ANTI-CD3 ANTIBODIES, BISPECIFIC ANTIGEN-BINDING MOLECULES THAT BIND CD3 AND CD20, AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to antibodies, and antigen-binding fragments thereof, which are specific for CD3, and methods of use thereof. The present invention also relates to bispecific antigen-binding molecules that bind CD3 and a target molecule such as CD20, and methods of use thereof.

BACKGROUND

[0002] 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.

[0003] CD20 is a non-glycosylated phosphoprotein expressed on the cell membranes of mature B cells. CD20 is considered a B cell tumor-associated antigen because it is expressed by more than 95% of B-cell non-Hodgkin lymphomas (NHLs) and other B-cell malignancies, but it is absent on precursor B-cells, dendritic cells and plasma cells. Methods for treating cancer by targeting CD20 are known in the art. For example, the chimeric anti-CD20 monoclonal antibody rituximab has been used or suggested for use in treating cancers such as NHL, chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). CD20 is believed to kill CD20-expressing tumor cells by complement dependent cytotoxicity (CDC), antibody-dependent cell mediated cytotoxicity (ADCC) and/or induction of apoptosis and sensitization to chemotherapy. Although anti-CD20 tumor targeting strategies have shown great promise in clinical settings, not all patients respond to anti-CD20 therapy, and some patients have been shown to develop resistance to or exhibit incomplete responses to anti-CD20 therapy (*e.g.*, resistance to rituximab).

[0004] Bispecific antigen-binding molecules that bind both CD3 and a target antigen (such as CD20) would be useful in therapeutic settings in which specific targeting and T cell-mediated killing of cells that express the target antigen is desired.

BRIEF SUMMARY OF THE INVENTION

[0005] In a first aspect, the present invention provides antibodies and antigen-binding

fragments thereof that bind human CD3. The antibodies according to this aspect of the invention are useful, *inter alia*, for targeting T cells expressing CD3, and for stimulating T cell activation, *e.g.*, under circumstances where T cell-mediated killing is beneficial or desirable. The anti-CD3 antibodies of the invention, or antigen-binding portions thereof, may be included as part of a bispecific antibody that directs CD3-mediated T cell activation to specific cell types such as tumor cells or infectious agents.

[0006] Exemplary anti-CD3 antibodies of the present invention are listed in Tables 1 and 2 herein. Table 1 sets forth the amino acid sequence identifiers of the heavy chain variable regions (HCVRs) and light chain variable regions (LCVRs), as well as heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3), and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) of the exemplary anti-CD3 antibodies. Table 2 sets forth the sequence identifiers of the nucleic acid molecules encoding the HCVRs, LCVRs, HCDR1, HCDR2 HCDR3, LCDR1, LCDR2 and LCDR3 of the exemplary anti-CD3 antibodies.

[0007] The present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCVR comprising an amino acid sequence selected from any of the HCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0008] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising an LCVR comprising an amino acid sequence selected from any of the LCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0009] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising an HCVR and an LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 1 paired with any of the LCVR amino acid sequences listed in Table 1. According to certain embodiments, the present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCVR/LCVR amino acid sequence pair contained within any of the exemplary anti-CD3 antibodies listed in Table 1. In certain embodiments, the HCVR/LCVR amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 2/10 (*e.g.*, H1H2712N); 114/122 (*e.g.*, H2M2609N); 514/522 (*e.g.*, H2M3563N); 770/778 (*e.g.*, H1H5778P); 1050/1234 (*e.g.*, H1H7195B); and 1090/1234 (*e.g.*, H1H7208B).

[0010] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0011] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from

any of the HCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0012] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from any of the HCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0013] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0014] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0015] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0016] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3 and an LCDR3 amino acid sequence pair (HCDR3/LCDR3) comprising any of the HCDR3 amino acid sequences listed in Table 1 paired with any of the LCDR3 amino acid sequences listed in Table 1. According to certain embodiments, the present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3/LCDR3 amino acid sequence pair contained within any of the exemplary anti-CD3 antibodies listed in Table 1. In certain embodiments, the HCDR3/LCDR3 amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 8/16 (*e.g.*, H1H2712N); 120/128 (*e.g.*, H2M2609N); 520/528 (*e.g.*, H2M3563N); 776/784 (*e.g.*, H1H5778P); 1056/1240 (*e.g.*, H1H7195B); and 1096/1240 (*e.g.*, H1H7208B).

[0017] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within any of the exemplary anti-CD3 antibodies listed in Table 1. In certain embodiments, the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set is selected from the group consisting of SEQ ID NOs: 4-6-8-12-14-16 (*e.g.*, H1H2712N); 116-118-120-124-126-128 (*e.g.*, H2M2609N); 516-518-520-524-526-528 (*e.g.*, H2M3563N); 772-774-776-780-782-784 (*e.g.*, H1H5778P); 1052-1054-1056-1236-1238-1240 (*e.g.*, H1H7195B); and 1092-1094-1096-1236-1238-1240 (*e.g.*, H1H7208B).

[0018] In a related embodiment, the present invention provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-

LCDR3) contained within an HCVR/LCVR amino acid sequence pair as defined by any of the exemplary anti-CD3 antibodies listed in Table 1. For example, the present invention includes antibodies, or antigen-binding fragments thereof, comprising the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set contained within an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10 (*e.g.*, H1H2712N); 114/122 (*e.g.*, H2M2609N); 514/522 (*e.g.*, H2M3563N); 770/778 (*e.g.*, H1H5778P); 1050/1234 (*e.g.*, H1H7195B); and 1090/1234 (*e.g.*, H1H7208B). Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See*, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol. 273*:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA 86*:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[0019] The present invention also provides nucleic acid molecules encoding anti-CD3 antibodies or portions thereof. For example, the present invention provides nucleic acid molecules encoding any of the HCVR amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0020] The present invention also provides nucleic acid molecules encoding any of the LCVR amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0021] The present invention also provides nucleic acid molecules encoding any of the HCDR1 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0022] The present invention also provides nucleic acid molecules encoding any of the HCDR2 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at

least 95%, at least 98% or at least 99% sequence identity thereto.

[0023] The present invention also provides nucleic acid molecules encoding any of the HCDR3 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0024] The present invention also provides nucleic acid molecules encoding any of the LCDR1 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0025] The present invention also provides nucleic acid molecules encoding any of the LCDR2 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0026] The present invention also provides nucleic acid molecules encoding any of the LCDR3 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0027] The present invention also provides nucleic acid molecules encoding an HCVR, wherein the HCVR comprises a set of three CDRs (*i.e.*, HCDR1-HCDR2-HCDR3), wherein the HCDR1-HCDR2-HCDR3 amino acid sequence set is as defined by any of the exemplary anti-CD3 antibodies listed in Table 1.

[0028] The present invention also provides nucleic acid molecules encoding an LCVR, wherein the LCVR comprises a set of three CDRs (*i.e.*, LCDR1-LCDR2-LCDR3), wherein the LCDR1-LCDR2-LCDR3 amino acid sequence set is as defined by any of the exemplary anti-CD3 antibodies listed in Table 1.

[0029] The present invention also provides nucleic acid molecules encoding both an HCVR and an LCVR, wherein the HCVR comprises an amino acid sequence of any of the HCVR amino acid sequences listed in Table 1, and wherein the LCVR comprises an amino acid sequence of any of the LCVR amino acid sequences listed in Table 1. In certain embodiments, the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto, and a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or

at least 99% sequence identity thereto. In certain embodiments according to this aspect of the invention, the nucleic acid molecule encodes an HCVR and LCVR, wherein the HCVR and LCVR are both derived from the same anti-CD3 antibody listed in Table 1.

[0030] The present invention also provides recombinant expression vectors capable of expressing a polypeptide comprising a heavy or light chain variable region of an anti-CD3 antibody. For example, the present invention includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the HCVR, LCVR, and/or CDR sequences as set forth in Table 1. Also included within the scope of the present invention are host cells into which such vectors have been introduced, as well as methods of producing the antibodies or portions thereof by culturing the host cells under conditions permitting production of the antibodies or antibody fragments, and recovering the antibodies and antibody fragments so produced.

[0031] The present invention includes anti-CD3 antibodies having a modified glycosylation pattern. In some embodiments, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fucose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0032] In another aspect, the invention provides a pharmaceutical composition comprising a recombinant human antibody or fragment thereof which specifically binds CD3 and a pharmaceutically acceptable carrier. In a related aspect, the invention features a composition which is a combination of an anti-CD3 antibody and a second therapeutic agent. In one embodiment, the second therapeutic agent is any agent that is advantageously combined with an anti-CD3 antibody. Exemplary agents that may be advantageously combined with an anti-CD3 antibody include, without limitation, other agents that bind and/or activate CD3 signaling (including other antibodies or antigen-binding fragments thereof, etc.) and/or agents which do not directly bind CD3 but nonetheless activate or stimulate immune cell activation. Additional combination therapies and co-formulations involving the anti-CD3 antibodies of the present invention are disclosed elsewhere herein.

[0033] In yet another aspect, the invention provides therapeutic methods for stimulating T cell activation using an anti-CD3 antibody or antigen-binding portion of an antibody of the invention, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an antibody or antigen-binding fragment of an antibody of the invention to a subject in need thereof. The disorder treated is any disease or condition which is improved, ameliorated, inhibited or prevented by stimulation of CD3 activity or signaling.

[0034] According to another aspect, the present invention provides bispecific antigen-binding molecules that bind CD3 and a target antigen. According to certain exemplary embodiments,

the bispecific antigen-binding molecules bind CD3 and CD20; such bispecific antigen-binding molecules are also referred to herein as "anti-CD3/anti-CD20 bispecific molecules." The anti-CD20 portion of the anti-CD3/anti-CD20 bispecific molecule is useful for targeting tumor cells that express CD20 (*e.g.*, B-cell tumors), and the anti-CD3 portion of the bispecific molecule is useful for activating T-cells. The simultaneous binding of CD20 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. The anti-CD3/anti-CD20 bispecific molecules of the invention are therefore useful, *inter alia*, for treating diseases and disorders related to or caused by CD20-expressing tumors (*e.g.*, lymphomas).

[0035] The bispecific antigen-binding molecules according to this aspect of the present invention comprise a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that specifically binds CD20. The present invention includes anti-CD3/anti-CD20 bispecific molecules (*e.g.*, bispecific antibodies) wherein each antigen-binding domain comprises a heavy chain variable region (HCVR) paired with a light chain variable region (LCVR). In certain exemplary embodiments of the invention, the anti-CD3 antigen-binding domain and the anti-CD20 antigen binding domain each comprise different, distinct HCVRs paired with a common LCVR. For example, as illustrated in Example 7 herein, bispecific antibodies were constructed comprising a first antigen-binding domain that specifically binds CD3, wherein the first antigen-binding domain comprises an HCVR/LCVR pair derived from an anti-CD3 antibody; and a second antigen-binding domain that specifically binds CD20, wherein the second antigen-binding domain comprises an HCVR derived from an anti-CD20 antibody paired with an LCVR derived from an anti-CD3 antibody (*e.g.*, the same LCVR that is included in the anti-CD3 antigen-binding domain). In other words, in the exemplary molecules disclosed herein, the pairing of an HCVR from an anti-CD20 antibody with an LCVR from an anti-CD3 antibody creates an antigen-binding domain that specifically binds CD20 (but does not bind CD3). In such embodiments, the first and second antigen-binding domains comprise distinct anti-CD3 and anti-CD20 HCVRs but share a common anti-CD3 LCVR.

[0036] The present invention provides anti-CD3/anti-CD20 bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises any of the HCVR amino acid sequences as set forth in Table 1 or Table 18. The first antigen-binding domain that specifically binds CD3 may also comprise any of the LCVR amino acid sequences as set forth in Table 1 or Table 19. According to certain embodiments, the first antigen-binding domain that specifically binds CD3 comprises any of the HCVR/LCVR amino acid sequence pairs as set forth in Table 1 or Table 17. The present invention also provides anti-CD3/anti-CD20 bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises any of the heavy chain CDR1-CDR2-CDR3 amino acid sequences as set forth in Table 1 or Table 18, and/or any of the light chain CDR1-CDR2-CDR3 amino acid sequences as set forth in Table 1 or Table 19.

[0037] According to certain embodiments, the present invention provides anti-CD3/anti-CD20

bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs:1250, 1266, 1282, 1298, 1314 and 1329 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0038] The present invention also provides anti-CD3/anti-CD20 bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs:1258, 1274, 1290, 1306, 1322 and 1333, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0039] The present invention also provides anti-CD3/anti-CD20 bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises a HCVR and LCVR (HCVR/LCVR) amino acid sequence pair selected from the group consisting of SEQ ID NOs:1250/1258, 1266/1274, 1282/1290, 1298/1306, 1314/1322, and 1329/1333.

[0040] The present invention also provides anti-CD3/anti-CD20 bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises a heavy chain CDR3 (HCDR3) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs:1256, 1272, 1288, 1304, 1320 and 1332, or a substantially similar sequence thereto having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR3 (LCDR3) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs:1264, 1280, 1296, 1312, 1328 and 1336, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0041] In certain embodiments, the first antigen-binding domain that specifically binds CD3 comprises a HCDR3/LCDR3 amino acid sequence pair selected from the group consisting of SEQ ID NOs: 1256/1264, 1272/1280, 1288/1296, 1304/1312, 1320/1328 and 1332/1336.

[0042] The present invention also provides anti-CD3/anti-CD20 bispecific antigen-binding molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises a heavy chain CDR1 (HCDR1) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs:1252, 1268, 1284, 1300, 1316 and 1330, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR2 (HCDR2) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs:1254, 1270, 1286, 1302, 1318 and 1331, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a light chain CDR1 (LCDR1) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs:1260, 1276, 1292, 1308, 1324 and 1334, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR2 (LCDR2) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs:1262, 1278, 1294, 1310, 1326 and

1335, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0043] Certain non-limiting, exemplary anti-CD3/anti-CD20 bispecific antigen-binding molecules of the invention include a first antigen-binding domain that specifically binds CD3 comprising HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, having the amino acid sequences selected from the group consisting of: SEQ ID NOs: 1252-1254-1256-1260-1262-1264 (*e.g.* BS3/20-001); 1268-1270-1272-1276-1278-1280 (*e.g.* BS3/20-002); 1284-1286-1288-1292-1294-1296 (*e.g.* BS3/20-003); 1300-1302-1304-1308-1310-1312 (*e.g.* BS3/20-004); 1316-1318-1320-1324-1326-1328 (*e.g.* BS3-20-005); and 1330-1331-1332-1334-1335-1336 (*e.g.* BS3/20-007).

[0044] The present invention also provides anti-CD3/anti-CD20 bispecific molecules, wherein the second antigen-binding domain that specifically binds CD20 comprises a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:1242, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0045] The present invention also provides anti-CD3/anti-CD20 bispecific molecules, wherein the second antigen-binding domain that specifically binds CD20 comprises a light chain variable region (LCVR) having the amino acid sequence selected from the group consisting of SEQ ID NOs:1258, 1274, 1290, 1306, 1322 and 1333, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0046] The present invention also provides anti-CD3/anti-CD20 bispecific molecules, wherein the second antigen-binding domain that specifically binds CD20 comprises a HCVR and LCVR (HCVR/LCVR) amino acid sequence pair selected from the group consisting of SEQ ID NOs: 1242/1258, 1242/1274, 1242/1290, 1242/1306, 1242/1322 and 1242/1333.

[0047] The present invention also provides anti-CD3/anti-CD20 bispecific molecules, wherein the second antigen-binding domain that specifically binds CD20 comprises a heavy chain CDR3 (HCDR3) domain having the amino acid sequence of SEQ ID NO:1248, or a substantially similar sequence thereto having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR3 (LCDR3) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1264, 1280, 1296, 1312, 1328 and 1336, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0048] In certain embodiments, the second antigen-binding domain that specifically binds CD20 comprises a HCDR3/LCDR3 amino acid sequence pair selected from the group consisting of SEQ ID NOs: 1248/1264, 1248/1280, 1248/1296, 1248/1312, 1248/1328 and 1248/1336.

[0049] The present invention also provides anti-CD3/anti-CD20 bispecific antigen-binding molecules, wherein the second antigen-binding domain that specifically binds CD20 comprises

a heavy chain CDR1 (HCDR1) domain having the amino acid sequence of SEQ ID NO:1244, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR2 (HCDR2) domain having the amino acid sequence of SEQ ID NO:1246, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a light chain CDR1 (LCDR1) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1260, 1276, 1292, 1308, 1324 and 1334, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR2 (LCDR2) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1262, 1278, 1294, 1310, 1326 and 1335, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0050] Certain non-limiting, exemplary anti-CD3/anti-CD20 bispecific antigen-binding molecules of the invention include a second antigen-binding domain that specifically binds CD20 comprising HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, having the amino acid sequences selected from the group consisting of: SEQ ID NOs: 1244-1246-1248-1260-1262-1264 (*e.g.* BS3/20-001); 1244-1246-1248-1276-1278-1280 (*e.g.* BS3/20-002); 1244-1246-1248-1292-1294-1296 (*e.g.* BS3/20-003); 1244-1246-1248-1308-1310-1312 (*e.g.* BS3/20-004); 1244-1246-1248-1324-1326-1328 (*e.g.* BS3-20-005); and 1244-1246-1248-1334-1335-1336 (*e.g.* BS3/20-007).

[0051] In a related embodiment, the invention includes anti-CD3/anti-CD20 bispecific antigen-binding molecules wherein the second antigen-binding domain that specifically binds CD20 comprises the heavy and light chain CDR domains contained within heavy and light chain variable region (HCVR/LCVR) sequences selected from the group consisting of SEQ ID NOs: 1242/1258, 1242/1274, 1242/1290, 1242/1306, 1242/1322 and 1242/1333.

