3	2.3	3	4.5	1	8.6	3
mAb16749	aBCMA	1.1	2	3.4	1	4.0	3
mAb16711	aBCMA	3.5	2	3.0	1	5.1	3
mAb16747	aBCMA	2.2	2	0.6	1	6.2	3
REGN960	scFv IsoC	1.1	2	1.0	1	1.0	3
mAb11810	IgG1 IsoC	1.0	2	1.3	1	1.1	3
mAb11810	IgG4s IsoC	0.9	2	1.2	1	1.0	3

Table 28: MFI fold change over cells incubated in media only (U266)

U266		67 nM		667 nM		DAPT	
		Average	n	Average	n	Average	n
mAb21581	aBCMA (parent to R5458)	1.8	2	2.3	1	6.7	6
REGN5458	BCMAxCD3	1.4	2	2.3	1	9.0	3
mAb16749	aBCMA	1.3	2	1.2	1	5.3	3
mAb16711	aBCMA	2.2	2	2.2	1	7.2	3
mAb16747	aBCMA	1.5	2	1.7	1	8.3	3
REGN960	scFv IsoC	1.0	2	1.0	1	1.0	3
mAb11810	IgG1 IsoC	1.0	2	1.1	1	1.1	3
mAb11810	IgG4s IsoC	1.1	2	1.1	1	1.4	3

Table 29: MFI fold change over cells incubated in media only (MM1S)

MM1S		67 nM		667 nM		DAPT	
		Average	n	Average	n	Average	n
mAb21581	aBCMA (parent to R5458)	7.3	2	7.0	2	11.9	2

Example 19: Autologous T Cell-Mediated Killing of Human and Cynomolgus Monkey Plasma Cells in the Presence of BCMAxCD3 Bispecific Antibodies

[0182] The specific killing of enriched $CD138^+$ human or cynomolgus monkey plasma cells by unstimulated autologous T cells was assessed by flow cytometry. Human or cynomolgus bone marrow aspirates and blood were provided within 24 hours of harvest. $CD138^+$ plasma cells were enriched from bone marrow by positive selection using the EasySep Human $CD138^+$ Positive Selection kit according to the manufacturer's instructions. PBMC from whole blood were isolated by density separation. PBMC were labeled with 1μM of Vybrant CFDA-SE fluorescent tracking dye. After labeling, 1×10^4 enriched $CD138^+$ plasma cells were plated in round-bottom 96 well plates at an E:T ratio of 10:1 with Vybrant CFDA-SE labeled PBMC and serial dilutions of REGN5458, CD3-binding control bsAb, or BCMA-binding control mAb for 72 hours at 37°C in complete media. At the end of the culture, surviving $CD138^+$ plasma cells were analyzed by flow cytometry, utilizing fixable LIVE/DEAD dye and plasma cell specific cell

surface markers. Percent viability was normalized to control condition (plasma cells in the presence of PBMC only). T cell activation was assessed by flow cytometry. Activation is reported as the percentage of $CD2^+/CD4^+$ or $CD2^+/CD8^+/CD16^-$ T cells expressing CD25. Percent T cell activation was normalized to control condition (plasma cells in the presence of PBMC only).

[0183] In vitro studies evaluated the effect of REGN5458 or negative controls (BCMA-binding control mAb or CD3-binding control bsAb) on primary human and cynomolgus monkey T cell activation and cytotoxicity of autologous plasma cells. The EC_{50} values for cytotoxicity and percent T cell activation for each donor are summarized in Table 30.

[0184] REGN5458 mediated cytotoxicity of primary human plasma cells from donors 1 and 2 in the presence autologous T cells in a concentration-dependent manner with EC_{50} values of 42.8pM and 191pM, respectively, and resulted in a maximum percent cytotoxicity of 91% and 89%, respectively. In parallel, REGN5458 mediated T cell activation in the presence of human plasma cells from donors 1 and 2 in a concentration-dependent manner with EC_{50} values of 214pM and 860pM for $CD8^+$ T cell activation, respectively, and maximum percent $CD8^+$ T cell activation of 2% and 36%, respectively. Cytotoxicity of plasma cells in both donors and increased $CD8^+$ T cell activation in donor 2 only was observed at nanomolar concentrations of CD3-binding control. No effect on cytotoxicity or T cell activation was observed with BCMA-binding control at any of the concentrations tested in either donor.

[0185] REGN5458 mediated cytotoxicity of primary cynomolgus plasma cells in both donors in a concentration-dependent manner; an EC_{50} of 1.31 nM was calculated for donor 1, however an EC_{50} could not be determined for donor 2. In both donors, REGN5458 treatment resulted in increased cytotoxicity of plasma cells (maximum percent cytotoxicity of 94% and 91% for donors 1 and 2, respectively). In parallel, REGN5458 mediated T cell activation in the presence of cynomolgus monkey plasma cells from donors 1 and 2 in a concentration-dependent manner with EC_{50} values of 28.1nM and 18.1nM for $CD4^+$ T cell activation and 22.4nM and 76.7nM for $CD8^+$ T cell activation, respectively. The resulting maximum percent T cell activation was 9% and 16% for $CD4^+$ T cells and 12% and 17% $CD8^+$ T cells for donors 1 and 2, respectively.

[0186] No target cell killing was observed with BCMA-binding control at any concentration tested in either of the cell lines evaluated. Some target cell killing and T cell activation in the presence of plasma cells from donor 2 was observed with CD3-binding control at nanomolar concentrations.