[0052] In another aspect, the present invention provides nucleic acid molecules encoding any of the HCVR, LCVR or CDR sequences of the anti-CD3/anti-CD20 bispecific antigen-binding molecules disclosed herein, including nucleic acid molecules comprising the polynucleotide sequences as set forth in Tables 20 and 21 herein, as well as nucleic acid molecules comprising two or more of the polynucleotide sequences as set forth in Tables 20 and 21 in any functional combination or arrangement thereof. Recombinant expression vectors carrying the nucleic acids of the invention, and host cells into which such vectors have been introduced, are also encompassed by the invention, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

[0053] The present invention includes anti-CD3/anti-CD20 bispecific antigen-binding molecules wherein any of the aforementioned antigen-binding domains that specifically bind CD3 is combined, connected or otherwise associated with any of the aforementioned antigen-binding domains that specifically bind CD20 to form a bispecific antigen-binding molecule that

binds CD3 and CD20.

[0054] The present invention includes anti-CD3/anti-CD20 bispecific antigen-binding molecules having a modified glycosylation pattern. In some applications, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fucose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0055] In another aspect, the invention provides a pharmaceutical composition comprising an anti-CD3/anti-CD20 bispecific antigen-binding molecule as disclosed herein and a pharmaceutically acceptable carrier. In a related aspect, the invention features a composition which is a combination of an anti-CD3/anti-CD20 bispecific antigen-binding molecule and a second therapeutic agent. In one embodiment, the second therapeutic agent is any agent that is advantageously combined with an anti-CD3/anti-CD20 bispecific antigen-binding molecule. Exemplary agents that may be advantageously combined with an anti-CD3/anti-CD20 bispecific antigen-binding molecule are discussed in detail elsewhere herein.

[0056] In yet another aspect, the invention provides therapeutic methods for targeting/killing tumor cells expressing CD20 using an anti-CD3/anti-CD20 bispecific antigen-binding molecule of the invention, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an anti-CD3/anti-CD20 bispecific antigen-binding molecule of the invention to a subject in need thereof.

[0057] The present invention also includes the use of an anti-CD3/anti-CD20 bispecific antigen-binding molecule of the invention in the manufacture of a medicament for the treatment of a disease or disorder related to or caused by CD20 expression.

[0058] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0059] **Figure 1** shows the tumor volume (in mm^3) over time in NOD/SCID mice implanted subcutaneously with a mixture of Raji tumor cells and PBMCs following tumor implantation and treatment, starting the day of tumor implantation, with either human Fc (hFc, solid line) or CD3xCD20 bispecific antibody (BS3/20-007, dashed line).

[0060] **Figure 2** shows the tumor volume (in mm^3) over time in NOD/SCID mice implanted subcutaneously with a mixture of Raji tumor cells and PBMCs following tumor implantation and treatment, starting 7 days after tumor implantation, with either human Fc (hFc, solid line) or CD3xCD20 bispecific antibody (BS3/20-007, dashed line).

[0061] **Figure 3** shows a plot of B-cell numbers (x1000/µL) over time in blood samples from cynomolgus monkeys treated with three different doses of bispecific antibody BS3/20-001 (0.01,

0.1 or 1.0 mg/kg); low-dose anti-CD20 control antibody (Control V, 0.01 mg/kg); or high-dose anti-CD20 control antibody (Control III (1.0 mg/kg).

[0062] **Figure 4** shows a plot of T-cell numbers (x1000/μL) over time in blood samples from cynomolgus monkeys treated with three different doses of bispecific antibody BS3/20-001 (0.01, 0.1 or 1.0 mg/kg); low-dose anti-CD20 control antibody (Control V, 0.01 mg/kg); or high-dose anti-CD20 control antibody (Control III (1.0 mg/kg).

[0063] **Figures 5A, 5B, 5C** and **5D** show the pre-dose and post-dose levels (pg/mL) of IFN-gamma, IL-2, IL-6, and TNF-alpha, respectively, for cynomolgous monkeys treated with a single dose of BS3/20-001 (0.01, 0.1 or 1.0 mg/kg), low dose anti-CD20 control antibody (0.01 mg/kg Control V), or high-dose anti-CD20 control antibody (1.0 mg/kg Control III).

[0064] **Figure 6** shows the CD20 expression profile (expressed in terms of Log2 fold change in expression) determined from blood samples taken at various time points from cynomolgus monkeys treated with 0.01 mg/kg Control V (anti-CD20 antibody); 1.0 mg/kg Control III (anti-CD20 antibody); and 0.01 mg/kg, 0.1 mg/kg and 1.0 mg/kg BS3/20-001 (anti-CD3xCD20 bispecific antibody).

[0065] **Figure 7** shows the total serum concentration (μg/mL) of CD3xCD20 bispecific antibody (BS3/20-001) over time in blood samples from cynomolgus monkeys treated with 1.0 mg/kg (open triangles), 0.1 mg/kg (open squares) or 0.01 mg/kg (open diamonds) of CD3xCD20 bispecific antibody.

DETAILED DESCRIPTION

[0066] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0067] 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.).

[0068] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

Definitions

[0069] 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:1370; human CD3-delta comprises the amino acid sequence as set forth in SEQ ID NO:1371. 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.

[0070] 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.

[0071] 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.

[0072] As used herein, the expression "anti-CD3 antibody" includes both monovalent antibodies with a single specificity, as well as bispecific antibodies comprising a first arm that binds CD3 and a second arm that binds a second (target) antigen, wherein the anti-CD3 arm comprises any of the HCVR/LCVR or CDR sequences as set forth in Table 1 or Tables 18/19 herein. Examples of anti-CD3 bispecific antibodies are described elsewhere herein. The term "antigen-binding molecule" includes antibodies and antigen-binding fragments of antibodies, including, *e.g.*, bispecific antibodies.

[0073] 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.*, 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). 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 amino-terminus 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-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.

[0074] 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.

[0075] 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.

[0076] 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.

[0077] In certain embodiments, 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 of the present invention 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 of the present invention 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)).

[0078] 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.

[0079] 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 cell-dependent cytotoxicity. Thus, the isotype of an antibody may be selected on the basis of whether it is desirable for the antibody to mediate cytotoxicity.

[0080] In certain embodiments of the invention, the anti-CD3 antibodies of the invention (monospecific or bispecific) 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. The human antibodies of the invention 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.

[0081] 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*.

[0082] 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.

[0083] 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.

[0084] The antibodies of the invention may be 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.

[0085] The present invention also includes one-arm antibodies that bind CD3. 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 of the present invention may comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 1 or Tables 18/19 herein.

[0086] The 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 invention 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 embodiments, 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 embodiments, 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 embodiments, 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 invention 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 invention.

[0087] The present invention also includes 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 invention includes 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 Table 1 herein.

[0088] 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.

[0089] 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.

[0090] 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 asparagine-glutamine. 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.

[0091] 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.

Bispecific Antigen-Binding Molecules

[0092] The antibodies of the present invention 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-CD3 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 binding specificity.

[0093] Use of the expression "anti-CD3 antibody" herein is intended to include both monospecific anti-CD3 antibodies as well as bispecific antibodies comprising a CD3-binding arm and a second arm that binds a target antigen. Thus, the present invention includes bispecific antibodies wherein one arm of an immunoglobulin binds human CD3, and the other arm of the immunoglobulin is specific for a target antigen. The target antigen that the other arm of the CD3 bispecific antibody binds can be any antigen expressed on or in the vicinity of a cell, tissue, organ, microorganism or virus, against which a targeted immune response is desired. The CD3-binding arm can comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 1 or Tables 18/19 herein. In certain embodiments, the CD3-binding arm binds human CD3 and induces human T cell proliferation.

[0094] In the context of bispecific antibodies of the present invention wherein one arm of the antibody binds CD3 and the other arm binds a target antigen, the target antigen can be a tumor-associated antigen. Non-limiting examples of specific tumor-associated antigens include, *e.g.*, AFP, ALK, BAGE proteins, β-catenin, brc-abl, BRCA1, BORIS, CA9, carbonic anhydrase IX, caspase-8, CCR5, CD19, CD20, CD30, CD40, CDK4, CEA, CTLA4, cyclin-B1, CYP1B1, EGFR, EGFRvIII, ErbB2/Her2, ErbB3, ErbB4, ETV6-AML, EpCAM, EphA2, Fra-1, FOLR1, GAGE proteins (*e.g.*, GAGE-1, -2), GD2, GD3, GloboH, glypican-3, GM3, gp100, Her2, HLA/B-raf, HLA/k-ras, HLA/MAGE-A3, hTERT, LMP2, MAGE proteins (*e.g.*, MAGE-1, -2, -3, -4, -6, and -12), MART-1, mesothelin, ML-IAP, Muc1, Muc2, Muc3, Muc4, Muc5, Muc16 (CA-125), MUM1, NA17, NY-BR1, NY-BR62, NY-BR85, NY-ESO1, OX40, p15, p53, PAP, PAX3, PAX5, PCTA-1, PLAC1, PRLR, PRAME, PSMA (FOLH1), RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, Steap-1, Steap-2, survivin, TAG-72, TGF-β, TMPRSS2, Tn, TRP-1, TRP-2, tyrosinase, and uroplakin-3.

[0095] In the context of bispecific antibodies of the present invention wherein one arm of the antibody binds CD3 and the other arm binds a target antigen, the target antigen can be an infectious disease-associated antigen. Non-limiting examples of infectious disease-associated antigens include, *e.g.*, an antigen that is expressed on the surface of a virus particle, or preferentially expressed on a cell that is infected with a virus, wherein the virus is selected from the group consisting of HIV, hepatitis (A, B or C), herpes virus (*e.g.*, HSV-1, HSV-2, CMV, HAV-6, VZV, Epstein Barr virus), adenovirus, influenza virus, flavivirus, echovirus, rhinovirus, coxsackie virus, coronavirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus, and arboviral encephalitis virus. Alternatively, the target antigen can be an antigen that is expressed on the surface of a bacterium, or preferentially expressed on a cell that is infected with a bacterium, wherein the bacterium is selected from the group consisting of chlamydia, rickettsia, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci, gonococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospira, and Lyme disease bacteria. In certain embodiments, the target antigen is an antigen that is expressed on the surface of a fungus, or preferentially expressed on a cell that is infected with a fungus, wherein the fungus is selected from the group consisting of Candida (albicans, krusei, glabrata, tropicalis, etc.), Crytococcus neoformans, Aspergillus (fumigatus, niger, etc.), Mucorales (mucor, absidia, rhizopus, etc.), Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis, and Histoplasma capsulatum. In certain embodiments, the target antigen is an antigen that is expressed on the surface of a parasite, or preferentially expressed on a cell that is infected with a parasite, wherein the parasite is selected from the group consisting of Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondii, Nippostrongylus brasiliensis, Taenia crassiceps, and Brugia malayi. Non-limiting examples of specific pathogen-associated antigens include, *e.g.*,HIV gp120, HIV CD4, hepatitis B glucoprotein L, hepatitis B glucoprotein M, hepatitis B glucoprotein S, hepatitis C E1, hepatitis C E2, hepatocyte-specific protein, herpes simplex virus gB, cytomegalovirus gB, and HTLV envelope protein.

[0096] According to certain exemplary embodiments, the present invention includes bispecific antigen-binding molecules that specifically bind CD3 and CD20. Such molecules may be referred to herein as, *e.g.*, "anti-CD3/anti-CD20," or "anti-CD3xCD20" or "CD3xCD20" bispecific molecules, or other similar terminology.

[0097] The term "CD20," as used herein, refers to the human CD20 protein unless specified as being from a non-human species (*e.g.*, "mouse CD20," "monkey CD20," etc.). The human CD20 protein has the amino acid sequence shown in SEQ ID NO:1369.

[0098] 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 embodiments, an antigen-binding molecule is an antibody or a fragment of an antibody, as those terms are defined elsewhere herein.

[0099] 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 (*e.g.*, CD3), and the second antigen-binding domain specifically binds a second, distinct antigen (*e.g.*, CD20).

[0100] 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 "A1" and the CDRs of the second antigen-binding domain may be designated with the prefix "A2". Thus, the CDRs of the first antigen-binding domain may be referred to herein as A1-HCDR1, A1-HCDR2, and A1-HCDR3; and the CDRs of the second antigen-binding domain may be referred to herein as A2-HCDR1, A2-HCDR2, and A2-HCDR3.

[0101] 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.

[0102] 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.

[0103] In certain embodiments, the multimerizing domain is an Fc fragment or an amino acid sequence of 1 to about 200 amino acids in length containing at least one cysteine residues. 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.

[0104] 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, IgG-scFv 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).

[0105] 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).

[0106] 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). 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.

[0107] 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 Provisional Application No. 61/759,578, filed February 1, 2013. 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

[0108] The antibodies and bispecific antigen-binding molecules of the present invention 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 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 invention 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 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 embodiments, 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 antigen-binding domain was originally derived. In other embodiments, 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 embodiments, 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 antigen-binding domain was originally derived). Furthermore, the antigen-binding domains 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, 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 invention.

[0109] The present invention also includes antigen-binding molecules wherein one or both antigen-binding domains comprise 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 invention includes antigen-binding molecules comprising an antigen-binding domain 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 disclosed herein. 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. 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 asparagine-glutamine. 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.

[0110] The present invention also includes antigen-binding molecules comprising an antigen-binding domain with an HCVR, LCVR, and/or CDR amino acid sequence that is substantially identical to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein. 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.

[0111] 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.

pH-Dependent Binding

[0112] The present invention includes anti-CD3 antibodies, and anti-CD3/anti-CD20 bispecific antigen-binding molecules, with pH-dependent binding characteristics. For example, an anti-CD3 antibody of the present invention may exhibit reduced binding to CD3 at acidic pH as compared to neutral pH. Alternatively, anti-CD3 antibodies of the invention may exhibit enhanced binding to CD3 at acidic pH as compared to neutral pH. The expression "acidic pH" includes pH values less than about 6.2, *e.g.*, about 6.0, 5.95, 5,9, 5.85, 5.8, 5.75, 5.7, 5.65, 5.6, 5.55, 5.5, 5.45, 5.4, 5.35, 5.3, 5.25, 5.2, 5.15, 5.1, 5.05, 5.0, or less. As used herein, the expression "neutral pH" means a pH of about 7.0 to about 7.4. The expression "neutral pH" includes pH values of about 7.0, 7.05, 7.1, 7.15, 7.2, 7.25, 7.3, 7.35, and 7.4.

[0113] In certain instances, "reduced binding ... at acidic pH as compared to neutral pH" is expressed in terms of a ratio of the K_D value of the antibody binding to its antigen at acidic pH to the K_D value of the antibody binding to its antigen at neutral pH (or vice versa). For example, an antibody or antigen-binding fragment thereof may be regarded as exhibiting "reduced binding to CD3 at acidic pH as compared to neutral pH" for purposes of the present invention if the antibody or antigen-binding fragment thereof exhibits an acidic/neutral K_D ratio of about 3.0 or greater. In certain exemplary embodiments, the acidic/neutral K_D ratio for an antibody or antigen-binding fragment of the present invention can be about 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 20.0. 25.0, 30.0, 40.0, 50.0, 60.0, 70.0, 100.0 or greater.

[0114] Antibodies with pH-dependent binding characteristics may be obtained, *e.g.*, by screening a population of antibodies for reduced (or enhanced) binding to a particular antigen at acidic pH as compared to neutral pH. Additionally, modifications of the antigen-binding domain at the amino acid level may yield antibodies with pH-dependent characteristics. For example, by substituting one or more amino acids of an antigen-binding domain (*e.g.*, within a CDR) with a histidine residue, an antibody with reduced antigen-binding at acidic pH relative to neutral pH may be obtained.

Antibodies Comprising Fc Variants

[0115] According to certain embodiments of the present invention, anti-CD3 antibodies, and anti-CD3/anti-CD20 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).

[0116] For example, the present invention includes anti-CD3 antibodies, and anti-CD3/anti-CD20 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 within the scope of the present invention.

Biological Characteristics of the Antibodies and Bispecific Antigen-Binding Molecules

[0117] The present invention includes antibodies and antigen-binding fragments thereof that bind human CD3 and induce T cell proliferation. For example, the present invention includes anti-CD3 antibodies that induce human T cell proliferation with an EC_{50} value of less than about 0.33 pM, as measured by an *in vitro* T cell proliferation assay, *e.g.*, using the assay format as defined in Example 4 herein (*e.g.*, assessing the proliferation of Jurkat cells or human PBMCs in the presence of anti-CD3 antibodies), or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments of the present invention induce human T cell proliferation (*e.g.*, Jurkat cell proliferation and/or PBMC proliferation) with an EC_{50} value of less than about 0.32 pM, less than about 0.31 pM, less than about 0.30 pM, less than about 0.28 pM, less than about 0.26 pM, less than about 0.24 pM, less than about 0.22 pM, or less than about 0.20 pM, as measured by an *in vitro* T cell proliferation assay, *e.g.*, using the assay format as defined in Example 4 herein, or a substantially similar assay.