Table 30: EC_{50} Values for Cytotoxicity and Percent T Cell Activation for Each Donor

Cell Lines		Cytotoxic Kill		T Cell Activation (% CD25 Upregulation)			
				$CD4^+$ T cells		$CD8^+$ T cells	
Effector Cells	Target Cells	EC_{50} (M)	Max % Cytotoxicity	EC_{50} (M)	% Activation	EC_{50} (M)	% Activation
Primary Human T Cells[a]	Human Donor 1 Plasma Cells	4.28×10^{-11}	91	NR	NR	214×10^{-10}	2
	Human Donor 2 Plasma Cells	1.91×10^{-10}	89	NR	NR	8.60×10^{-10}	36
Primary Cynomolgus Monkey T Cells[a]	Cynomolgus Monkey Donor 1 Plasma Cells	1.31×10^{9}	94	2.81×10^{-8}	9	224×10^{-8}	12

Cell Lines		Cytotoxic Kill		T Cell Activation (% CD25 Upregulation)			
				$CD4^+$ T cells		$CD8^+$ T cells	
Effector Cells	Target Cells	EC_{50} (M)	Max % Cytotoxicity	EC_{50} (M)	% Activation	EC_{50} (M)	% Activation
	Cynomolgus Monkey Donor 2 Plasma Cells	ND	91	$\sim 1.81\times10^{-8}$	16	7.67×10^{-8}	17
[a] Autologous plasma cells were tested for each donor.							

Example 20: Anti-BCMA × Anti-CD3 Bispecific Antibodies Act Synergistically with Anti-PD-1 Antibodies to Enhance Anti-Tumor Efficacy *In Vivo*

[0187] To determine whether BCMAxCD3 bispecific antibodies (Abs) synergize with PD-1 blockade to provide superior anti-tumor efficacy *in vivo,* a syngenic tumor study was performed in mice expressing human CD3. The results demonstrate that combining REGN5458 plus PD-1 blockade provides superior anti-tumor efficacy than either REGN5458 or PD-1 blockade alone.

[0188] *Implantation and measurement of syngenic tumors:* C57BL/6 mice that express human CD3deg in place of murine CD3deg (CD3-humanized mice) were subcutaneously implanted with 1×10^6 MC38 colon carcinoma cells that have been engineered to express full-length human BCMA (MC38/BCMA). The tumors were allowed to establish for 3 days, at which time the mice (n= 6 or 7 per group) were administered a CD3-binding control bispecific Ab (G; H4sH17664D) at a dose of 0.4 mg/kg or a BCMAxCD3 (G; REGN5458) bispecific Ab at doses of either 0.04 mg/kg or 0.24 mg/kg, along with either a surrogate anti-mouse PD-1 antibody (Clone RPM1-14) at 4 mg/kg or an isotype control Ab (Clone 2A3) at 4 mg/kg. The specific treatment groups are shown in Table 31, below.

Table 31: Treatment Groups

Group	Bispecific Treatment	Antibody	n
1	H4SH17664D (0.24 mg/kg)	Isotype (4 mg/kg)	7
2	H4SH17664D (0.24 mg/kg)	RPM1-14 (4 mg/kg)	7
3	REGN5458 (0.04 mg/kg)	Isotype (4 mg/kg)	7
4	REGN5458 (0.04 mg/kg)	RPM1-14 (4 mg/kg)	7
5	REGN5458 (0.24 mg/kg)	Isotype (4 mg/kg)	6
6	REGN5458 (0.24 mg/kg)	RPM1-14 (4 mg/kg)	6

[0189] The mice were administered these Abs twice more on days 7 and 11 for a total of three doses, and tumor growth was assessed throughout the experiment.

[0190] *Calculation of syngenic tumor growth and inhibition:* In order to determine tumor volume by external caliper, the greatest longitudinal diameter (length in mm) and the greatest transverse diameter (width in mm) were determined. Tumor volumes based on caliper measurements were calculated by the formula: Volume (mm^3) = (length × $width^2$)/2.

[0191] The results demonstrate that combining REGN5458 plus PD-1 blockade provides superior anti-tumor efficacy than either REGN5458 or PD-1 blockade alone. In particular, the results demonstrate that at day 24 (the

last day for which data was collected for all treatment groups) the combination of BCMAxCD3 bispecific antibody and anti-PD-1 antibody produced a statistically significant synergistic therapeutic effect in the inhibition of tumor growth (Table 32, BCMAxCD3 at 0.04 mg/kg and anti-PD-1 at 4 mg/kg). Using a 2-way ANOVA test at day 24, $p<0.0001$ between (i) REGN5458 (0.04 mg/kg) + Isotype and the combination of REGN5458 (0.04 mg/kg) + anti-PD-1 antibody (Group 3 vs. Group 4), (ii) REGN5458 (0.24 mg/kg) + Isotype and the combination of REGN5458 (0.24 mg/kg) + anti-PD-1 antibody (Group 5 vs. Group 6), (iii) anti-PD-1 and the combination of REGN5458 (0.04 mg/kg) + anti-PD-1 antibody (Group 2 vs. Group 6). Using a 2-way ANOVA test at day 24, $p=0.0005$ between anti-PD-1 and the combination of REGN5458 (0.04 mg/kg) + anti-PD-1 antibody (Group 2 vs. Group 4). Increasing the dose of BCMAxCD3 bispecific antibody (0.24 mg/kg) in combination with PD-1 blockade resulted in tumor inhibition comparable to the lower bispecific antibody dose plus PD-1 blockade in this experiment. The demonstrated synergy with the lower dose bispecific antibody is advantageous because the use of a lower dose reduces the risk of any adverse side effects. Similarly, the combination of BCMAxCD3 bispecific antibody and anti-PD-1 antibody showed a synergistic therapeutic effect at both doses of bispecific antibody (0.04 mg/kg and 0.24 mg/kg) in the number of tumor-free mice at the end of the experiment (day 28), as shown in Table 33.