[0118] The present invention also includes antibodies and antigen-binding fragments thereof that bind human CD3 and induce T cell-mediated killing of tumor cells. For example, the present invention includes anti-CD3 antibodies that induce T cell-mediated killing of tumor cells with an EC_{50} of less than about 2.3 pM, as measured in an *in vitro* T cell-mediated tumor cell killing assay, *e.g.*, using the assay format as defined in Example 6 herein (*e.g.*, assessing the extent of U937 tumor cell killing by human PBMCs in the presence of anti-CD3 antibodies), or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding

fragments of the present invention induce T cell-mediated tumor cell killing (*e.g.*, PBMC-mediated killing of U937 cells) with an EC_{50} value of less than about 2.3 pM, less than about 2.2 pM, less than about 2.1 pM, less than about 2.0 pM, less than about 1.8 pM, less than about 1.6 pM, less than about 1.4 pM, less than about 1.2 pM, less than about 1.0 pM, less than about 0.8 pM, less than about 0.6 pM, or less than about 0.5 pM, as measured by an *in vitro* T cell-mediated tumor cell killing assay, *e.g.*, using the assay format as defined in Example 6 herein, or a substantially similar assay.

[0119] The present invention includes antibodies and antigen-binding fragments thereof that bind human CD3 with high affinity. The present invention 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. 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.*, CD20), 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. 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.

[0120] According to certain embodiments, the present invention includes antibodies and antigen-binding fragments of antibodies that bind human CD3 (*e.g.*, at 25°C) with a K_D of less than about 15 nM as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein. In certain embodiments, the antibodies or antigen-binding fragments of the present invention bind CD3 with a K_D of less than about 5 nM, less than about 2 nM, less than about 1 nM, less than about 800 pM, less than about 600 pM, less than about 500 pM, less than about 400 pM, less than about 300 pM, less than about 200 pM, less than about 180 pM, less than about 160 pM, less than about 140 pM, less than about 120 pM, less than about 100 pM, less than about 80 pM, less than about 60 pM, less than about 40 pM, less than about 20 pM, or less than about 10 pM, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein (*e.g.*, mAb-capture or antigen-capture format), or a substantially similar assay.

[0121] The present invention also includes antibodies and antigen-binding fragments thereof that bind CD3 with a dissociative half-life (t½) of greater than about 10 minutes as measured by surface plasmon resonance at 25°C or 37°C, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments of the present invention bind CD3 with a t½ of 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 200 minutes, greater than about 300 minutes, greater than about 400 minutes, greater than about 500

minutes, greater than about 600 minutes, greater than about 700 minutes, greater than about 800 minutes, greater than about 900 minutes, greater than about 1000 minutes, or greater than about 1200 minutes, as measured by surface plasmon resonance at 25°C or 37°C, *e.g.*, using an assay format as defined in Example 3 herein (*e.g.*, mAb-capture or antigen-capture format), or a substantially similar assay.

[0122] The present invention includes bispecific antigen-binding molecules (*e.g.*, bispecific antibodies) which are capable of simultaneously binding to human CD3 and human CD20. According to certain embodiments, the bispecific antigen-binding molecules of the invention specifically interact with cells that express CD3 and/or CD20. The extent to which a bispecific antigen-binding molecule binds cells that express CD3 and/or CD20 can be assessed by fluorescence activated cell sorting (FACS), as illustrated in Example 8 herein. For example, the present invention includes bispecific antigen-binding molecules which specifically bind human T-cell lines which express CD3 but not CD20 (*e.g.*, Jurkat), human B-cell lines which express CD20 but not CD3 (*e.g.*, Raji), and/or primate T-cells (*e.g.*, cynomolgus peripheral blood mononuclear cells [PBMCs]). The present invention includes bispecific antigen-binding molecules which bind any of the aforementioned cells and cell lines with an EC_{50} value of from about $9.0x10^{-6}$ to about $2.0x10^{-9}$, or less, as determined using a FACS assay as set forth in Example 8 or a substantially similar assay.

[0123] The present invention also includes anti-CD3/anti-CD20 bispecific antigen-binding molecules which bind to CD3-expressing human T-cells (*e.g.*, Jurkat) with an EC_{50} value of between 1.0 pM and 1000 nM. In certain embodiments, the anti-CD3/anti-CD20 bispecific antigen-binding molecules bind to CD3-expressing human T-cells with an EC50 value of between 1 nM and 60 nM. For example, the present invention includes anti-CD3/anti-CD20 bispecific antigen-binding molecules which bind to CD3-expressing human T-cells (*e.g.*, Jurkat) with an EC_{50} value of about 1 pM. about 10 pM, about 100 pM, about 500 pM, about 1 nM, about 2 nM, about 5 nM, about 10 nM, about 20 nM, about 30 nM, about 40 nM, about 50 nM about 60 nM, about 70 nM, about 80 nM, about 90 nM, about 100 nM, about 200 nM, about 300 nM, about 500 nM, about 800 nM, about 1000 nM, or more.

[0124] The present invention also includes anti-CD3/anti-CD20 bispecific antigen-binding molecules which exhibit one or more characteristics selected from the group consisting of: (a) inducing PBMC proliferation in vitro (*see*, *e.g.*, Example 9 herein); (b) activating T-cells, inducing IFN-gamma release and CD25 up-regulation in human whole blood (*see*, *e.g.*, Example 10 herein); (c) inducing T-cell mediated cytotoxicity on anti-CD20-resistant cell lines (*see*, *e.g.*, Example 11 herein); (d) inducing cytotoxicity to human B-cells (*e.g.*, Raji; *see*, *e.g.*, Example 13 herein); (e) depleting B-cells (*e.g.*, CD19+ B-cells) in mice reconstituted with human immune cells (*see*, *e.g.*, Example 14 herein); and (f) decreasing B-cell tumor volume (*e.g.*, Raji tumor volume) in mouse xenografts (*see*, *e.g.*, Example 15).

[0125] The present invention includes anti-CD3/anti-CD20 bispecific antigen-binding

molecules which are capable of depleting B cells in a subject (*see*, *e.g.*, Example 16). For example, according to certain embodiments, anti-CD3/anti-CD20 bispecific antigen-binding molecules are provided, wherein a single administration of the bispecific antigen-binding molecule to a subject (*e.g.*, at a dose of about 0.1 mg/kg, about 0.08 mg/kg, about 0.06 mg/kg about 0.04 mg/kg, about 0.04 mg/kg, about 0.02 mg/kg, about 0.01 mg/kg, or less) causes a reduction in the number of B cells in the subject (*e.g.*, in a blood sample taken from the subject) below detectable levels. In certain embodiments, a single administration of the anti-CD3/anti-CD20 bispecific antigen-binding molecule at a dose of about 0.1 mg/kg causes a reduction in the number of B cells in the subject below detectable levels by about day 7, about day 6, about day 5, about day 4, about day 3, about day 2, or about day 1 after administration of the bispecific antigen-binding molecule to the subject. According to certain embodiments, a single administration of an anti-CD3/anti-CD20 bispecific antigen-binding molecule of the invention, at a dose of about 0.01 mg/kg, causes the number of B-cells to remain below detectable levels until at least about 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days or more, following the administration. As used herein, the expression "below detectable levels" means that no B cells can be directly or indirectly detected in a blood sample drawn from a subject using standard B-cell detection assays, *e.g.*, a FACS assay for B-cell markers, as set forth in Example 16, herein.

[0126] In related embodiments, an anti-CD3/anti-CD20 bispecific antigen-binding molecule is provided, wherein the number of B-cells per microliter of blood drawn from a subject at about day 1 through about day 28 after administration of a single dose of about 0.01 mg/kg of the antigen-binding molecule to the subject is less than 25% the number of B-cells per microliter of blood drawn from the subject prior to the administration. In certain other embodiments, an anti-CD3/anti-CD20 bispecific antigen-binding molecule is provided, wherein the number of B-cells per microliter of blood drawn from a subject at about day 1 through about day 56 after administration of a single dose of about 0.01 mg/kg of the antigen-binding molecule to the subject is less than 50% the number of B-cells per microliter of blood drawn from the subject prior to the administration.

[0127] The present invention also provides anti-CD3/anti-CD20 bispecific antigen-binding molecules that, when administered to a subject, cause no more than a transient decrease in T cells. For example, anti-CD3/anti-CD20 bispecific antigen-binding molecules are provided that, when administered to a subject at a dose of about 0.01 mg/kg cause the number of T cells to decline at day 1 following administration, but wherein the number of T cells per microliter of blood rebounds at timepoints thereafter (*e.g.*, by about day 2, day 7, day 14, day 28, day 42, day 56 or later following the administration). For example the present invention provides an anti-CD3/anti-CD20 bispecific antigen-binding molecule, wherein the number of T cells per microliter of blood drawn from the subject at about day 14 through about day 56 after administration of the antigen binding molecule to the subject at a dose of about 0.01 mg/kg is

equal to or greater than the number of T cells per microliter of blood drawn from the subject prior to administration of the bispecific antigen-binding molecule.

Epitope Mapping and Related Technologies

[0128] The epitope on CD3 to which the antigen-binding molecules of the present invention bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids of a CD3 protein. Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) of CD3. The antibodies of the invention may interact with amino acids contained within a single CD3 chain (*e.g.*, CD3-epsilon, CD3-delta or CD3-gamma), or may interact with amino acids on two or more different CD3 chains. The term "epitope," as used herein, 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.

[0129] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antigen-binding domain of an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, *e.g.*, routine cross-blocking assay such as that described Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY), alanine scanning mutational analysis, peptide blots analysis (Reineke, 2004, Methods Mol Biol 248:443-463), and peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer, 2000, Protein Science 9:487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antigen-binding domain of an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water to allow hydrogen-deuterium exchange to occur at all residues except for the residues protected by the antibody (which remain deuterium-labeled). After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehring (1999) *Analytical Biochemistry* 267(2):252-259; Engen and Smith (2001) *Anal. Chem. 73*:256A-265A. X-ray crystallography of the antigen/antibody complex may also be used for epitope mapping purposes.

[0130] The present invention further includes anti-CD3 antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 1 herein). Likewise, the present invention also includes anti-CD3 antibodies that compete for binding to CD3 with any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 1 herein).

[0131] The present invention also includes bispecific antigen-binding molecules comprising a first antigen-binding domain that specifically binds human CD3, and a second antigen binding domain that specifically binds human CD20, wherein the first antigen-binding domain binds to the same epitope on CD3 as any of the specific exemplary CD3-specific antigen-binding domains described herein, and/or wherein the second antigen-binding domain binds to the same epitope on CD20 as any of the specific exemplary CD20-specific antigen-binding domains described herein.

[0132] Likewise, the present invention also includes bispecific antigen-binding molecules comprising a first antigen-binding domain that specifically binds human CD3, and a second antigen binding domain that specifically binds human CD20, wherein the first antigen-binding domain competes for binding to CD3 with any of the specific exemplary CD3-specific antigen-binding domains described herein, and/or wherein the second antigen-binding domain competes for binding to CD20 with any of the specific exemplary CD20-specific antigen-binding domains described herein.

[0133] One can easily determine whether a particular antigen-binding molecule (*e.g.*, antibody) or antigen-binding domain thereof binds to the same epitope as, or competes for binding with, a reference antigen-binding molecule of the present invention by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope on CD3 (or CD20) as a reference bispecific antigen-binding molecule of the present invention, the reference bispecific molecule is first allowed to bind to a CD3 protein (or CD20 protein). Next, the ability of a test antibody to bind to the CD3 (or CD20) molecule is assessed. If the test antibody is able to bind to CD3 (or CD20) following saturation binding with the reference bispecific antigen-binding molecule, it can be concluded that the test antibody binds to a different epitope of CD3 (or CD20) than the reference bispecific antigen-binding molecule. On the other hand, if the test antibody is not able to bind to the CD3 (or CD20) molecule following saturation binding with the reference bispecific antigen-binding molecule, then the test antibody may bind to the same epitope of CD3 (or CD20) as the epitope bound by the reference bispecific antigen-binding molecule of the invention. Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference bispecific antigen-binding molecule or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA,

Biacore, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art. In accordance with certain embodiments of the present invention, two antigen-binding proteins bind to the same (or overlapping) epitope if, *e.g.*, a 1-, 5-, 10-, 20- or 100-fold excess of one antigen-binding protein inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, e.g., Junghans et al., Cancer Res. 1990:50:1495-1502). Alternatively, two antigen-binding proteins are deemed to bind to the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antigen-binding protein reduce or eliminate binding of the other. Two antigen-binding proteins are deemed to have "overlapping epitopes" if only a subset of the amino acid mutations that reduce or eliminate binding of one antigen-binding protein reduce or eliminate binding of the other.

[0134] To determine if an antibody or antigen-binding domain thereof competes for binding with a reference antigen-binding molecule, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antigen-binding molecule is allowed to bind to a CD3 protein (or CD20 protein) under saturating conditions followed by assessment of binding of the test antibody to the CD3 (or CD20) molecule. In a second orientation, the test antibody is allowed to bind to a CD3 (or CD20) molecule under saturating conditions followed by assessment of binding of the reference antigen-binding molecule to the CD3 (or CD20) molecule. If, in both orientations, only the first (saturating) antigen-binding molecule is capable of binding to the CD3 (or CD20) molecule, then it is concluded that the test antibody and the reference antigen-binding molecule compete for binding to CD3 (or CD20). As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antigen-binding molecule may not necessarily bind to the same epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Preparation of Antigen-Binding Domains and Construction of Bispecific Molecules

[0135] 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 CD20), 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 of the present invention 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 CD20) 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 of the present invention.

[0136] 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 for a detailed discussion of such engineered mice and the use thereof to produce bispecific antigen-binding molecules).

Bioequivalents

[0137] The present invention encompasses antigen-binding molecules having amino acid sequences that vary from those of the exemplary molecules disclosed herein but that retain the ability to bind CD3 and/or CD20. Such variant molecules may comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described bispecific antigen-binding molecules.

[0138] The present invention includes antigen-binding molecules that are bioequivalent to any of the exemplary antigen-binding molecules set forth herein. Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single does or multiple dose. Some antigen-binding proteins will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, e.g., chronic use, and are considered medically insignificant for the particular drug product studied.

[0139] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[0140] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be

switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[0141] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[0142] Bioequivalence may be demonstrated by in vivo and in vitro methods. Bioequivalence measures include, e.g., (a) an in vivo test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an in vitro test that has been correlated with and is reasonably predictive of human in vivo bioavailability data; (c) an in vivo test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antigen-binding protein.

[0143] Bioequivalent variants of the exemplary bispecific antigen-binding molecules set forth herein may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antigen-binding proteins may include variants of the exemplary bispecific antigen-binding molecules set forth herein comprising amino acid changes which modify the glycosylation characteristics of the molecules, *e.g.*, mutations which eliminate or remove glycosylation.

Species Selectivity and Species Cross-Reactivity

[0144] According to certain embodiments of the invention, antigen-binding molecules are provided which bind to human CD3 but not to CD3 from other species. Also provided are antigen-binding molecules which bind to human CD20 but not to CD20 from other species. The present invention also includes antigen-binding molecules that bind to human CD3 and to CD3 from one or more non-human species; and/or antigen-binding molecules that bind to human CD20 and to CD20 from one or more non-human species.

[0145] According to certain exemplary embodiments of the invention, antigen-binding molecules are provided which bind to human CD3 and/or human CD20 and may bind or not bind, as the case may be, to one or more of mouse, rat, guinea pig, hamster, gerbil, pig, cat, dog, rabbit, goat, sheep, cow, horse, camel, cynomolgus, marmoset, rhesus or chimpanzee CD3 and/or CD20. For example, in a particular exemplary embodiment of the present invention bispecific antigen-binding molecules are provided comprising a first antigen-binding domain that binds human CD3 and cynomologous CD3, and a second antigen-binding domain that

specifically binds human CD20.

Immunoconjugates

[0146] The present invention encompasses antigen-binding molecules conjugated to a therapeutic moiety ("immunoconjugate"), such as a cytotoxin, a chemotherapeutic drug, an immunosuppressant or a radioisotope. Cytotoxic agents include any agent that is detrimental to cells. Examples of suitable cytotoxic agents and chemotherapeutic agents for forming immunoconjugates are known in the art, (see for example, WO 05/103081).

Therapeutic Formulation and Administration

[0147] 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.

[0148] 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).

[0149] 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.

[0150] 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.

[0151] 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.

[0152] 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.

[0153] 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.

[0154] 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

[0155] The present invention includes methods comprising administering to a subject in need thereof a therapeutic composition comprising an anti-CD3 antibody or a bispecific antigen-binding molecule that specifically binds CD3 and a target antigen (*e.g.*, CD20). The therapeutic composition can comprise any of the antibodies or bispecific antigen-binding molecules as disclosed herein and 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 CD20 activity or a depletion of CD20+ B cells.

[0156] The antibodies and 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-CD3/anti-CD20 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 CD20 expression or activity or the proliferation of CD20+ B cells. The mechanism of action by which the therapeutic methods of the invention are achieved include killing of the cells expressing CD20 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 CD20 which can be inhibited or killed using the bispecific antigen-binding molecules of the invention include, for example, tumorigenic B cells.

[0157] The antigen-binding molecules of the present invention may be used to treat, *e.g.*, primary and/or metastatic tumors arising in the brain and meninges, oropharynx, lung and bronchial tree, gastrointestinal tract, male and female reproductive tract, muscle, bone, skin and appendages, connective tissue, spleen, immune system, blood forming cells and bone marrow, liver and urinary tract, and special sensory organs such as the eye. In certain embodiments, the bispecific antigen-binding molecules of the invention are used to treat one or more of the following cancers: renal cell carcinoma, pancreatic carcinoma, breast cancer, head and neck cancer, prostate cancer, malignant gliomas, osteosarcoma, colorectal cancer, gastric cancer (*e.g.*, gastric cancer with MET amplification), malignant mesothelioma, multiple myeloma, ovarian cancer, small cell lung cancer, non-small cell lung cancer, synovial sarcoma, thyroid cancer, or melanoma. According to certain exemplary embodiments, the bispecific antigen-binding molecules of the present invention are used to treat a B cell cancer (*e.g.*, Hodgkin's lymphoma, non-Hodgkin's lymphoma [NHL], precursor B cell lymphoblastic leukemia/lymphoma, mature B cell neoplasms, B cell chronic lymphocytic leukemia/small lymphocytic lymphoma, B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, mantle cell lymphoma, follicular lymphoma, cutaneous follicle center lymphoma, marginal zone B cell lymphoma, hairy cell leukemia, diffuse large B cell lymphoma, Burkitt's lymphoma, plasmacytoma, plasma cell myeloma, post-transplant lymphoproliferative disorder, Waldenstrom's macroglobulinemia, and anaplastic large-cell lymphoma).

[0158] According to certain embodiments of the present invention, the antigen-binding molecules are useful for treating a patient afflicted with a B-cell lymphoma (*e.g.*, NHL) that is resistant to, or incompletely responsive to anti-CD20 therapy alone (*e.g.*, resistant to rituximab therapy). According to other related embodiments of the invention, methods are provided comprising administering an anti-CD3/anti-CD20 bispecific antigen-binding molecule as disclosed herein to a patient who is afflicted with a B-cell lymphoma (*e.g.*, NHL) that is refractory to anti-CD20 therapy (*e.g.*, a patient with a rituximab-refractory tumor or with relapsed or refractory B-cell lymphoma). Analytic/diagnostic methods known in the art, such as tumor scanning, etc., may be used to ascertain whether a patient harbors as tumor that is resistant to, incompletely responsive to, or refractory to anti-CD20 therapy alone.

[0159] The present invention also includes methods 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.

[0160] According to certain aspects, the present invention provides methods for treating a disease or disorder associated with CD20 expression (*e.g.*, B cell lymphoma) comprising administering one or more of the bispecific antigen-binding molecules described elsewhere herein to a subject after the subject has received anti-CD20 mono-therapy (*e.g.*, after administration of a pharmaceutical composition comprising an anti-CD20 antibody such as rituximab). For example, the present invention includes methods for treating B cell lymphoma comprising administering an anti-CD3/anti-CD20 bispecific antigen-binding molecule to a patient 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 anti-CD20 mono-therapy (*e.g.*, rituximab treatment or an equivalent treatment thereof). In other aspects, a bispecific antigen-binding molecule of the invention (an anti-CD3/anti-CD20 bispecific antigen-binding molecule) comprising an IgG4 Fc domain is initially administered to a subject at one or more time points (*e.g.*, to provide robust initial depletion of B cells), followed by administration of an equivalent bispecific antigen-binding molecule comprising a different IgG domain, such as an IgG1 Fc domain, at subsequent time points.

Combination Therapies and Formulations

[0161] The present invention provides methods which comprise administering a pharmaceutical composition comprising any of the exemplary antibodies and bispecific antigen-binding molecules described herein 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 EGFR antagonist (*e.g.*, an anti-EGFR antibody [*e.g.*, cetuximab or panitumumab] or small molecule inhibitor of EGFR [*e.g.*, gefitinib or erlotinib]), an antagonist of another EGFR family member such as Her2/ErbB2, ErbB3 or ErbB4 (*e.g.*, anti-ErbB2, anti-ErbB3 or anti-ErbB4 antibody or small molecule inhibitor of ErbB2, ErbB3 or ErbB4 activity), an antagonist of EGFRvIII (*e.g.*, an antibody that specifically binds EGFRvIII), a cMET anagonist (*e.g.*, an anti-cMET antibody), an IGF1R antagonist (*e.g.*, an anti-IGF1R antibody), a B-raf inhibitor (*e.g.*, vemurafenib, sorafenib, GDC-0879, PLX-4720), a PDGFR-α inhibitor (*e.g.*, an anti-PDGFR-α antibody), a PDGFR-β inhibitor (*e.g.*, an anti-PDGFR-β antibody), a VEGF antagonist (*e.g.*, a VEGF-Trap, *see*, *e.g.*, US 7,087,411 (also referred to herein as a "VEGF-inhibiting fusion protein"), anti-VEGF antibody (*e.g.*, bevacizumab), a small molecule kinase inhibitor of VEGF receptor (*e.g.*, sunitinib, sorafenib or pazopanib)), a DLL4 antagonist (*e.g.*, an anti-DLL4 antibody disclosed in US 2009/0142354 such as REGN421), an Ang2 antagonist (*e.g.*, an anti-Ang2 antibody disclosed in US 2011/0027286 such as H1H685P), a FOLH1 antagonist (*e.g.*, an anti-FOLH1 antibody), a PRLR antagonist (*e.g.*, an anti-PRLR antibody), a STEAP1 or STEAP2 antagonist (*e.g.*, an anti-STEAP1 antibody or an anti-STEAP2 antibody), a TMPRSS2 antagonist (*e.g.*, an anti-TMPRSS2 antibody), a MSLN antagonist (*e.g.*, an anti-MSLN antibody), a CA9 antagonist (*e.g.*, an anti-CA9 antibody), a uroplakin antagonist (*e.g.*, an anti-uroplakin antibody), a

monovalent CD20 antagonist (*e.g.*, a monovalent anti-CD20 antibody such as rituximab), etc. 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-CD3/anti-CD20 bispecific antigen-binding molecule as disclosed herein) may also be administered as part of a therapeutic regimen comprising one or more therapeutic combinations selected from "ICE": ifosfamide (*e.g.*, Ifex®), carboplatin (*e.g.*, Paraplatin®), etoposide (*e.g.*, Etopophos®, Toposar®, VePesid®, VP-16); "DHAP": dexamethasone (*e.g.*, Decadron®), cytarabine (*e.g.*, Cytosar-U®, cytosine arabinoside, ara-C), cisplatin (*e.g.*, Platinol®-AQ); and "ESHAP": etoposide (*e.g.*, Etopophos®, Toposar®, VePesid®, VP-16), methylprednisolone (*e.g.*, Medrol®), high-dose cytarabine, cisplatin (*e.g.*, Platinol®-AQ).

[0162] The present invention also includes therapeutic combinations comprising any of the antigen-binding molecules mentioned herein 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, 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.

[0163] 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).

[0164] 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

[0165] According to certain embodiments of the present invention, multiple doses of an antigen-binding molecule (*e.g.*, an anti-CD3 antibody or a bispecific antigen-binding molecule that specifically binds CD20 and CD3) 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.

[0166] 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").

[0167] 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.

[0168] 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-CD3 antibody or a bispecific antigen-binding molecule that specifically binds CD20 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.

[0169] 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

[0170] The anti-CD3 antibodies of the present invention may also be used to detect and/or measure CD3, or CD3-expressing cells in a sample, *e.g.*, for diagnostic purposes. For example, an anti-CD3 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 CD3. Exemplary diagnostic assays for CD3 may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-CD3 antibody of the invention, wherein the anti-CD3 antibody is labeled with a detectable label or reporter molecule. Alternatively, an unlabeled anti-CD3 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, beta-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure CD3 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).
Samples that can be used in CD3 diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient which contains detectable quantities of CD3 protein, or fragments thereof, under normal or pathological conditions. Generally, levels of CD3 in a particular sample obtained from a healthy patient (*e.g.*, a patient not afflicted with a disease or condition associated with abnormal CD3 levels or activity) will be measured to initially establish a baseline, or standard, level of CD3. This baseline level of CD3 can then be compared against the levels of CD3 measured in samples obtained from individuals suspected of having a CD3 related disease or condition.

EXAMPLES

[0171] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. 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-CD3 Antibodies

[0172] Anti-CD3 antibodies were obtained by immunizing a VELOCIMMUNE® mouse (*i.e.*, an engineered mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions) with cells expressing CD3 or with DNA encoding CD3. The antibody immune response was monitored by a CD3-specific immunoassay. When a desired immune response was achieved splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce CD3-specific antibodies. Using this technique several anti-CD3 chimeric antibodies (*i.e.*, antibodies possessing human variable domains and mouse constant domains) were obtained. In addition, several fully human anti-CD3 antibodies were isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in US 2007/0280945A1.

[0173] Certain biological properties of the exemplary anti-CD3 antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2. Heavy and Light Chain Variable Region Amino Acid and Nucleic Acid Sequences

[0174] Table 1 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs of selected anti-CD3 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**
H1H2712N	2	4	6	8	10	12	14	16
H1M2692N	18	20	22	24	26	28	30	32
H1M3542N	34	36	38	40	42	44	46	48
H1M3544N	50	52	54	56	58	60	62	64
H1M3549N	66	68	70	72	74	76	78	80
H1M3613N	82	84	86	88	90	92	94	96
H2M2689N	98	100	102	104	106	108	110	112
H2M2690N	114	116	118	120	122	124	126	128
H2M2691N	130	132	134	136	138	140	142	144
H2M2704N	146	148	150	152	154	156	158	160
H2M2705N	162	164	166	168	170	172	174	176
H2M2706N	178	180	182	184	186	188	190	192

H2M2707N	194	196	198	200	202	204	206	208
H2M2708N	210	212	214	216	218	220	222	224
H2M2709N	226	228	230	232	234	236	238	240
H2M2710N	242	244	246	248	250	252	254	256
H2M2711N	258	260	262	264	266	268	270	272
H2M2774N	274	276	278	280	282	284	286	288
H2M2775N	290	292	294	296	298	300	302	304
H2M2776N	306	308	310	312	314	316	318	320
H2M2777N	322	324	326	328	330	332	334	336
H2M2778N	338	340	342	344	346	348	350	352
H2M2779N	354	356	358	360	362	364	366	368
H2M2789N	370	372	374	376	378	380	382	384
H2M2862N	386	388	390	392	394	396	398	400
H2M2885N	402	404	406	408	410	412	414	416
H2M2886N	418	420	422	424	426	428	430	432
H2M3540N	434	436	438	440	442	444	446	448
H2M3541N	450	452	454	456	458	460	462	464
H2M3543N	466	468	470	472	474	476	478	480
H2M3547N	482	484	486	488	490	492	494	496
H2M3548N	498	500	502	504	506	508	510	512
H2M3563N	514	516	518	520	522	524	526	528
H1H5751P	530	532	534	536	538	540	542	544
H1H5752P	546	548	550	552	554	556	558	560
H1H5753B	562	564	566	568	570	572	574	576
H1H5754B	578	580	582	584	586	588	590	592
H1H5755B	594	596	598	600	602	604	606	608
H1H5756B	610	612	614	616	618	620	622	624
H1H5757B	626	628	630	632	634	636	638	640
H1H5758B	642	644	646	648	650	652	654	656
H1H5761P	658	660	662	664	666	668	670	672
H1H5763P	674	676	678	680	682	684	686	688
H1H5764P	690	692	694	696	698	700	702	704
H1H5769P	706	708	710	712	714	716	718	720
H1H5771P	722	724	726	728	730	732	734	736
H1H5772P	738	740	742	744	746	748	750	752
H1H5777P	754	756	758	460	762	764	766	768
H1H5778P	770	772	774	776	778	780	782	784
H1H5780P	786	788	790	792	794	796	798	800
H1H5781P	802	804	806	808	810	812	814	816
H1H5782P	818	820	822	824	826	828	830	832
H1H5785B	834	836	838	840	842	844	846	848
H1H5786B	850	852	854	856	858	860	862	864
H1H5788P	866	868	870	872	874	876	878	880
H1H5790B	882	884	886	888	890	892	894	896
H1H5791B	898	900	902	904	906	908	910	912

H1H5792B	914	916	918	920	922	924	926	928
H1H5793B	930	932	934	936	938	940	942	944
H1H5795B	946	948	950	952	954	956	958	960
H1H5796B	962	964	966	968	970	972	974	976
H1H5797B	978	980	982	984	986	988	990	992
H1H5798B	994	996	998	1000	1002	1004	1006	1008
H1H5799P	1010	1012	1014	1016	1018	1020	1022	1024
H1H5801B	1026	1028	1030	1032	1034	1036	1038	1040
H1H7194B	1042	1044	1046	1048	1234	1236	1238	1240
H1H7195B	1050	1052	1054	1056	1234	1236	1238	1240
H1H7196B	1058	1060	1062	1064	1234	1236	1238	1240
H1H7198B	1066	1068	1070	1072	1234	1236	1238	1240
H1H7203B	1074	1076	1078	1080	1234	1236	1238	1240
H1H7204B	1082	1084	1086	1088	1234	1236	1238	1240
H1H7208B	1090	1092	1094	1096	1234	1236	1238	1240
H1H7211B	1098	1100	1102	1104	1234	1236	1238	1240
H1H7221B	1106	1108	1110	1112	1234	1236	1238	1240
H1H7223B	1114	1116	1118	1120	1234	1236	1238	1240
H1H7226B	1122	1124	1126	1128	1234	1236	1238	1240
H1H7232B	1130	1132	1134	1136	1234	1236	1238	1240
H1H7233B	1138	1140	1142	1144	1234	1236	1238	1240
H1H7241B	1146	1148	1150	1152	1234	1236	1238	1240
H1H7242B	1154	1156	1158	1160	1234	1236	1238	1240
H1H7250B	1162	1164	1166	1168	1234	1236	1238	1240
H1H7251B	1170	1172	1174	1176	1234	1236	1238	1240
H1H7254B	1178	1180	1182	1184	1234	1236	1238	1240
H1H7258B	1186	1188	1190	1192	1234	1236	1238	1240
H1H7269B	1194	1196	1198	1200	1234	1236	1238	1240
H1H7279B	1202	1204	1206	1208	1234	1236	1238	1240
H1xH7221G	1210	1212	1214	1216	1234	1236	1238	1240
H1xH7221G3	1218	1220	1222	1224	1234	1236	1238	1240
H1xH7221G5	1226	1228	1230	1232	1234	1236	1238	1240

Table 2: Nucleic Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H1H2712N	1	3	5	7	9	11	13	15
H1M2692N	17	19	21	23	25	27	29	31
H1M3542N	33	35	37	39	41	43	45	47
H1M3544N	49	51	53	55	57	59	61	63
H1M3549N	65	67	69	71	73	75	77	79
H1M3613N	81	83	85	87	89	91	93	95
H2M2689N	97	99	101	103	105	107	109	111
H2M2690N	113	115	117	119	121	123	125	127
H2M2691N	129	131	133	135	137	139	141	143
H2M2704N	145	147	149	151	153	155	157	159
H2M2705N	161	163	165	167	169	171	173	175
H2M2706N	177	179	181	183	185	187	189	191

H2M2707N	193	195	197	199	201	203	205	207
H2M2708N	209	211	213	215	217	219	221	223
H2M2709N	225	227	229	231	233	235	237	239
H2M2710N	241	243	245	247	249	251	253	255
H2M2711N	257	259	261	263	265	267	269	271
H2M2774N	273	275	277	279	281	283	285	287
H2M2775N	289	291	293	295	297	299	301	303
H2M2776N	305	307	309	311	313	315	317	319
H2M2777N	321	323	325	327	329	331	333	335
H2M2778N	337	339	341	343	345	347	349	351
H2M2779N	353	355	357	359	361	363	365	367
H2M2789N	369	371	373	375	377	379	381	383
H2M2862N	385	387	389	391	393	395	397	399
H2M2885N	401	403	405	407	409	411	413	415
H2M2886N	417	419	421	423	425	427	429	431
H2M3540N	433	435	437	439	441	443	445	447
H2M3541N	449	451	453	455	457	459	461	463
H2M3543N	465	467	469	471	473	475	477	479
H2M3547N	481	483	485	487	489	491	493	495
H2M3548N	497	499	501	503	505	507	509	511
H2M3563N	513	515	517	519	521	523	525	527
H1H5751P	529	531	533	535	537	539	541	543
H1H5752P	545	547	549	551	553	555	557	559
H1H5753B	561	563	565	567	569	571	573	575
H1H5754B	577	579	581	583	585	587	589	591
H1H5755B	593	595	597	599	601	603	605	607
H1H5756B	609	611	613	615	617	619	621	623
H1H5757B	625	627	629	631	633	635	637	639
H1H5758B	641	643	645	647	649	651	653	655
H1H5761P	657	659	661	663	665	667	669	671
H1H5763P	673	675	677	679	681	683	685	687
H1H5764P	689	691	693	695	697	699	701	703
H1H5769P	705	707	709	711	713	715	717	719
H1H5771P	721	723	725	727	729	731	733	735
H1H5772P	737	739	741	743	745	747	749	751
H1H5777P	753	755	757	759	761	763	765	767
H1H5778P	769	771	773	775	777	779	781	783
H1H5780P	785	787	789	791	793	795	797	799
H1H5781P	801	803	805	807	809	811	813	815
H1H5782P	817	819	821	823	825	827	829	831
H1H5785B	833	835	837	839	841	843	845	847
H1H5786B	849	851	853	855	857	859	861	863
H1H5788P	865	867	869	871	873	875	877	879
H1H5790B	881	883	885	887	889	891	893	895
H1H5791B	897	899	901	903	905	907	909	911
H1H5792B	913	915	917	919	921	923	925	927
H1H5793B	929	931	933	935	937	939	941	943
H1H5795B	945	947	949	951	953	955	957	959
H1H5796B	961	963	965	967	969	971	973	975
H1H5797B	977	979	981	983	985	987	989	991
H1H5798B	993	995	997	999	1001	1003	1005	1007
H1H5799P	1009	1011	1013	1015	1017	1019	1021	1023
H1H5801B	1025	1027	1029	1031	1033	1035	1037	1039
H1H7194B	1041	1043	1045	1047	1233	1235	1237	1239
H1H7195B	1049	1051	1053	1055	1233	1235	1237	1239