Table 32: Average Tumor Size at Various Time Points

Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 3**
CD3-binding control H4SH17664D (0.24 mg/kg) + Isotype (4 mg/kg)	16.30 ± 1.50 n=7
CD3-binding control H4SH17664D (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	14.34 ± 1.17 n=7
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	15.62 ± 1.61 n=7
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	19.20 ± 2.94 n=7
BCMAxCD3 REGN5458 (0.24 mg/kg) + Isotype (4 mg/kg)	13.13 ± 3.12 n=6
BCMAxCD3 REGN5458 (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	20.41 ± 3.15 n=6
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 7**
CD3-binding control H4SH17664D (0.24 mg/kg) + Isotype (4 mg/kg)	55.78 ± 6.61 n=7
CD3-binding control H4SH17664D (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	43.59 ± 8.32 n=7
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	37.98 ± 3.93 n=7
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	30.30 ± 6.47 n=7
BCMAxCD3 REGN5458 (0.24 mg/kg) + Isotype (4 mg/kg)	29.27 ± 5.00 n=6
BCMAxCD3 REGN5458 (0.24 mg/kg)	29.18 ± 3.65
+ PD-1-blocking RPM1-14 (4 mg/kg)	n=6
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 11**
CD3-binding control H4SH17664D (0.24 mg/kg) + Isotype (4 mg/kg)	145.74 ± 21.37 n=7
CD3-binding control H4SH17664D (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	45.33 ± 11.46 n=7
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	112.53 ± 17.39 n=7

Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 11**
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	8.81 ± 0.88 n=7
BCMAxCD3 REGN5458 (0.24 mg/kg) + Isotype (4 mg/kg)	36.63 ± 14.89 n=6
BCMAxCD3 REGN5458 (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	12.99 ± 4.35 n=6
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 14**
CD3-binding control H4SH17664D (0.24 mg/kg) + Isotype (4 mg/kg)	414.28 ± 46.72 n=7
CD3-binding control H4SH17664D (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	49.50 ± 17.02 n=7
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	438.16 ± 59.56 n=7
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	6.86 ± 3.90 n=7
BCMAxCD3 REGN5458 (0.24 mg/kg) + Isotype (4 mg/kg)	224.33 ± 47.04 n=6
BCMAxCD3 REGN5458 (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	22.75 ± 17.62 n=6
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 18**
CD3-binding control H4SH17664D (0.24 mg/kg) + Isotype (4 mg/kg)	1035.43 ± 123.41 n=6
CD3-binding control H4SH17664D (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	100.83 ± 41.62 n=7
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	1040.12 ± 61.95 n=7
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	7.81 ± 7.81 n=7
BCMAxCD3 REGN5458 (0.24 mg/kg) + Isotype (4 mg/kg)	515.15 ± 115.38 n=6
BCMAxCD3 REGN5458 (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	57.79 ± 43.62 n=6
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 21**
CD3-binding control H4SH17664D (0.24 mg/kg)	1834.87 ± 639.56
+ Isotype (4 mg/kg)	n=2
CD3-binding control H4SH17664D (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	208.29 ± 91.80 n=7
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	2133.12 ± 129.26 n=6
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	21.13 ± 21.13 n=7
BCMAxCD3 REGN5458 (0.24 mg/kg) + Isotype (4 mg/kg)	1225.47 ± 289.39 n=6
BCMAxCD3 REGN5458 (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	113.69 ± 85.39 n=6

Antibody Treatment	Average Tumor Size (mm3) ± SEM on Day 24
CD3-binding control H4SH17664D (0.24 mg/kg) + Isotype (4 mg/kg)	2358.81 ± 0.00 n=1
CD3-binding control H4SH17664D (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	534.03 ± 205.49 n=7
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	3648.37 ± 536.71 n=3
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	53.52 ± 53.52 n=7
BCMAxCD3 REGN5458 (0.24 mg/kg) + Isotype (4 mg/kg)	1493.26 ± 973.01 n=2
BCMAxCD3 REGN5458 (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	54.29 ± 54.29 n=5
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 28**
CD3-binding control H4SH17664D (0.24 mg/kg) + Isotype (4 mg/kg)	All Animals Euthanized n=0
CD3-binding control H4SH17664D (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	1196.57 ± 467.34 n=7
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	All Animals Euthanized n=0
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	141.68 ± 141.68 n=7
BCMAxCD3 REGN5458 (0.24 mg/kg) + Isotype (4 mg/kg)	1371.17 ± 0.00 n=1
BCMAxCD3 REGN5458 (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	104.44 ± 104.44 n=5

Table 33: Tumor-Free Mice at End of Experiment

Antibody Treatment	Number of Mice Tumor-Free at End of Experiment (Day 28)
CD3-binding control H4SH17664D (0.24 mg/kg) + Isotype (4 mg/kg)	0 of 7
CD3-binding control H4SH17664D (0.24 mg/kg)	2 of 7
+ PD-1-blocking RPM1-14 (4 mg/kg)	
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	0 of 7
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	6 of 7
BCMAxCD3 REGN5458 (0.24 mg/kg) + Isotype (4 mg/kg)	0 of 6
BCMAxCD3 REGN5458 (0.24 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	4 of 6

Example 21: Anti-BCMA × Anti-CD3 Bispecific Antibodies Act Synergistically with Anti-PD-1 Antibodies to Enhance Anti-Tumor Efficacy *In Vivo*

[0192] Similar results were obtained in a second experiment, identical to that discussed above in Example 20, except that the number of mice per group = 10, and the higher dose of BCMAxCD3 REGN5458 was 0.4 mg/kg. The specific treatment groups for the second experiment are shown in Table 34, below.

Table 34: Treatment Groups

Group	Bispecific Treatment	Antibody	n
1	H4SH17664D (0.4 mg/kg)	Isotype (4 mg/kg)	10
2	H4SH17664D (0.4 mg/kg)	RPM1-14 (4 mg/kg)	10
3	REGN5458 (0.04 mg/kg)	Isotype (4 mg/kg)	10
4	REGN5458 (0.04 mg/kg)	RPM1-14 (4 mg/kg)	10
5	REGN5458 (0.4 mg/kg)	Isotype (4 mg/kg)	10
6	REGN5458 (0.4 mg/kg)	RPM1-14 (4 mg/kg)	10

[0193] The results demonstrate that combining REGN5458 plus PD-1 blockade provides superior anti-tumor efficacy than either REGN5458 or PD-1 blockade alone. In particular, the results demonstrate that at day 21 (the last day for which data was collected for all treatment groups) the combination of BCMAxCD3 bispecific antibody and anti-PD-1 antibody produced a synergistic therapeutic effect in the inhibition of tumor growth (Table 35, BCMAxCD3 at 0.04 mg/kg and anti-PD-1 at 4 mg/kg). Using a 2-way ANOVA test at day 21, $p<0.0001$ between (i) REGN5458 (0.04 mg/kg) + Isotype and the combination of REGN5458 (0.04 mg/kg) + anti-PD-1 antibody (Group 3 vs. Group 4), (ii) anti-PD-1 and the combination of REGN5458 (0.04 mg/kg) + anti-PD-1 antibody (Group 2 vs. Group 4), (iii) anti-PD-1 and the combination of REGN5458 (0.4 mg/kg) + anti-PD-1 antibody (Group 2 vs. Group 6). As discussed above in Example 20, increasing the dose of BCMAxCD3 bispecific antibody (0.4 mg/kg) in combination with PD-1 blockade resulted in tumor inhibition comparable to the lower bispecific antibody dose combined with PD-1 blockade in this experiment. The demonstrated synergy with the lower dose bispecific antibody is advantageous because the use of a lower dose reduces the risk of any adverse side effects. Similarly, the combination of BCMAxCD3 bispecific antibody and anti-PD-1 antibody showed a synergistic therapeutic effect at both doses of bispecific antibody (0.04 mg/kg and 0.4 mg/kg) in the number of tumor-free mice at the end of the experiment (day 25), as shown in Table 36.

Table 35: Average Tumor Size at Various Time Points

Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 3**
CD3-binding control H4SH17664D (0.4 mg/kg) + Isotype (4 mg/kg)	9.85 ± 0.61 n=10
CD3-binding control H4SH17664D (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	13.44 ± 1.44 n=10
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	12.41 ± 2.56 n=10
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	9.73 ± 1.25 n=10
BCMAxCD3 REGN5458 (0.4 mg/kg) + Isotype (4 mg/kg)	11.22 ± 0.68 n=10
BCMAxCD3 REGN5458 (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	9.59 ± 1.78 n=10
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 6**
CD3-binding control H4SH17664D (0.4 mg/kg) + Isotype (4 mg/kg)	40.43 ± 4.07 n=10
CD3-binding control H4SH17664D (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	44.52 ± 2.80 n=10
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	38.79 ± 3.52 n=10
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	36.42 ± 3.51 n=10
BCMAxCD3 REGN5458 (0.4 mg/kg) + Isotype (4 mg/kg)	16.11 ± 1.27 n=10
BCMAxCD3 REGN5458 (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	24.34 ± 1.86 n=10

Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 10**
CD3-binding control H4SH17664D (0.4 mg/kg) + Isotype (4 mg/kg)	149.41 ± 17.08 n=10
CD3-binding control H4SH17664D (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	107.34 ± 13.73 n=10
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	116.32 ± 19.99 n=10
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	23.48 ± 3.24 n=10
BCMAxCD3 REGN5458 (0.4 mg/kg)	24.27 ± 6.74
+ Isotype (4 mg/kg)	n=10
BCMAxCD3 REGN5458 (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	3.60 ± 1.92 n=10
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 13**
CD3-binding control H4SH17664D (0.4 mg/kg) + Isotype (4 mg/kg)	386.55 ± 48.49 n=10
CD3-binding control H4SH17664D (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	186.87 ± 41.06 n=10
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	319.91 ± 53.05 n=10
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	10.60 ± 2.34 n=10
BCMAxCD3 REGN5458 (0.4 mg/kg) + Isotype (4 mg/kg)	50.93 ± 20.00 n=10
BCMAxCD3 REGN5458 (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	0.74 ± 0.74 n=10
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 18**
CD3-binding control H4SH17664D (0.4 mg/kg) + Isotype (4 mg/kg)	1809.29 ± 242.64 n=9
CD3-binding control H4SH17664D (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	688.52 ± 152.20 n=10
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	1314.27 ± 211.22 n=10
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	6.28 ± 4.55 n=10
BCMAxCD3 REGN5458 (0.4 mg/kg) + Isotype (4 mg/kg)	248.51 ± 107.21 n=10
BCMAxCD3 REGN5458 (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	3.93 ± 2.67 n=10
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 21**
CD3-binding control H4SH17664D (0.4 mg/kg) + Isotype (4 mg/kg)	3094.87 ± 482.38 n=8
CD3-binding control H4SH17664D (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	1425.22 ± 338.49 n=10
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	2446.35 ± 395.48 n=10
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	15.03 ± 10.35 n=10
BCMAxCD3 REGN5458 (0.4 mg/kg) + Isotype (4 mg/kg)	453.43 ± 174.75 n=10
BCMAxCD3 REGN5458 (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	9.34 ± 7.59 n=10
Antibody Treatment	**Average Tumor Size (mm3) ± SEM on Day 25**
CD3-binding control H4SH17664D (0.4 mg/kg) + Isotype (4 mg/kg)	Animals Euthanized n=0
CD3-binding control H4SH17664D (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	1918.27 ± 571.19 n=6
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	2411.64 ± 451.96 n=3

Antibody Treatment	Average Tumor Size (mm3) ± SEM on Day 25
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	38.96 ± 21.47 n=10
BCMAxCD3 REGN5458 (0.4 mg/kg) + Isotype (4 mg/kg)	661.70 ± 331.60 n=8
BCMAxCD3 REGN5458 (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	32.02 ± 24.67 n=10

Table 36: Tumor-Free Mice at End of Experiment

Antibody Treatment	Number of Mice Tumor-Free at End of Experiment (Day 25)
CD3-binding control H4SH17664D (0.4 mg/kg) + Isotype (4 mg/kg)	0 of 10
CD3-binding control H4SH17664D (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	1 of 10
BCMAxCD3 REGN5458 (0.04 mg/kg) + Isotype (4 mg/kg)	0 of 10
BCMAxCD3 REGN5458 (0.04 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	7 of 10
BCMAxCD3 REGN5458 (0.4 mg/kg) + Isotype (4 mg/kg)	2 of 10
BCMAxCD3 REGN5458 (0.4 mg/kg) + PD-1-blocking RPM1-14 (4 mg/kg)	8 of 10

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PATENTKRAV

1. Isoleret, bispecifikt antigenbindingsmolekyle, der omfatter:

(a) et første antigenbindingsdomæne, der specifikt binder et humant B-cellemodningsantigen (BCMA), som henholdsvis omfatter HCDR1-, HCDR2-, HCDR3-domæner, omfattende aminosyresekvenserne ifølge henholdsvis SEQ ID NO: 68, 70, 72 og LCDR1-, LCDR2-, LCDR3-domæner, der omfatter aminosyresekvenserne ifølge SEQ ID NO: 84, 86, 88; og

(b) et andet antigenbindingsdomæne, der specifikt binder humant CD3, som omfatter henholdsvis HCDR1-, HCDR2-, HCDR3-domæner, der omfatter aminosyresekvenserne ifølge henholdsvis SEQ ID NO: 92, 94, 96 og LCDR1-, LCDR2-, LCDR3-domæner, der omfatter aminosyresekvenserne ifølge SEQ ID NO: 84, 86, 88.

2. Isoleret, bispecifikt antigenbindingsmolekyle, der omfatter:

(a) et første antigenbindingsdomæne, der specifikt binder et humant B-cellemodningsantigen (BCMA), som omfatter henholdsvis HCDR1-, HCDR2-, HCDR3-domæner, der omfatter aminosyresekvenserne ifølge henholdsvis SEQ ID NO: 68, 70, 72 og LCDR1-, LCDR2-, LCDR3-domæner, der omfatter aminosyresekvenserne ifølge SEQ ID NO: 84, 86, 88; og

(b) et andet antigenbindingsdomæne, der specifikt binder human CD3, som omfatter henholdsvis HCDR1-, HCDR2-, HCDR3-domæner, der omfatter aminosyresekvenserne ifølge henholdsvis SEQ ID NO: 100, 102, 104 og LCDR1-, LCDR2-, LCDR3-domæner, der omfatter aminosyresekvenserne ifølge SEQ ID NO: 84, 86, 88.

3. Isoleret, bispecifikt antigenbindingsmolekyle ifølge krav 1, hvor:

(a) det første antigenbindingsdomæne omfatter et HCVR, der omfatter aminosyresekvensen ifølge SEQ ID NO: 66, og et LCVR, der omfatter aminosyresekvensen ifølge SEQ ID NO: 82; og

(b) det andet antigenbindingsdomæne omfatter et HCVR, der omfatter aminosyresekvensen ifølge SEQ ID NO: 90, og et LCVR, der omfatter aminosyresekvensen ifølge SEQ ID NO: 82.