H1H7196B	1057	1059	1061	1063	1233	1235	1237	1239
H1H7198B	1065	1067	1069	1071	1233	1235	1237	1239
H1H7203B	1073	1075	1077	1079	1233	1235	1237	1239
H1H7204B	1081	1083	1085	1087	1233	1235	1237	1239
H1H7208B	1089	1091	1093	1095	1233	1235	1237	1239
H1H7211B	1097	1099	1101	1103	1233	1235	1237	1239
H1H7221B	1105	1107	1109	1111	1233	1235	1237	1239
H1H7223B	1113	1115	1117	1119	1233	1235	1237	1239
H1H7226B	1121	1123	1125	1127	1233	1235	1237	1239
H1H7232B	1129	1131	1133	1135	1233	1235	1237	1239
H1H7233B	1137	1139	1141	1143	1233	1235	1237	1239
H1H7241B	1145	1147	1149	1151	1233	1235	1237	1239
H1H7242B	1153	1155	1157	1159	1233	1235	1237	1239
H1H7250B	1161	1163	1165	1167	1233	1235	1237	1239
H1H7251B	1169	1171	1173	1175	1233	1235	1237	1239
H1H7254B	1177	1179	1181	1183	1233	1235	1237	1239
H1H7258B	1185	1187	1189	1191	1233	1235	1237	1239
H1H7269B	1193	1195	1197	1199	1233	1235	1237	1239
H1H7279B	1201	1203	1205	1207	1233	1235	1237	1239
H1xH7221G	1209	1211	1213	1215	1233	1235	1237	1239
H1xH7221G3	1217	1219	1221	1223	1233	1235	1237	1239
H1xH7221G5	1225	1227	1229	1231	1233	1235	1237	1239

[0175] Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (*e.g.* "H1H," "H1M," "H2M," etc.), followed by a numerical identifier (*e.g.* "2712," "2692," etc., as shown in Table 1), followed by a "P," "N," or "B" suffix. Thus, according to this nomenclature, an antibody may be referred to herein as, *e.g.*, "H1H2712N," "H1M2692N," "H2M2689N," etc. The H1H, H1M and H2M prefixes on the antibody designations used herein indicate the particular Fc region isotype of the antibody. For example, an "H1H" antibody has a human IgG1 Fc, an "H1M" antibody has a mouse IgG1 Fc, and an "H2M" antibody has a mouse IgG2 Fc, (all variable regions are fully human as denoted by the first 'H' in the antibody designation). As will be appreciated by a person of ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (*e.g.*, an antibody with a mouse IgG1 Fc can be converted to an antibody with a human IgG4, etc.), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Table 1 – will remain the same, and the binding properties are expected to be identical or substantially similar regardless of the nature of the Fc domain.

Control Constructs Used in the Following Examples

[0176] Various control constructs (anti-CD3 antibodies) were included in the following experiments for comparative purposes: "OKT-3," a mouse monoclonal antibody against human T-cell surface antigens available from the American Type Culture Collection (ATCC) under catalog no. CRL-8001; and "SP34," a commercially available mouse monoclonal antibody obtained from Biolegend, San Diego, CA (Cat. No. 302914), reactive against the epsilon chain of the T3 complex on human T lymphocyte cells.

Example 3. Surface Plasmon Resonance Derived Binding Affinities and Kinetic Constants of Human Monoclonal Anti-CD3 Antibodies

[0177] Binding affinities and kinetic constants of human monoclonal anti-CD3 antibodies were determined by surface plasmon resonance at 25°C using either an antibody-capture format (Tables 3, 5 and 7) or an antigen-capture format (Tables 4, 6 and 8). Measurements were conducted on a T200 Biacore instrument.

[0178] In the antibody-capture format, the Biacore sensor surface was derivatized with a rabbit anti-mouse Fc for hybridoma capture (antibody prefix H1M or H2M) or a mouse anti-human Fc surface for human IgG formatted antibodies (antibody prefix H1H). Soluble heterodimeric CD3 protein (hCD3-epsilon/hCD3-delta; SEQ ID NOs:1370/1371) with either a human Fc tag (hFcΔAdp/hFc; SEQ ID NOs:1372/1373) or a mouse Fc tag (mFcΔAdp/mFc; SEQ ID NOs:1374/1375) was injected over the antibody captured surface and the binding response was recorded. Heterodimeric CD3 protein was purified using the method described in Davis *et al.* (US2010/0331527).

[0179] In the antigen-capture format, heterodimeric CD3 protein was captured using a rabbit anti-mouse Fc or mouse anti-human Fc and the respective antibodies were injected over the captured antigen.

[0180] Antibodies were analyzed in their conventional divalent format (Tables 3 to 6) or in a monovalent 1-arm configuration (Tables 7 and 8) in which the second Fab from the antibody was removed and only the Fc portion (CH2-CH3) was expressed.

[0181] Kinetic association (k_a) and dissociation (k_d) rate constants were determined by processing and fitting the data to a 1:1 binding model using Scrubber 2.0 curve fitting software. Binding dissociation equilibrium constants (K_D) and dissociative half-lives ($t_{1/2}$) were calculated from the kinetic rate constants as: K_D (M) = k_d / k_a; and $t_{1/2}$ (min) = (ln2/(60*k_d). NT = not tested; NB = no binding observed.

Table 3: Biacore Binding Affinities of Hybridoma mAbs (H1M and H2M)

Binding at 25ºC / Antibody-Capture Format				
Antibody	**ka (Ms^{-1})**	**kd (s^{-1})**	**K_D (Molar)**	**T½ (min)**
H2M2689N	7.73E+05	3.23E-03	4.18E-09	4
H2M2690N	9.70E+03	2.02E-04	2.09E-08	57
H2M2691N	1.03E+04	2.07E-04	2.01E-08	56
H1M2692N	8.05E+03	4.34E-04	5.39E-08	27
H2M2704N	3.46E+04	6.92E-04	2.00E-08	17
H2M2705N	6.62E+04	9.10E-04	1.37E-08	13
H2M2706N	3.29E+04	4.44E-03	1.35E-07	3
H2M2707N	2.95E+04	1.87E-03	6.35E-08	6
H2M2708N	6.94E+04	6.12E-04	8.82E-09	19

H2M2709N	NT	NT	NT	NT
H2M2710N	6.72E+04	7.53E-04	1.12E-08	15
H2M2711N	6.72E+04	7.67E-04	1.14E-08	15
H1M2712N	9.32E+03	2.19E-04	2.35E-08	53
H2M2774N	7.79E+04	9.18E-04	1.18E-08	13
H2M2775N	6.97E+04	6.26E-04	8.98E-09	18
H2M2776N	6.29E+04	6.39E-04	1.02E-08	18
H2M2777N	3.70E+04	1.63E-03	4.39E-08	7
H2M2778N	2.13E+04	1.89E-04	8.90E-09	61
H2M2779N	2.18E+04	2.28E-04	1.05E-08	51
H2M2789N	NT	NT	NT	NT
H2M2862N	3.72E+04	3.00E-03	8.07E-08	4
H2M2885N	6.82E+04	6.51E-04	9.54E-09	18
H2M2886N	7.29E+04	6.53E-04	8.96E-09	18
H2M3540N	3.77E+04	6.11E-04	1.62E-08	19
H2M3541N	7.10E+03	1.35E-03	1.89E-07	9
H1M3542N	2.37E+04	5.08E-04	2.14E-08	23
H2M3543N	7.53E+03	2.26E-04	3.00E-08	51
H1M3544N	9.69E+03	1.42E-04	1.46E-08	82
H2M3547N	2.18E+04	3.47E-04	1.59E-08	33
H2M3548N	3.87E+04	5.04E-03	1.30E-07	2
H1M3549N	1.18E+04	9.19E-04	7.76E-08	13
H2M3563N	3.24E+04	1.19E-04	3.66E-09	97
H1M3613N	1.93E+04	3.04E-04	1.57E-08	38

Table 4: Biacore Binding Affinities of Hybridoma mAbs (H1M and H2M)

Binding at 25ºC / Antigen-Capture Format				
Antibody	**ka (Ms^{-1})**	**kd (s^{-1})**	**K_D (Molar)**	**T½ (min)**
H2M2689N	1.71E+06	9.97E-05	5.83E-11	116
H2M2690N	7.51E+04	6.35E-06	7.99E-11	1820
H2M2691N	3.94E+04	9.98E-06	2.54E-10	1158
H1M2692N	4.19E+04	9.90E-06	2.38E-10	1167
H2M2704N	1.32E+06	2.48E-04	1.87E-10	47
H2M2705N	2.43E+06	3.41E-04	1.40E-10	34
H2M2706N	5.63E+05	3.06E-04	5.44E-10	38
H2M2707N	3.99E+05	2.85E-04	7.15E-10	41
H2M2708N	1.73E+06	2.27E-04	1.31E-10	51
H2M2709N	NT	NT	NT	NT
H2M2710N	1.59E+06	2.43E-04	1.53E-10	48

H2M2711N	1.59E+06	2.40E-04	1.51E-10	48
H1M2712N	4.75E+04	1.37E-05	2.95E-10	846
H2M2774N	2.49E+06	3.36E-04	1.35E-10	34
H2M2775N	1.56E+06	2.16E-04	1.38E-10	53
H2M2776N	1.58E+06	2.22E-04	1.40E-10	52
H2M2777N	5.80E+05	3.21E-04	5.54E-10	36
H2M2778N	1.50E+05	6.57E-06	4.68E-11	1758
H2M2779N	1.28E+05	1.23E-05	9.38E-11	941
H2M2789N	NT	NT	NT	NT
H2M2862N	5.91E+05	3.21E-04	5.41E-10	36
H2M2885N	1.37E+06	1.52E-04	1.11E-10	76
H2M2886N	1.42E+06	1.36E-04	9.56E-11	85
H2M3540N	2.55E+06	5.87E-04	2.31E-10	20
H2M3541N	8.40E+04	1.16E-03	1.38E-08	10
H1M3542N	4.37E+05	2.00E-04	4.57E-10	58
H2M3543N	1.22E+05	7.96E-05	6.53E-10	145
H1M3544N	5.74E+04	5.98E-05	1.04E-09	193
H2M3547N	4.70E-05	1.00E-05	2.15E-11	1155
H2M3548N	NT	NT	NT	NT
H1M3549N	2.81E+05	2.89E-04	1.03E-09	40
H2M3563N	6.16E+05	4.77E-05	7.73E-11	242
H1M3613N	2.20E+05	9.60E-05	4.35E-10	120

Table 5: Biacore Binding Affinities of Human Fc mAbs (H1H)

Binding at 25ºC / Antibody-Capture Format				
Antibody	**ka (Ms^{-1})**	**kd (s^{-1})**	**K_D (Molar)**	**T½ (min)**
H1H2690N	NT	NT	NT	NT
H1H2712N	3.06E+03	2.70E-04	8.82E-08	43
H1H5751P	4.01E+03	5.18E-04	1.29E-07	22
H1H5752P	NB	NB	NB	NB
H1H5753B	NT	NT	NT	NT
H1H5755B	8.21E+03	4.72E-04	5.75E-08	24
H1H5756B	8.15E+03	2.66E-04	3.26E-08	43
H1H5757B	6.63E+03	7.85E-04	1.18E-07	15
H1H5758B	5.02E+03	1.17E-03	2.33E-07	10
H1H5761P	4.72E+03	2.44E-02	5.16E-06	0
H1H5763P	1.85E+04	5.40E-02	2.92E-06	0
H1H5764P	4.16E+03	1.59E-02	3.82E-06	1
H1H5769P	7.80E+03	9.41E-04	1.21E-07	12

H1H5771P	3.00E+04	6.26E-04	2.09E-08	18
H1H5772S	1.56E+04	1.55E-03	9.96E-08	7
H1H5777P	1.35E+04	3.02E-03	2.24E-07	4
H1H5778P	5.52E+03	1.54E-04	2.78E-08	75
H1H5780P	1.31E+04	3.99E-04	3.04E-08	29
H1H5781P	8.61E+03	4.97E-04	5.77E-08	23
H1H5782P	NB	NB	NB	NB
H1H5785B	NT	NT	NT	NT
H1H5786B	1.26E+04	1.08E-03	8.54E-08	11
H1H5788P	2.88E+03	2.91E-04	1.01E-07	40
H1H5790B	1.82E+04	5.17E-04	2.83E-08	22
H1H5791B	1.09E+04	7.90E-04	7.25E-08	15
H1H5792B	NT	NT	NT	NT
H1H5793B	8.54E+03	3.82E-04	4.47E-08	30
H1H5795B	1.73E+04	5.76E-04	3.33E-08	20
H1H5796B	1.47E+04	8.91E-04	6.05E-08	13
H1H5797B	NT	NT	NT	NT
H1H5798B	NT	NT	NT	NT
H1H5799P	1.36E+04	7.88E-03	5.79E-07	1
H1H5801B	6.57E+03	1.62E-03	2.46E-07	7
OKT3	2.10E+06	2.00E+00	1.00E-06	0.35 sec

Table 6: Biacore Binding Affinities of Human Fc mAbs (H1H)

Binding at 25ºC / Antigen-Capture Format				
Antibody	**ka (Ms^{-1})**	**kd (s^{-1})**	**K_D (Molar)**	**T½ (min)**
H1H2690N	NT	NT	NT	NT
H1H2712N	8.93E+04	8.68E-05	9.71E-10	133
H1H5751P	7.24E+04	2.47E-04	3.42E-09	47
H1H5752P	NB	NB	NB	NB
H1H5753B	NT	NT	NT	NT
H1H5755B	2.15E+05	2.01E-04	9.36E-10	57
H1H5756B	1.44E+05	1.11E-04	7.67E-10	105
H1H5757B	1.80E+05	2.95E-04	1.64E-09	39
H1H5758B	1.42E+05	5.62E-04	3.97E-09	21
H1H5761P	2.11E+05	1.13E-02	5.34E-08	1
H1H5763P	1.84E+05	1.70E-02	9.24E-08	1
H1H5764P	3.50E+05	7.36E-03	2.10E-08	2
H1H5769P	1.19E+05	5.23E-04	4.41E-09	22
H1H5771P	9.23E+05	3.42E-04	3.71E-10	34
H1H5772S	5.19E+05	8.69E-04	1.67E-09	13
H1H5777P	4.83E+05	1.70E-03	3.52E-09	7
H1H5778P	3.99E+05	3.42E-05	8.56E-11	338

H1H5780P	4.78E+05	1.71E-04	3.58E-10	68
H1H5781P	1.40E+05	2.68E-04	1.92E-09	43
H1H5782P	NB	NB	NB	NB
H1H5785B	NT	NT	NT	NT
H1H5786B	3.00E+06	4.24E-04	1.41E-10	27
H1H5788P	7.06E+04	1.64E-04	2.33E-09	70
H1H5790B	9.25E+05	2.36E-04	2.54E-10	49
H1H5791B	7.86E+05	3.40E-04	4.33E-10	34
H1H5792B	NT	NT	NT	NT
H1H5793B	4.78E+05	1.59E-04	3.33E-10	73
H1H5795B	1.58E+06	2.29E-04	1.45E-10	50
H1H5796B	1.05E+05	2.44E-04	2.32E-09	47
H1H5797B	NT	NT	NT	NT
H1H5798B	NT	NT	NT	NT
H1H5799P	7.18E+05	5.64E-03	7.85E-09	2
H1H5801B	3.31E+05	1.12E-03	3.38E-09	10
OKT3	3.94E+06	2.18E-02	5.53E-09	0.5

Table 7: Biacore Binding Affinities of monovalent 1-arm mAbs

Binding at 25°C / Antibody-Capture Format				
Antibody	**ka (Ms^{-1})**	**kd (s^{-1})**	**K_D (Molar)**	**T½ (min)**
H1H7194P	1.16E+04	1.51E-04	1.30E-08	76
H1H7195P	3.13E+04	9.89E-05	3.16E-09	117
H1H7196P	1.07E+04	4.43E-04	4.13E-08	26
H1H7198P	2.63E+04	1.58E-04	6.02E-09	73
H1H7203P	1.46E+04	2.67E-04	1.83E-08	43
H1H7204P	1.43E+04	3.62E-04	2.53E-08	32
H1H7208P	NT	NT	NT	NT
H1H7211P	1.41E+04	1.59E-04	1.13E-08	73
H1H7221P	1.07E+04	2.92E-04	2.75E-08	40
H1H7223P	1.60E+04	3.07E-04	1.92E-08	38
H1H7226P	1.30E+04	3.55E-04	2.72E-08	33
H1H7232P	8.03E+03	1.77E-03	2.20E-07	7
H1H7233P	1.11E+04	2.69E-04	2.42E-08	43
H1H7241P	1.34E+04	2.95E-04	2.20E-08	39
H1H7242P	2.15E+04	6.64E-04	3.09E-08	17
H1H7250P	2.34E+04	2.47E-04	1.05E-08	47
H1H7251P	2.56E+04	1.07E-03	4.17E-08	11
H1H7254P	2.60E+04	3.88E-04	1.49E-08	30
H1H7258P	1.26E+04	3.02E-04	2.40E-08	38
H1H7269P	2.57E+04	6.24E-03	2.43E-07	2
H1H7279P	NB	NB	NB	NB
H1xH7221G	NT	NT	NT	NT
H1xH7221G3	NB	NB	NB	NB
H1xH7221G5	NB	NB	NB	NB

Table 8: Biacore Binding Affinities of monovalent 1-arm mAbs

Binding at 25°C / Antigen-Capture Format				
Antibody	**ka (Ms^{-1})**	**kd (s^{-1})**	**K_D (Molar)**	**T½ (min)**
H1H7194P	3.50E+05	8.43E-05	2.41E-10	137
H1H7195P	5.66E+05	7.14E-05	1.26E-10	162
H1H7196P	1.85E+05	4.61E-04	2.49E-09	25
H1H7198P	6.28E+05	7.07E-05	1.12E-10	163
H1H7203P	4.79E+05	2.38E-04	4.98E-10	48
H1H7204P	1.73E+05	3.65E-04	2.12E-09	32
H1H7208P	NT	NT	NT	NT
H1H7211P	3.45E+05	9.61E-05	2.79E-10	120
H1H7221P	1.36E+05	2.39E-04	1.75E-09	48
H1H7223P	1.87E+05	2.86E-04	1.53E-09	40
H1H7226P	4.18E+05	2.36E-04	5.65E-10	49
H1H7232P	1.49E+05	1.49E-03	1.00E-08	8
H1H7233P	1.61E+05	2.04E-04	1.27E-09	57
H1H7241P	1.87E+05	2.36E-04	1.26E-09	49
H1H7242P	3.83E+05	1.01E-03	2.63E-09	11
H1H7250P	2.31E+05	1.89E-04	8.20E-10	61
H1H7251P	4.47E+05	1.19E-03	2.67E-09	10
H1H7254P	4.33E+05	3.30E-04	7.62E-10	35
H1H7258P	1.33E+05	2.90E-04	2.18E-09	40
H1H7269P	2.77E+05	6.89E-03	2.49E-08	2
H1H7279P	NB	NB	NB	NB
H1xH7221G	NT	NT	NT	NT
H1xH7221G3	NB	NB	NB	NB
H1xH7221G5	NB	NB	NB	NB

[0182] As shown in Tables 3-8, Several anti-CD3 antibodies of the present invention bind CD3, in either antibody-capture or antigen-capture formats, with high affinity.