4. Isoleret, bispecifikt antigenbindingsmolekyle ifølge krav 2, hvor:

(a) det første antigenbindingsdomæne omfatter et HCVR, der omfatter aminosyresekvensen ifølge SEQ ID NO: 66, og et LCVR, der omfatter aminosyresekvensen ifølge SEQ ID NO: 82; og

(b) det andet antigenbindingsdomæne omfatter et HCVR, der omfatter aminosyresekvensen ifølge SEQ ID NO: 98, og et LCVR, der omfatter aminosyresekvensen ifølge SEQ ID NO: 82.

5. Isoleret, bispecifikt antigenbindingsmolekyle ifølge et hvilket som helst af kravene 1-4, som er et bispecifikt antistof.

6. Isoleret, bispecifikt antigenbindingsmolekyle ifølge krav 5, hvor:

(a) det bispecifikke antistof omfatter et konstant område af tungkæden af humant IgG af isotype IgG1 eller IgG4; og/eller

(b) det bispecifikke antistof omfatter et kimærisk hængsel, der reducerer Fcγ-receptorbinding i forhold til et vildtypehængsel af den samme isotype.

7. Farmaceutisk sammensætning, der omfatter det bispecifikke antigenbindingsmolekyle ifølge et hvilket som helst af kravene 1-6 og en farmaceutisk acceptabel bærer eller fortynder.

8. Nukleinsyremolekyle, der omfatter en nukleotidsekvens, der koder for et bispecifikt antigenbindingsmolekyle ifølge et hvilket som helst af kravene 1-6.

9. Ekspressionsvektor, der omfatter nukleinsyremolekylet ifølge krav 8.

10. Værtscelle, der omfatter ekspressionsvektoren ifølge krav 9.

11. Isoleret, bispecifikt antigenbindingsmolekyle ifølge et hvilket som helst af kravene 1-6 eller farmaceutisk sammensætning ifølge krav 7 til anvendelse i en fremgangsmåde til hæmning af en plasmacelles tumorvækst hos en person.

12. Isoleret, bispecifikt antigenbindingsmolekyle ifølge et hvilket som helst af kravene 1-6 eller farmaceutisk sammensætning ifølge krav 7 til anvendelse i en fremgangsmåde til behandling af multipelt myelom, eller en anden BCMA-udtrykkende B-cellemalignitet.

13. Isoleret, bispecifikt antigenbindingsmolekyle eller farmaceutisk sammensætning til anvendelse ifølge krav 12, hvor den BCMA-udtrykkende B-cellemalignitet vælges fra gruppen bestående af Waldenströms makroglobulinæmi, Burkitts lymfom, diffust stort B-cellelymfom, non-Hodgkins lymfom, kronisk lymfocytisk leukæmi, follikulært lymfom, mantelcellelymfom, marginalzonelymfom, lymfoplasmacytisk lymfom og Hodgkins lymfom.

14. Isoleret, bispecifikt antigenbindingsmolekyle eller farmaceutisk sammensætning til anvendelse ifølge krav 11, 12 eller krav 13, hvor det isolerede, bispecifikke antigenbindingsmolekyle eller den farmaceutiske sammensætning skal administreres i kombination med et/en andet/anden terapeutisk middel eller terapi.

15. Isoleret, bispecifikt antigenbindingsmolekyle eller farmaceutisk sammensætning til anvendelse ifølge krav 14, hvor det/den andet/anden terapeutiske middel eller terapi omfatter et kemoterapeutisk lægemiddel, DNA-alkylatorer, immunmodulatorer, proteasomhæmmere, histondeacetylasehæmmere, radioterapi, et stamcelletransplantat, et andet bispecifikt antistof, der interagerer med et andet tumorcelleoverfladeantigen, og et T-celle- eller immuncelleantigen, et antistoflægemiddelkonjugat, et bispecifikt antistof konjugeret til et antitumormiddel, en PD-1-, PD-L1- eller CTLA-4-checkpoint-hæmmer eller kombinationer deraf.

16. Isoleret, bispecifikt antigenbindingsmolekyle ifølge et hvilket som helst af kravene 1-6 eller farmaceutisk sammensætning ifølge krav 7 til anvendelse i en fremgangsmåde til behandling af en BCMA-udtrykkende tumor, hvor det isolerede, bispecifikke antigenbindingsmolekyle, eller den farmaceutiske sammensætning, skal administreres til personen i kombination med et anti-PD-1-antistof eller antigenbindingsfragment deraf.

17. Isoleret, bispecifikt antigenbindingsmolekyle eller farmaceutisk sammensætning til anvendelse ifølge krav 16, hvor anti-PD-1-antistoffet eller antigenbindingsfragment an anti-PD-1-antistof.

18. Isoleret, bispecifikt antigenbindingsmolekyle eller farmaceutisk sammensætning til anvendelse ifølge krav 17, hvor anti-PD-1-antistoffet er cemiplimab (REGN2810).

DRAWINGS

Drawing

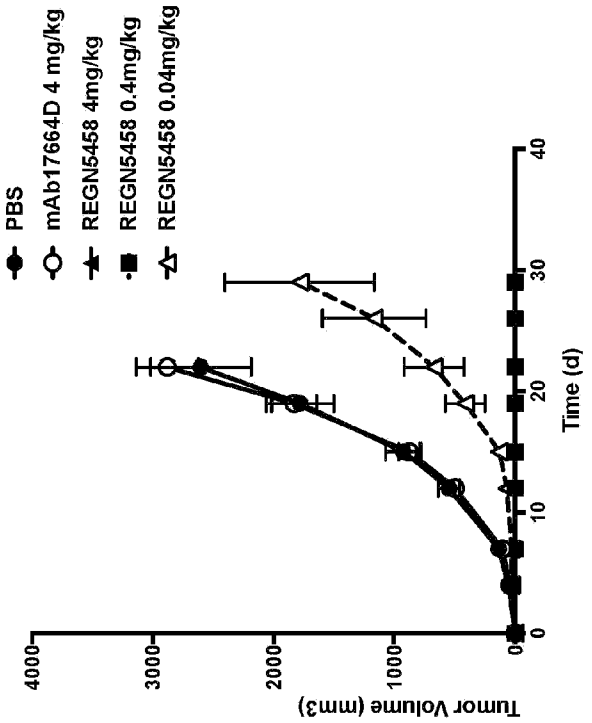
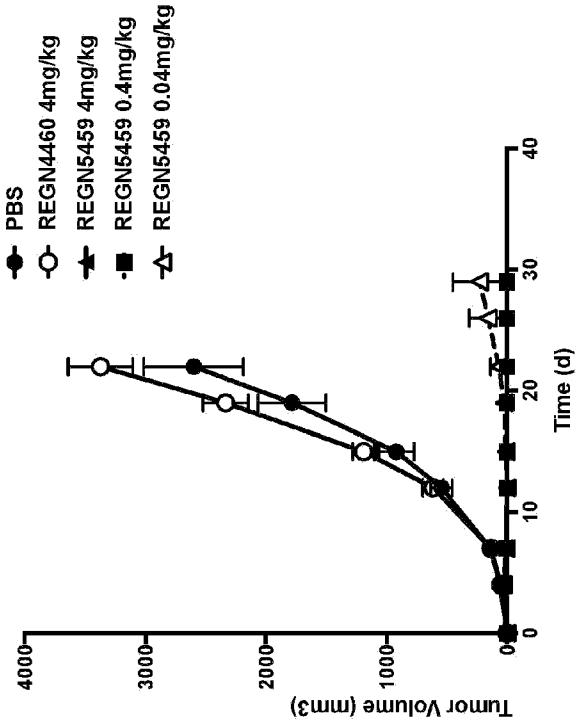


Figure 1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REGN5459 4mg/kg
REGN5459 0.4mg/kg
REGN5459 0.04mg/kg
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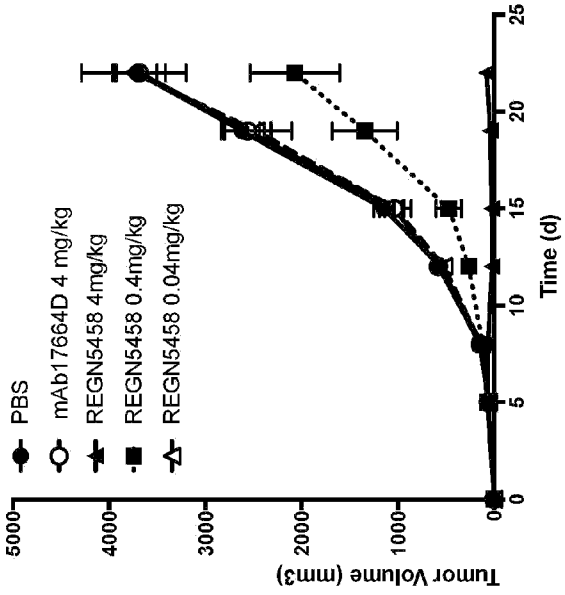
3000
2000
1000
0
0
10
20
30
40
Time (d)

Figure 2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REGN5458 4mg/kg
REGN5458 0.4mg/kg
REGN5458 0.04mg/kg
Tumor Volume (mm3)
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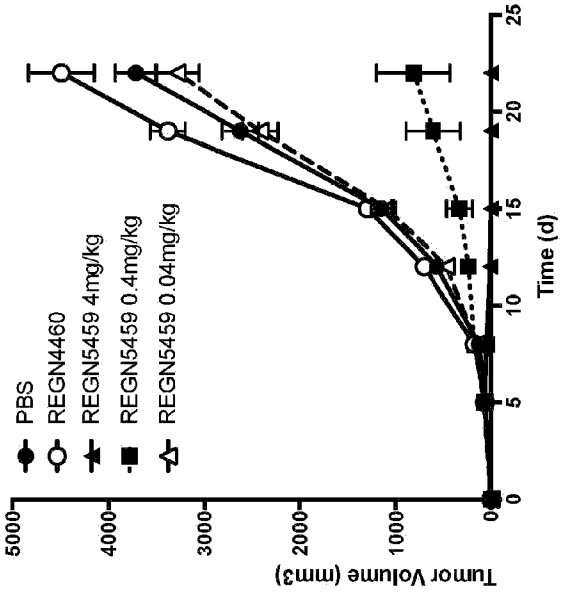
4000
3000
2000
1000
0
0
5
10
15
20
25
Time (d)

Figure 3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REGN4460
REGN5459 4mg/kg
REGN5459 0.4mg/kg
REGN5459 0.04mg/kg
Tumor Volume (mm3)
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4000
3000
2000
1000
0
0
5
10
15
20
25
Time (d)