Example 4. Anti-CD3 Antibodies Bind and Proliferate Human T-Cells

[0183] Anti-CD3 antibodies of the present invention were tested for their ability to bind to human T-cells and induce their proliferation. Binding was assessed using Jurkat cells (a CD3+ human T-cell line), while proliferation of Peripheral Blood Mononuclear Cells (PBMC) was assessed using ATP catalyzed quantification (CellTiter Glo®). Anti-CD3 antibody OKT3 acted as a positive control and irrelevant isotype matched antibodies served as negative controls.

[0184] FACS data was acquired using the following protocol: Cells at $2x10^5$ per well were incubated with serially diluted antibodies for 30 min on ice. Post incubation, cells were washed and secondary antibody was added and incubated for an additional 30 minutes. After incubation, cells were washed, re-suspended in cold PBS containing 1% BSA and analyzed by flow cytometry with viable Jurkat cells gated by side and forward scatters. The EC_{50}s for cell binding titration were determined using Prism software with values calculated using a 4-

parameter non-linear regression analysis.

[0185] Proliferation data was acquired using the following protocol: Human PBMC ($5x10^4$ / well) were incubated with a 3-fold serial dilution of anti-CD3 and a fixed concentration of a commercial anti-CD28 antibody (200ng/ml) in 96 well plates for 72 h at 37°C. Following incubation, CellTiter Glo® was added and luminescence was measured using a VICTOR X5 multi-label plate reader (PerkinElmer). The EC_{50} of cell viability (ATP catalyzed quantification) was calculated using a 4-parameter non-linear regression analysis in GraphPad Prism.

[0186] Results of the binding and proliferation experiments are summarized in Tables 9-11.

Table 9: Hybridoma Anti-CD3 mAbs Bind & Proliferate Human T-Cells

Antibody	EC50 [M] FACS JURKAT	EC50 [M] hPBMC Proliferation
H2M2689N	NB	0.00E+00
H2M2690N	4.37E-09	5.37E-12
H2M2691N	6.77E-09	3.43E-11
H1M2692N	5.99E-09	1.42E-10
H2M2704N	8.45E-10	2.93E-12
H2M2705N	2.96E-10	1.76E-11
H2M2706N	2.37E-09	3.86E-12
H2M2707N	1.24E-07	1.92E-12
H2M2708N	6.58E-10	2.69E-08
H2M2709N	7.11E-10	2.48E-11
H2M2710N	7.10E-10	2.11E-10
H2M2711N	1.16E-09	6.48E-10
H1M2712N	2.19E-08	1.28E-10
H2M2774N	3.52E-10	4.92E-10
H2M2775N	1.32E-09	1.09E-09
H2M2776N	4.91E-10	2.84E-11
H2M2777N	2.16E-09	2.51E-11
H2M2778N	3.62E-09	0.00E+00
H2M2779N	NT	0.00E+00
H2M2789N	NT	2.85E-08
H2M2862N	7.68E-09	6.72E-13
H2M2885N	2.09E-09	2.49E-12
H2M2886N	3.97E-09	2.69E-12
H2M3540N	3.99E-09	3.16E-12
H2M3541N	3.70E-09	6.40E-12
H1M3542N	2.01E-09	0.00E+00
H2M3543N	5.63E-09	6.12E-12
H1M3544N	2.32E-08	0.00E+00
H2M3547N	2.71E-09	5.02E-12
H2M3548N	1.10E-09	1.89E-12
H1M3549N	2.30E-09	0.00E+00
H2M3563N	1.07E-09	7.74E-12
H1M3613N	1.03E-08	0.00E+00

Isotype Ctrl	NB	0.00E+00

NB: No Binding; NT: Not Tested

Table 10: Human Fc Anti-CD3 mAbs Bind & Proliferate Human T-Cells

Antibody	EC50 [M] FACS JURKAT	EC50 [M] hPBMC Proliferation
H1H5751P	2.12E-09	9.29E-12
H1H5752P	3.43E-10	1.09E-12
H1H5753B	NB	9.14E-11
H1H5755B	1.23E-09	4.24E-12
H1H5756B	NB	0.00E+00
H1H5757B	3.38E-09	4.86E-12
H1H5758B	1.90E-09	2.13E-12
H1H5761P	2.10E-09	3.62E-13
H1H5763P	2.76E-09	3.11E-13
H1H5764P	8.80E-10	3.27E-13
H1H5769P	4.10E-09	6.17E-12
H1H5771P	NT	6.35E-12
H1H5772S	6.64E-10	4.42E-12
H1H5777P	5.71E-10	3.04E-12
H1H5778P	6.85E-10	5.04E-12
H1H5780P	7.62E-10	3.44E-12
H1H5781P	1.23E-09	6.08E-12
H1H5782P	NB	5.17E-12
H1H5785B	NB	0.00E+00
H1H5786B	1.10E-09	1.79E-12
H1H5788P	3.53E-09	4.62E-12
H1H5790B	3.55E-09	2.71E-12
H1H5791B	3.77E-09	1.75E-12
H1H5792B	5.87E-09	6.47E-12
H1H5793B	4.62E-09	3.28E-12
H1H5795B	2.04E-09	3.09E-12
H1H5796B	9.82E-09	4.37E-12
H1H5797B	3.96E-08	1.07E-11
H1H5798B	5.57E-09	2.59E-12
H1H5799P	NT	1.63E-13
H1H5801B	1.55E-08	1.09E-12
OKT3	1.96E-10	3.30E-13
Isotype Ctrl	NB	0.00E+00

NB: No Binding; NT: Not Tested

Table 11: Monovalent 1-arm Anti-CD3 mAbs Bind & Proliferate Human T-Cells

Antibody	EC50 [M] FACS JURKAT	EC50 [M] hPBMC Proliferation
H1H7194P	1.50E-09	2.37E-12
H1H7195P	3.42E-10	2.42E-12
H1H7196P	3.44E-08	1.27E-12

H1H7198P	7.26E-10	2.55E-12
H1H7203P	3.24E-09	1.64E-12
H1H7204P	2.29E-09	1.51E-12
H1H7208P	5.19E-08	1.46E-12
H1H7211P	7.01E-10	2.75E-12
H1H7221P	1.40E-09	2.60E-12
H1H7223P	9.37E-10	1.07E-12
H1H7226P	7.95E-10	9.52E-13
H1H7232P	1.50E-09	1.03E-12
H1H7233P	7.15E-10	7.34E-13
H1H7241P	1.01E-09	1.05E-12
H1H7242P	1.83E-09	2.13E-12
H1H7250P	1.37E-09	2.43E-12
H1H7251P	1.45E-09	1.30E-12
H1H7254P	1.09E-09	2.80E-12
H1H7258P	1.07E-09	2.17E-12
H1H7269P	1.95E-09	1.15E-12
H1H7279P	NB	0.00E+00
Isotype Ctrl	NB	0.00E+00

NB: No Binding; NT: Not Tested

[0187] As shown in Tables 7-9, the vast majority of anti-CD3 antibodies of the invention bound human T-cells and induced T-cell proliferation.

Example 5. Anti-CD3 Antibodies Bind and Proliferate Monkey T-Cells

[0188] A subset of anti-CD3 antibodies of the invention was tested for the ability to bind to and induce proliferation of monkey T-cells.

[0189] FACS data was acquired using the following protocol: Cells at $2x10^5$ per well were incubated with serially diluted antibodies for 30 min on ice. Post incubation, cells were washed and secondary antibodies were added and incubated for an additional 30 minutes. After incubation, cells were washed, re-suspended in cold PBS containing 1% BSA and analyzed by flow cytometry. CD4+ monkey T cells were gated by side and forward scatters, and on the CD2+CD4+CD20- population. The EC_{50}s for cell binding titration were calculated using a 4-parameter non-linear regression analysis in GraphPad Prism.

[0190] Proliferation data was acquired using the following protocol: Freshly isolated cynomolgus monkey derived PBMC ($5x10^4$ / well) were incubated with a 3-fold serial dilution of anti-CD3 antibody and a fixed concentration of a commercial anti-CD28 antibody (500 ng/ml) antibody in 96 well plates for 72 h at 37°C. Following incubation, CellTiter Glo® was added and luminescence was measured using a VICTOR X5 multi-label plate reader (PerkinElmer). The EC_{50} of cell viability (ATP catalyzed quantification) was calculated using a 4-parameter non-linear regression analysis in GraphPad Prism.

[0191] Results of the binding and proliferation experiments are summarized in Tables 12 and 13.

Table 12: Anti-CD3 mAbs Bind & Proliferate monkey PBMCs

Antibody	EC50 [M] FACS PBMCs	EC50 [M] mfPBMC Proliferation
H1H2690N	5.66E-09	2.71E-12
H1H2712N	2.29E-09	2.72E-12
H2M3547N	1.12E-10	NT
H2M3563N	1.65E-10	NT
H1H5761P	NT	2.81E-09
H1H5763P	NT	0.00E+00
H1H5764P	NT	4.06E-10
H1H5769P	NT	8.33E-13
H1H5771P	NT	2.74E-12
H1H5772S	NT	1.47E-12
H1H5778P	NT	5.93E-13
H1H5780P	NT	3.13E-13
H1H5781P	NT	7.92E-13
H1H5788P	NT	2.01E-12
OKT3	NB	NT
SP34	7.03E-11	1.71E-12

NB: No Binding; NT: not tested

Table 13: Monovalent 1-arm Anti-CD3 mAbs Bind & Proliferate Monkey PBMCs

Antibody	EC50 [M] FACS PBMCs	EC50 [M] mfPBMC Proliferation
H1H7194P	NT	4.84E-12
H1H7195P	NT	1.36E-12
H1H7196P	NT	1.40E-08
H1H7198P	NT	2.29E-12
H1H7203P	NT	4.97E-13
H1H7204P	NT	1.26E-11
H1H7208P	NT	7.02E-12
H1H7211P	NT	2.81E-13
H1H7221P	NT	1.72E-12
H1H7223P	NT	6.75E-11
H1H7226P	NT	2.26E-11
H1H7232P	NT	4.90E-11
H1H7233P	NT	4.35E-12
H1H7241P	NT	2.05E-11
H1H7242P	NT	1.38E-11
H1H7250P	NT	7.27E-11
H1H7251P	NT	1.83E-11
H1H7254P	NT	8.88E-11
H1H7258P	NT	1.11E-11

NB: No Binding; NT: not tested

[0192] As shown in Tables 12 and 13, several anti-CD3 antibodies of the invention bound

CD2+CD4+ monkey T-cells and induced their proliferation. OKT3 did not drive monkey PBMC proliferation, while SP34 was active against monkey PBMCs.

Example 6. Anti-CD3 mAbs Support T-Cell-Mediated Killing of Tumor Cells

[0193] The ability of anti-CD3 antibodies to redirect T-cell mediated killing via Fc/FcR interactions was studied using a calcein based U937 killing assay. Briefly, human PBMC were isolated over Ficoll-Paque and activated over a course of several days with media containing human IL-2 (30 U/ml) and T-cell activation beads (anti-CD3/CD28). U937 cells were labeled with calcein, and then incubated with activated T-cells at a 10:1 effector: target ratio using 3-fold serial dilutions of antibodies over a course of 3 hours at 37°C. Following incubation, the plates were centrifuged and supernatants were transferred to a translucent black clear bottom plate for fluorescence analysis. EC_{50} values, defined as the molar concentration of CD3 antibody that induces 50% cytotoxicity, were calculated using a 4-parameter non-linear regression analysis in GraphPad Prism. Results using hybridoma antibodies, human Fc antibodies, and monovalent one-arm antibodies are shown in Tables 14, 15 and 16, respectively.

Table 14: Hybridoma Anti-CD3 mAbs Redirect T-Cell Killing to U937 Cells

Antibody	U937 Cytotoxicity Human T-cells [M]
H2M2689N	0.00E+00
H2M2690N	2.79E-11
H2M2691N	2.34E-11
H1M2692N	3.59E-10
H2M2704N	2.49E-12
H2M2705N	1.73E-12
H2M2706N	7.91E-12
H2M2707N	7.21E-12
H2M2708N	3.27E-12
H2M2709N	3.47E-12
H2M2710N	3.97E-12
H2M2711N	3.66E-12
H1M2712N	3.14E-10
H2M2774N	2.46E-12
H2M2775N	3.38E-12
H2M2776N	4.06E-12
H2M2777N	4.86E-12
H2M2778N	0.00E+00
H2M2779N	6.75E-10
H2M2789N	NT
H2M2862N	7.66E-12
H2M2885N	3.71E-12
H2M2886N	8.06E-12
H2M3540N	1.25E-11
H2M3541N	5.39E-11

H1M3542N	2.92E-11
H2M3543N	1.31E-11
H1M3544N	1.72E-10
H2M3547N	3.17E-11
H2M3548N	5.50E-12
H1M3549N	1.07E-10
H2M3563N	4.05E-11
H1M3613N	8.66E-10
Isotype Ctrl	0.00E+00

NT: Not Tested

Table 15: Human Fc formatted Anti-CD3 mAbs Redirect T-Cell Killing to U937 Cells

Antibody	U937 Cytotoxicity Human T-cells [M]
H1H5751P	1.30E-10
H1H5752P	1.85E-11
H1H5753B	3.79E-10
H1H5755B	5.16E-11
H1H5756B	7.69E-11
H1H5757B	9.65E-11
H1H5758B	8.86E-08
H1H5761P	2.00E-12
H1H5763P	NT
H1H5764P	NT
H1H5769P	5.65E-11
H1H5771P	NT
H1H5772S	6.89E-13
H1H5777P	4.87E-13
H1H5778P	3.41E-13
H1H5780P	4.03E-12
H1H5781P	1.83E-12
H1H5782P	5.18E-12
H1H5785B	4.43E-11
H1H5786B	6.10E-11
H1H5788P	1.54E-11
H1H5790B	8.71E-11
H1H5791B	8.01E-11
H1H5792B	1.40E-10
H1H5793B	8.85E-11
H1H5795B	6.74E-11
H1H5796B	5.03E-10
H1H5797B	5.76E-10
H1H5798B	1.81E-10
H1H5799P	NT
H1H5801B	9.23E-11
OKT3	2.35E-12
Isotype Ctrl	0.00E+00

NT: Not Tested

Table 16: Monovalent 1-arm Anti-CD3 mAbs Redirect T-Cell Killing to U937 Cells

Antibody	U937 Cytotoxicity Human T-cells [M]
H1H7194P	4.71E-12
H1H7195P	6.10E-12
H1H7196P	1.96E-11
H1H7198P	5.21E-12
H1H7203P	5.47E-12
H1H7204P	1.08E-11
H1H7208P	4.59E-11
H1H7211P	7.89E-12
H1H7221P	9.21E-12
H1H7223P	5.30E-12
H1H7226P	1.04E-11
H1H7232P	9.96E-12
H1H7233P	1.19E-11
H1H7241P	1.23E-11
H1H7242P	7.50E-12
H1H7250P	5.91E-12
H1H7251P	1.81E-12
H1H7254P	4.18E-12
H1H7258P	1.53E-11
H1H7269P	1.08E-11
H1H7279P	0.00E+00
Isotype Ctrl	0.00E+00

NT: Not Tested

As shown in Tables 14-16, most anti-CD3 antibodies, as well as OKT3, supported redirected T-cell mediated killing in this assay system. The observed killing, believed to be dependent on the antibody's Fc engagement with the Fc Receptor on U937 cells leading to clustering of CD3 on adjacent T-cells, was squelched by addition of non-specific human IgG (data not shown).