Figure 4

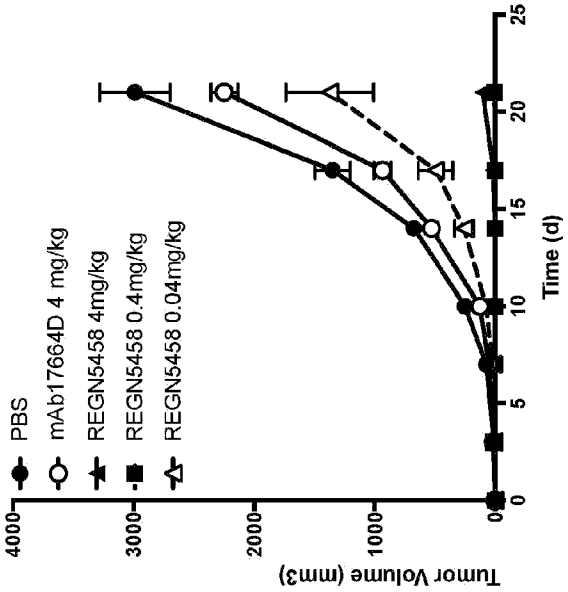
PBS
mAb17664D 4 mg/kg
REGN5458 4mg/kg
REGN5458 0.4mg/kg
REGN5458 0.04mg/kg
Tumor Volume (mm3)
4000
3000
2000
1000
0
0
5
10
15
20
25
Time (d)

Figure 5

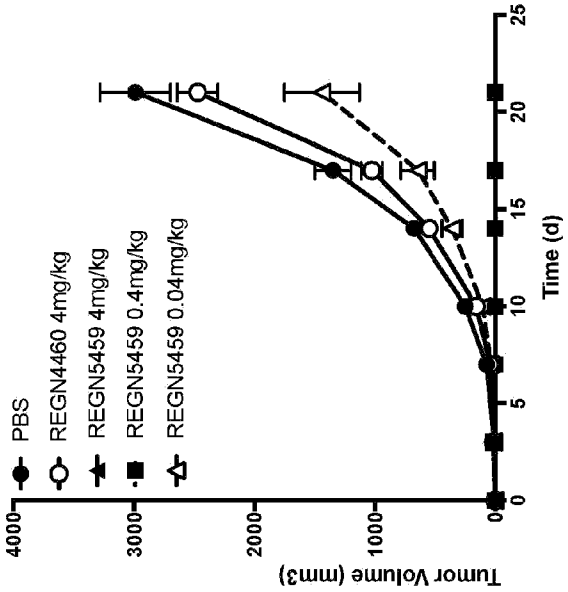
PBS
REGN4460 4mg/kg
REGN5459 4mg/kg
REGN5459 0.4mg/kg
REGN5459 0.04mg/kg
Tumor Volume (mm3)
4000
3000
2000
1000
0
0
5
10
15
20
25
Time (d)

Figure 6

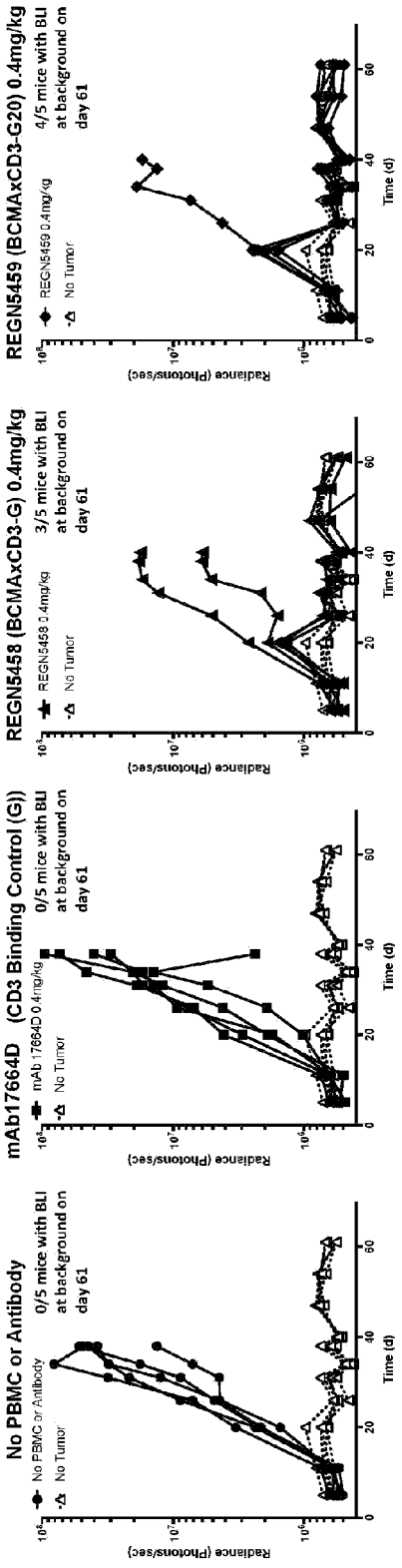
No PBMC or Antibody
0/5 mice with BLI at background on day 61
No PBMC or Antibody
No Tumor
mAb17664D (CD3 Binding Control (G))
0/5 mice with BLI at background on day 61
mAb 17664D 0.4mg/kg
No Tumor
REGN5458 (BCMAxCD3-G) 0.4mg/kg
3/5 mice with BLI at background on day 61
REGN5458 0.4mg/kg
No Tumor
REGN5459 (BCMAxCD3-G20) 0.4mg/kg
4/5 mice with BLI at background on day 61
REGN5459 0.4mg/kg
No Tumor
Radiance (Photons/sec)
Time (d)

Figure 7

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.