Example 7. Generation of Bispecific Antibodies that Bind CD3 and CD20

[0194] Bispecific antibodies comprising an anti-CD3-specific binding domain and an anti-CD20-specific binding domain were constructed using standard methodologies wherein a heavy chain and a light chain from an anti-CD3 antibody were combined with a heavy chain from an anti-CD20 antibody. The anti-CD3 antibodies used to construct the bispecific antibodies of this example were obtained by immunizing a VelocImmune® mouse with cells expressing CD3 or with DNA encoding CD3, or in the case of BS3/20-007 and -009, from a known anti-CD3 antibody (*i.e.*, the anti-CD3 antibody "L2K" as set forth in WO2004/106380). The anti-CD20 antibodies used to construct the bispecific antibodies of this example are as set forth in US 7,879,984.

[0195] The bispecific antibodies created in accordance with the present Example comprise

two separate antigen-binding domains (*i.e.*, binding arms). The first antigen-binding domain comprises a heavy chain variable region derived from an anti-CD20 antibody ("CD20-VH"), paired with a light chain variable region derived from an anti-CD3 antibody ("CD3-VL"). The CD20-VH/CD3-VL pairing creates an antigen-binding domain that specifically recognizes CD20. The second antigen-binding domain comprises a heavy chain variable region derived from an anti-CD3 antibody ("CD3-VH"), paired with a light chain variable region derived from an anti-CD3 antibody ("CD3-VL"). The CD3-VH/CD3-VL pairing creates an antigen-binding domain that specifically recognizes CD3. The same CD20-VH was used in all bispecific antibodies created in this example and is designated "CD20-VH-A" (except for BS3/20-009, which used a different CD20-VH called "CD20-VH-B"). However, several different CD3-VH and CD3-VL components (designated "CD3-VH-A, CD3-VH-B, etc. and CD3-VL-A, CD3-VL-B, etc., derived from different anti-CD3 antibodies) were used in the different bispecific antibodies of the following Examples.

[0196] A summary of the component parts of the antigen-binding domains of the various bispecific antibodies made in accordance with this Example is set forth in Table 17.

Table 17

Bispecific Antibody Identifier	Anti-CD20 Antigen-Binding Domain		Anti-CD3 Antigen-Binding Domain	
	Heavy Chain Variable Region	Light Chain Variable Region	Heavy Chain Variable Region	Light Chain Variable Region
BS3/20-001	CD20-VH-A	CD3-VL-A	CD3-VH-A	CD3-VL-A
BS3/20-002	CD20-VH-A	CD3-VL-B	CD3-VH-B	CD3-VL-B
BS3/20-003	CD20-VH-A	CD3-VL-C	CD3-VH-C	CD3-VL-C
BS3/20-004	CD20-VH-A	CD3-VL-D	CD3-VH-D	CD3-VL-D
BS3/20-005	CD20-VH-A	CD3-VL-E	CD3-VH-E	CD3-VL-E
BS3/20-007	CD20-VH-A	CD3-VL-F[#]	CD3-VH-F[#]	CD3-VL-F[#]
BS3/20-009*	CD20-VH-B	CD3-VL-F[#]	CD3-VH-F[#]	CD3-VL-F[#]

[#] The heavy and light chain variable regions of CD3-VH-F and CD3-VL-F were derived from the anti-CD3 antibody designated "L2K" as set forth in WO2004/106380.

* The anti-CD20 arm of BS3/20-009, comprising the CD20-VH-B/CD3-VL-F pairing, is non-functional; *i.e.*, it does not bind CD20. However, the anti-CD3 arm (comprising the CD3-VH-F/CD3-VL-F pairing) specifically binds CD3. Thus, BS3/20-009 retains the same general "bispecific" structure of the other bispecific molecules generated in this example, but it only binds CD3.

[0197] Tables 18 and 19 set out the amino acid sequence identifiers for the various heavy chain variable regions (Table 18) and light chain variable regions (Table 19), and their corresponding CDRs, of the bispecific antibodies of this Example.

Table 18 (Heavy Chain Variable Region Amino Acid Sequences)

	SEQ ID NOs			
Heavy Chain Identifier	HCVR	HCDR1	HCDR2	HCDR3
CD20-VH-A	1242	1244	1246	1248
CD20-VH-B	1338	1340	1342	1344
CD3-VH-A	1250	1252	1254	1256

CD3-VH-B	1266	1268	1270	1272
CD3-VH-C	1282	1284	1286	1288
CD3-VH-D	1298	1300	1302	1304
CD3-VH-E	1314	1316	1318	1320
CD3-VH-F	1329	1330	1331	1332

Table 19 (Light Chain Variable Region Amino Acid Sequences)

	SEQ ID NOs			
Light Chain Identifier	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
CD3-VL-A	1258	1260	1262	1264
CD3-VL-B	1274	1276	1278	1280
CD3-VL-C	1290	1292	1294	1296
CD3-VL-D	1306	1308	1310	1312
CD3-VL-E	1322	1324	1326	1328
CD3-VL-F	1333	1334	1335	1336

[0198] In addition, Tables 20 and 21 set out the sequence identifiers for the nucleotide sequences encoding the heavy chain variable regions (Table 20) and light chain variable regions (Table 21), and their corresponding CDRs, of the bispecific antibodies of this Example.

Table 20 (Nucleotide Sequences Encoding Heavy Chain Variable Region Sequences)

	SEQ ID NOs			
Heavy Chain Identifier	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**
CD20-VH-A	1241	1243	1245	1247
CD20-VH-B	1337	1339	1341	1343
CD3-VH-A	1249	1251	1253	1255
CD3-VH-B	1265	1267	1269	1271
CD3-VH-C	1281	1283	1285	1287
CD3-VH-D	1297	1299	1301	1303
CD3-VH-E	1313	1315	1317	1319

Table 21 (Nucleotide Sequences Encoding Light Chain Variable Region Sequences)

	SEQ ID NOs			
Light Chain Identifier	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
CD3-VL-A	1257	1259	1261	1263
CD3-VL-B	1273	1275	1277	1279
CD3-VL-C	1289	1291	1293	1295
CD3-VL-D	1305	1307	1309	1311
CD3-VL-E	1321	1323	1325	1327

[0199] In addition to the bispecific antibodies described above, the following control antibodies were also used in certain of the experiments set out in the Examples that follow:

[0200] Control I: Monoclonal antibody "OKT-3" against human T-cell surface antigens as set forth in US 4,361,549 and available from hybridoma CRL-8001 (American Type Culture Collection, Manassas, VA).

[0201] Control II: Antibody "SP34" reactive against the epsilon chain of the T3 complex on human T lymphocyte cells, available from BD Pharmagen, Cat # 55052.

[0202] Control III: anti-CD20 therapeutic antibody, with heavy and light chain sequences of Rituxan (Rituximab) as disclosed in US 5,736,137.

[0203] Control IV: Monoclonal anti-CD20 antibody designated "3B9-10" as disclosed in US 7,879,984, and set forth herein as an antibody comprising the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 1242/1346 and HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences of SEQ ID NOs: 1244-1246-1248-1348-1350-1352.

[0204] Control V: Monoclonal anti-CD20 antibody designated "10F2-13" as disclosed in US 7,879,984, and set forth herein as an antibody comprising the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 1354/1362 and HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences of SEQ ID NOs: 1356-1358-1360-1364-1366-1368.

Example 8. CD20 x CD3 Bispecific Antibodies Selectively Bind Jurkat, Raji and Monkey T-Cells

[0205] CD20 x CD3 bispecific antibodies and Control constructs, as set forth in Example 1, were tested via FACS for their ability to bind to Jurkat (CD3+, CD20 - human T-cell line), Raji (CD3-, CD20+ Human B-cell line), or cynomolgus PBMCs ("mkT cells").

[0206] FACS data was acquired using the following protocol: Cells at $2x10^5$ per well were incubated with serially diluted antibodies for 30 min on ice. Post incubation, cells were washed and appropriate secondary (Jurkat, RAJI cells) or cocktail of secondary antibodies (for cyno PBMC) was added and incubated for an additional 30 minutes. After incubation, cells were washed, re-suspended in cold PBS containing 1% BSA and analyzed by flow cytometry on a BD FACS Canto II. Jurkat and Raji cells were gated by side and forward scatters, while cynomolgus T cells were also gated in a CD2+CD4+ population. The EC_{50}s for cell binding titration were determined using Prism software with values calculated using a 4-parameter non-linear regression analysis. Results are shown in Table 22.

Table 22. EC50 Binding Values (Molar) for CD3xCD20 Bispecific Antibodies

Antibody	FACS – Jurkat	FACS – RAJI	FACS – mkT cells
Control I (anti-CD3)	1.96E-10	NB	NB
Control II (anti-CD3)	(+)	NB	7.03E-11
Control IV (anti-CD20)	No Binding	(+)	NB
BS3/20-001	3.85E-08	5.99E-08	8.74E-06
BS3/20-002	5.62E-08	1.15E-08	NT
BS3/20-003	5.67E-08	9.24E-08	2.48E-08
BS3/20-004	4.89E-08	1.02E-08	NT
BS3/20-005	1.95E-09	8.17E-08	NT

(+) EC_{50} values not determined, but binding observed; NB no binding; NT not tested

[0207] As shown in Table 22, the panel of tested antibodies showed a range of binding affinities on the various cell lines, depending on their specificities. Bispecific antibodies (BS3/20-001, -002, -003, -004 and -005) showed the ability to bind both human target lines. A

subset of antibodies also showed the ability to bind to cynomolgus cells (Control II, BS3/20-001 and BS3/20-003). Anti-CD3 Control I (OKT3), anti-CD3 Control II (SP34), and anti-CD20 Control IV bound to Jurkat, cynomolgus T cells, and RAJI, respectively.

Example 9. CD20 x CD3 Bispecific Antibodies Induce PBMC Proliferation *in vitro*

[0208] The ability of selected CD20 x CD3 bispecific antibodies and Control constructs to stimulate Peripheral Blood Mononuclear Cells (PBMC) and induce proliferation was assessed using ATP catalyzed quantification (CellTiter Glo®). The activation of PBMCs results in the release of cytokines, which drive cellular proliferation.

[0209] Proliferation data was acquired using the following protocol: Human or cynomolgus monkey derived PBMC ($5x10^5$ / well) were incubated with a 3-fold serial dilution of anti-CD3xCD20 or Control antibody in 96 well plates for 72 h at 37°C. Following incubation, CellTiter Glo® was added and luminescence was measured using a VICTOR X5 multi-label plate reader (PerkinElmer). The EC_{50} of cell viability (ATP catalyzed quantification) was determined using Prism software. Values were calculated using a 4-parameter non-linear regression analysis and are shown in Table 23.

Table 23. EC_{50}s for human and cynomolgus PBMC proliferation induced by anti-CD20 x CD3 bispecific antibodies

Antibody	Human PBMC Proliferation EC_{50} [M]	Cyno PBMC Proliferation EC_{50} [M]
Control I	3.30E-13	NA
Control II	8.93E-12	1.71E-12
BS3/20-001	1.08E-11*	4.02E-11*
BS3/20-002	8.59E-12*	2.60E-11*
BS3/20-003	9.55E-12*	2.78E-11*
BS3/20-004	1.45E-12*	NT
BS3/20-005	1.05E-12*	NT

(*) Data are median values of 3 or more independent assays. Data without a (*) are representative/average values of 1 or 2 independent assays. NA = no activity; NT = not tested.

[0210] As shown in Table 23, all CD20 x CD3 bispecific antibodies of the invention were activators of human or cynomolgus PBMCs. In general, anti-CD3 mono specific bivalent parental antibodies (Contros I and II) were 2-10 fold more potent than the bispecific counterparts. Control I (OKT3) did not drive monkey PBMC proliferation, while Control II (SP34) was active against both human and monkey PBMCs.

Example 10. CD20 x CD3 Bispecific Antibodies Activate T-cells and Induce IFN-gamma Release and CD25 Upregulation in Human Whole Blood

[0211] Selected CD20 x CD3 bispecific antibodies were tested for their ability to activate T-cells in human whole blood. The extent of T-cell activation was determined by measuring interferon-gamma (IFNγ) secretion as well as the upregulation of CD25 on CD8+ T cells.

[0212] Interferon-gamma (IFNγ) secretion was quantified by combining heparinized whole blood with 5-fold serial dilutions of bispecific antibodies in 96-well plates. After 20 hours, the

plates were centrifuged for 5 minutes and plasma was removed for ELISA analysis to determine IFNγ levels. Extrapolated IFNγ concentrations were plotted versus antibody concentration, and EC_{50} values were calculated using a 4-parameter non-linear regression analysis using Prism software.

[0213] For analysis of CD25 expression on CD8+ T-cells, following incubation with antibodies and removal of plasma, 150 µl of blood was transferred to a deep well plate and lysed for 15 minutes with 1.5 mL RBC lysis buffer. Cells were washed twice, blocked for 10 minutes at room temperature with hFcR blocking reagent, and then incubated for 30 min at 4°C with antibodies conjugated directly to CD2, CD19, CD4, CD8, and CD25. Next, cells were washed twice before analysis with a FACSCanto cytometer and FlowJo software.

[0214] The percentage of CD2+CD8+ T cells expressing the activation marker CD25 was plotted versus antibody concentration, and EC_{50} values were calculated using a 4-parameter non-linear regression analysis using Prism software. Results are shown in Table 24.

Table 24: EC_{50} values of Bispecific antibody mediated upregulation of CD25 and IFNγ production in whole blood

Bispecific Antibody	EC_{50} of CD25 Upregulation [M]	EC_{50} of IFNγ Production [M]	Max IFNγ (pg/mL)
BS3/20-001	1.3E-10	3.9E-10	1815
BS3/20-003	1.7E-10	5.7E-10	1693
BS3/20-004	2.9E-10	2.3E-09	5810

Median values of at least 3 independent experiments (except IFN-gamma expression of BS3/20-003, which is n=2)

[0215] As shown in Table 24, the CD20 x CD3 bispecific antibodies mediated the upregulation of CD25 on CD8+ T cells in whole blood with EC_{50} values ranging from 130-290 pM with corresponding EC_{50} values for IFNγ that were slightly higher ranging from 390 pM to 2 nM. BS3/20-004 was less slightly less potent then BS3/20-001 and BS3/20-003 in mediating CD25 upregulation and IFNγ production as determined by EC_{50}, however BS3/20-004 could induce greater levels of IFNγ in whole blood cultures.

Example 11. CD20 x CD3 Bispecific Antibodies Induce T-cell Mediated Cytotoxicity on Rituximab Resistant Cell Lines

[0216] The ability of selected CD20 x CD3 bispecific antibodies and Control constructs to mediate complement-dependent cytotoxicity (CDC) and T-cell mediated cytotoxicity was evaluated using parental Raji cells and Raji SCID lines. The later (Raji SCID lines) were derived from individual anti-CD20 resistant tumors isolated from immunodeficient mice injected subcutaneously with Raji cells following treatment with the anti-CD20 mAb Rituximab. Four lines (Raji SCID 1-4) were used in this Example.

[0217] The expression of CD20 and the complement inhibitory molecules CD55 and CD59 on Raji cell lines was determined by FACS. Briefly, $1x10^6$ cells were incubated in individual tubes for 30 minutes with antibodies directly conjugated to CD20, CD55 and CD59. Cells were

washed twice before FACS acquisition by a FACSCanto cytometer and analysis with FlowJo software.

[0218] To determine the ability of anti-CD20 and anti-CD3xCD20 antibodies to mediate T-cell directed killing of Raji cell lines, calcein labeled Raji cells were incubated for 2 hours at 37°C with pre-activated T cells (ficoll-isolated human PBMC activated with rhIL-2 (30U/mL) and anti-CD3/CD28 activation beads) and 3-fold serial dilutions of antibodies starting at 2 nM. Following incubation, plates were centrifuged and supernatants were transferred to a translucent black clear bottom plate for 530nm fluorescence detection at 485nm emission. Percent cytotoxicity was determined based on spontaneous (target cells alone) and maximum release (target cells lysed with detergent) values. EC_{50} values were calculated using a 4-parameter non-linear regression analysis using Prism software.

[0219] To determine the activity of the antibodies to mediate CDC, Raji cell lines were incubated with 5% normal human serum complement and 3-fold serial dilutions of antibodies starting at 100 nM. After incubation for 4.5 hours at 37C, cell death was determined using CellTiter Glo®. Percent cytotoxicity was determined based on spontaneous (target cells alone) and maximum release (target cells lysed with detergent) values. EC_{50} values were calculated using a 4-parameter non-linear regression analysis using Prism software.

[0220] Results are shown in Table 25.

Table 25. EC50 values for antibody mediated CDC and T-cell mediated cytotoxicity

Cell Line	CD20 MFI	% CD55/ CD59+	CDC			T-Cell Mediated Cytotoxicity	
			BS3/20-007	Control IV (anti-CD20)	Control III (anti-CD20)	BS3/20-007	Control IV (anti-CD20)
Raji	1709	8.81	2.62E-09	2.47E-10	9.66E-11	1.66E-12	No Activity
Raji SCID1	570	80.7	1.01E-07	5.19E-08	8.56E-08	1.11E-12	No Activity
Raji SCID2	1373	9.1	8.83E-09	2.29E-10	5.87E-11	6.52E-13	No Activity
Raji SCID3	1151	97.3	3.77E-08	5.71E-09	2.55E-08	2.93E-13	No Activity
Raji SCID4	1717	64.6	1.40E-07	1.14E-09	5.29E-09	1.53E-12	No Activity

[0221] Compared to parental Raji cells, 2 of 4 Raji SCID lines showed reduced expression of CD20 (Table 25; lines Raji SCID 1 and 3), with significantly higher percentage of cells expressing the complement inhibitory molecules CD55 and CD59. The sensitivity of the Raji SCID cells to CDC mediated by either anti-CD20 or anti-CD20 x CD3 antibodies was dependent on the percentage of CD55/CD59 expressing cells, but not on the levels of CD20, such that increased expression of CD55/CD59 on target cells inhibited CDC.

[0222] The anti-CD20 antibodies (Control IV & Control III [Rituximab]) were more potent than the anti-CD20 x CD3 (BS3/20-007) in mediating CDC, as the bispecific is monovalent for CD20.

However, in contrast to CDC, T-cell mediated cytotoxicity was not dependent on CD20 or CD55/CD59 levels, as all cell lines were equally susceptible to cell death by activated T-cells in the presence of anti-CD20 x CD3 bispecific antibody. Additionally, the bispecific antibody was 100-1000 fold more potent in mediating T-cell dependent killing of Raji cells than the anti-CD20 antibody in the CDC assay.

Example 12. CD25 Upregulation on CD8+ T-cells is Dependent on CD20 Concentration when in the Presence of CD20 x CD3 Bispecific Antibodies

[0223] To evaluate if higher concentrations of target cell (CD20+ lymphomas) would lead to an increased potency of CD20 x CD3 bispecific antibodies, human peripheral blood mononuclear cells (PBMCs) were co-cultured in the presence of a Burkitt's lymphoma-derived cell line, *i.e.*, Raji.

[0224] CD25 upregulation on CD8+ T-cells was determined using the following protocol: Human PBMCs ($5x10^5$/mL), isolated via centrifugation of mononuclear-cell enriched leukapharesis-derived blood over Ficoll, were incubated in the presence ($1x10^5$/mL) or absence of Raji cells, at 37°C in 96-well flat bottom plates with 5-fold serial dilutions of the bispecific antibodies. After 48 hours, cells were washed 2x, blocked for 10 minutes at room temperature with hFcR blocking reagent, and then incubated for 30 minutes at 4°C with directly conjugated antibodies to CD2, CD19, CD4, CD8, and CD25. After staining, cells were washed twice before FACS acquisition by a FACSCanto cytometer and analysis with FlowJo software. The percentage of activated CD2+CD8+ T cells expressing CD25 was plotted versus antibody concentration, and EC_{50} values were calculated using a 4-parameter non-linear regression analysis using Prism software. Results are shown in Table 26.

Table 26. CD25 upregulation on CD8+ T-cells following incubation of human PBMC with CD20 x CD3 bispecific antibodies plus or minus Raji cells

	PBMC		PBMC + Raji	
Antibody	**EC_{50} (M)**	**Max % CD25+**	**EC_{50} (M)**	**Max % CD25+**
BS3/20-001	1.12E-10	14.2	1.35E-12	92.2
BS3/20-003	3.65E-10	21.1	3.38E-13	94.4

[0225] As shown in Table 26, activated T-cells when cultured in the presence of Raji (target) cells showed an upregulation of CD25, and a subsequent 100-fold decrease in their EC_{50} values.

Example 13. CD20 x CD3 Bispecific Antibodies Induce Cytotoxicity to Raji cells in the Presence of Activated T-cells

[0226] The ability of CD20 x CD3 bispecific antibodies to redirect T-cell mediated killing to CD20-expressing Raji cells was tested in an *in vitro* cytotoxicity assay. In addition, the ability of both bispecific and parental anti-CD3 antibodies to kill U937 cells via Fc/FcR interactions was also studied.

[0227] Calcein killing assays were carried out using the following protocol: Human and

cynomolgus PBMC were isolated over ficoll-Plaque or via Lympholyte Mammal cell separation media, respectively. The isolated PBMCs were activated over a course of several days with media containing recombinant human IL-2 (30U/ml) and T-cell activation beads (anti-CD3/CD28 for human PBMC, anti-CD2/CD3/CD28 for cynomolgus PBMC).

[0228] Target cells (Raji for CD20 mediated killing and U937 for FcR mediated killing) were labeled with calcein, and incubated with activated T-cells at a 10:1 effector: target ratio using 3-fold serial dilutions of antibodies over a course of 3 hours at 37°C. Following incubation, the plates were centrifuged and supernatants were transferred to a translucent black clear bottom plate for fluorescence analysis. EC_{50}s defined as the molar concentration of bispecific antibody that induces 50% cytotoxicity was determined using Prism. Values were calculated using a 4-parameter non-linear regression analysis. Results are summarized in Table 27.

Table 27. EC_{50} values for CD20 x CD3-Induced Cytotoxicity to Raji and U937 cells

Antibody	Raji Cytotoxicity Human T-cells [M]	U937 Cytotoxicity Human T-cells [M]	Raji Cytotoxicity Monkey T-cells [M]
Control I (anti-CD3)	NA	3.04E-12	NA
BS3/20-001	5.63E-11*	8.86E-11*	1.27E-12*
BS3/20-002	7.71E-11*	8.24E-10	NT
BS3/20-003	7.38E-11*	8.10E-11*	4.36E-14
BS3/20-004	1.29E-11*	6.07E-11	NT
BS3/20-005	1.95E-11	1.48E-10	NT

(*) Data are median values of 3 or more independent assays. Data without a (*) are representative/average values of 1 or 2 independent assays. NA = No Activity; NT = Not Tested.

[0229] As shown in Table 27, bispecific CD20 x CD3 antibodies containing human-specific or human/cynomolgus cross reactive anti-CD3 arms were able to specifically redirect cytotoxicity to Raji cells in the presence of human activated T cells. In the presence of cynomolgus activated T cells, Raji were killed when they were incubated with BS3/20-001 or BS3/20-003, bispecific antibodies that have anti-CD3 arms that activate monkey T-cells. All bispecific antibodies as well as Control I, an anti-CD3 mAb, showed activity in the U937 Fc/FcR dependent killing assay. This activity could be blocked by the addition of blocking non-specific human IgG to the reaction (Data not shown).

Example 14. CD3 x CD20 Bispecific Antibodies Can Deplete CD19+ B-cells in Mice Reconstituted with Human Immune Cells

[0230] To determine the *in vivo* potency of CD3xCD20 bispecific antibody administration, changes in CD19+ B-cell and CD2+ T-cell levels were examined via FACS after administration of 10 µg or 0.1 µg of anti-CD3xCD20 bispecific antibody into mice, which were reconstituted with human immune cells.

[0231] Briefly, newborn BALB/Rag2^{null}/γ_c^{null} mice were irradiated with 2 x 150 Rads and reconstituted with 4x10^5 human CD34$^+$ hematopoietic progenitor cells via intrahepatic injection. After 12 weeks, the composition of reconstituted human immune system in peripheral blood was

determined by flow cytometry. Typically by three months post reconstitution, between 10%-60% percent of peripheral white blood cells are human CD45+ of which 40%-70% are B cells, 15%-40% are T-cells and the remaining are small populations of natural killer and dendritic cells.

[0232] Five months post-reconstitution, mice were injected intraperitoneally with 10 µg or 0.1 µg of anti-CD3xCD20 bispecific antibody BS3/20-007, 10 µg of a monovalent 1-arm CD3 antibody (BS3/20-009, see Table 1) or 10 µg of an irrelevant hIgG isotype control. One, eight, and twenty-five days post injection, mice were bled retro-orbitally and immune cell populations in the peripheral blood were determined by flow cytometry (FACS).

[0233] For FACS analysis, 100 µl of blood was incubated with 1.5 ml RBC lysis buffer in Eppendorf tubes for three minutes. Cells were centrifuged for five minutes at 0.4xg, washed 2x with FACS wash (PBS+3%FBS), and blocked for 10 minutes at room temperature with mouse Fc blocking reagent. Cells were then incubated for 30 minutes at 4°C with directly conjugated antibodies to CD2, CD3, CD19, CD4, CD8, hCD45, hHLA-DR, and mCD45. After staining, cells were washed two times before FACS acquisition by a FACSCanto cytometer and analysis with FlowJo software. Results are shown in Table 28.

Table 28: Percentage of circulating CD45, CD19 and CD2 positive cells in mice reconstituted with human immune cells

		Isotype Ctrl (10 µg)		BS3/20-007 (10 µg)		BS3/20-007 (0.1 µg)			BS3/20-009 [one-arm CD3] (10 µg)	
Mouse ID		1	2	3	4	5	6	7	8	9
	Day									
%huCD45+	Pre	13.7	14.8	16.1	30.9	37.2	22.5	25.5	26.6	33.3
	1d	7.7	10.8	0.01	0.13	1.7	1.2	0.8	2.7	8.9
	8d	14.1	12.7	0.12	0.16	3.3	7.7	3.9	3.2	4.5
	25d	13.0	7.3	0.15	0.12	9.0	1.2	1.0	2.8	5.1
%CD19+ (of huCD45+)	1d	58.7	66.8	0.00	7.69	20.2	7.0	5.2	75.3	87.1
	8d	66.2	56.2	0.00	0.00	21.3	0.4	0.0	70.4	76.6
	25d	37.3	62.8	9.7	2.6	58.3	0.7	0.6	38.9	51.3
%CD2+ (of huCD45+)	1d	58.7	66.8	0.00	7.69	20.2	7.0	5.2	75.3	87.1
	8d	66.2	56.2	0.00	0.00	21.3	0.4	0.0	70.4	76.6
	25d	37.3	62.8	9.7	2.6	58.3	0.7	0.6	38.9	51.3

[0234] As shown in Table 28, a single 10 µg dose of anti-CD3xCD20 bispecific antibody BS3/20-007 resulted in a disappearance of circulating hCD45+ cells in 2 of 2 treated mice which did not recover over the length of the experiment. A single 0.1 µg dose of BS3/20-007 reduced circulating hCD45+ cells, including CD19+ B-cells and CD2+ T-cells 24 hours post injection in 2 of 3 treated mice. Once depleted, the percentage of hCD45+ cells did not recover significantly in the responding mice treated with 0.1 µg BS3/20-007. However, what cells remained in these mice were predominantly hCD2+ T-cells, and CD19+ B cells were not present in the responding mice even at 25 days post treatment. A single 10 µg dose of a monovalent 1-arm CD3 antibody

(BS3/20-009) also resulted in a persistent but modest reduction in CD45+ cells, notably CD2+ T-cells, in 2 of 2 treated mice. A single 10 µg dose of an irrelevant hIgG1 control had no significant effect on the percentage of circulating hCD45+, hCD19+, or hCD2+ cells.

Example 15. Treatment with CD20 x CD3 Bispecific Antibody Decreases Raji Tumor Volume in NOD/SCID Mice

[0235] To assess the efficacy of selected anti-CD3xCD20 bispecific antibodies in reducing Raji tumor growth, NOD/SCID mice (Taconic) were implanted subcutaneously with a mixture of $2x10^6$ Raji tumor cells and $8x10^6$ human PBMC. Mice were treated three times per week, starting on the day of tumor implantation, with either human Fc (hFc) or CD3xCD20 bispecific antibody (BS3/20-007) at a dose of 1 µg per mouse (N=20 mice per treatment group). Reagents were delivered by intraperitoneal (i.p.) injection. Tumor size was measured three times per week using calipers, and tumor volume calculated as Volume = (length x width^2)/2. Results are shown in Figure 1.

[0236] In a second experiment, NOD/SCID mice were implanted subcutaneously with a mixture of $2x10^6$ Raji tumor cells and $4x10^6$ human PBMC. Treatment with CD3xCD20 bispecific antibody (BS3/20-007) or control reagent (hFc) began 7 days post tumor implantation to allow tumors to become palpable. Mice were treated two times per week at a dose of 1 µg per mouse (N=6 mice per treatment group). Reagents were injected subcutaneously, away from the site of tumor implantation. Tumor size was measured two times per week using calipers, and tumor volume calculated as Volume = (length x width^2)/2. Results are shown in Figure 2.

[0237] This Example demonstrates that treatment with CD3xCD20 bispecific antibody BS3/20-007 was effective in inhibiting tumor growth both at the time of tumor implantation and once tumors were established. Tumor volume in mice was decreased 25 days post implantation in both studies, relative to control.

Example 16. CD20 x CD3 Bispecific Antibodies Deplete B-cell Populations in Cynomolgus Monkeys and Have a Pharmacokinetic Profile Typical of Monoclonal Antibodies

[0238] A pilot non-GLP toxicology and pharmacology study was performed in cynomolgus monkeys (*Macaca fascicularis*) to determine the ability of the CD3xCD20 bispecific antibodies to deplete B-cell populations in these animals. Male animals were organized into three cohorts. Cohort 1 received bispecific antibody BS3/20-001 and included three different dosing groups (0.01, 0.10 and 1.00 mg/kg) with 3-4 animals per dosing group. Cohort 2 was a two-animal cohort that received a low dose of anti-CD20 control antibody (Control V; 0.01 mg/kg). Cohort 3 was a four-animal cohort that received a high dose of anti-CD20 control antibody (Control III; 1.0 mg/kg). Blood was drawn at day -7 and immediately prior to dosing in order to establish baseline levels for B and T cells in these animals. Doses of drug at 0.01, 0.10, or 1.00 mg/kg were administered by i.v. infusion and blood was drawn at 5 minutes, 5 hours, and 1, 4, 7, and

14 days post dosing. Following day 14 post-dose, blood was drawn every two weeks until the conclusion of the study. Blood samples were analyzed by FACS for B and T cell markers and the absolute number of these cell types was determined. Serum samples were also analyzed for cytokine levels (IFNγ, IL-2, IL-6 and TNFα) using standard analytic methods. Results are shown in Figure 3 (B-cells), Figure 4 (T-cells), and Figures 5A-5D (cytokines).

[0239] As shown in this Example, administration of the CD3xCD20 bispecific antibody resulted in depletion of circulating B-cells to baseline levels by the first time point measured (day 1). This depletion was not seen in the control animal cohort. B-cell depletion in the bispecific cohort was maintained until two weeks after dosing and in the 0.01 and 0.10 mg/kg dose cohorts was followed by a gradual recovery of B-cell levels until the experiment was concluded at around 11 weeks post dosing. In the 1.0 mg/kg cohort, however, no recovery of B-cell levels was seen for the duration of the experiment (11 weeks). T-cell levels were also monitored in this experiment. A transient loss of circulating T-cells was observed at day 1 post-dose in the bispecific cohorts. T-cell levels returned to baseline levels in these cohorts by the day 4 time-point and maintained at those baseline levels until the end of the experiment. In addition, serum cytokine levels for BS3/20-001 at 5 hours exhibited a dose- and time-dependent response that is consistent with T-cell activation (see Figures 5A-5D).

[0240] Gene expression levels in the peripheral blood were also analyzed during this experiment. Blood samples were obtained from animals at two pre-dose time points (Day 7 pre-dose and immediately pre-dose) and at 5, 24, 72, 96, and 168 hours post-dosing. RNA was isolated from these samples and analyzed by microarray. When compared to pre-dose levels and gene expression levels from the control group, a notable decrease in the gene expression of B-cell markers in animals treated with the bispecific antibody was observed; this effect was similar to the effect observed in samples obtained from animals treated with 1.0 mg/kg Control III (anti-CD20 antibody corresponding to Rituximab). The observed change in B-cell marker expression corresponds to the loss of B-cells detected in the blood of treated animals. The expression of T-cell marker genes in samples from animals treated with the CD3xCD20 bispecific antibody showed an initial decrease followed by a return to normal levels by the 24 hour time point. In addition, genes associated with an inflammatory response showed an initial upregulation in animals in the bispecific cohort but returned to normal or below normal levels after 24 hours. Finally, examination of the raw intensity of the CD20 gene expression signal suggests that a greater depletion of B-cells arises from treatment of animals with the CD3xCD20 bispecific antibody than with the control anti-CD20 antibodies. (*See* Figure 6 and Table 29).

Table 29. CD20 Gene Expression Levels at Day 7

Antibody	Dose mg/kg	CD20 Expression (Raw Intensity)
Control V (anti-CD20)	0.01	26485.44
	0.01	24335.17
Control III (anti-CD20)	1.0	1813.46
	1.0	47.09
	1.0	98.88
	1.0	70.52
BS3/20-001	0.01	24.93
	0.01	226.45
	0.01	4.78
	0.01	8.12
	0.1	8.26
	0.1	5.62
	0.1	4.82
	0.1	23.61
	1.0	9.38
	1.0	9.19
	1.0	8.22

[0241] As shown in Table 29, at seven days post-dosing the raw intensity of CD20 signal remained at background levels in all but one of the CD3xCD20 animals while 3 of 4 animals treated with 1 mg/kg of Control III showed either marginal or detectable CD20 signal levels.

[0242] In the same experiment the pharmacokinetic profile of the bispecific antibody (Figure 7) was evaluated by obtaining blood samples at pre-dose and at 0.083, 5, 24, 48, 72, 168, 336, 504 and 840 hours. The resultant serum samples were analyzed by a direct enzyme linked immunosorbent assay (ELISA) to determine the concentration of total bispecific antibody. Serum total bispecific (BS3/20-001) concentration data were analyzed by non-compartmental analysis (Phoenix WinNonLin) to determine pharmacokinetic parameters Results are shown in Table 30 (AUC = area under the curve vs. time; C_{max} = maximum concentration of compound observed in matrix of interest).

Table 30: Pharmacokinetic Parameters of BS3/20-001 in Cynomolgus Monkey

Parameter	Units	0.01 mg/kg		0.10 mg/kg		1.0 mg/kg	
		Mean	SD	Mean	SD	Mean	SD
C_{max}	µg/mL	0.261	0.0413	2.32	0.274	33.4	4.20
C_{max}/Dose	kg*µg/mL/mg	26.1	4.13	23.2	2.74	33.4	4.20
t_{max}	hr	0.083	0.00	0.083	0.00	0.083	0.00
AUC_{all}	µg*hr/mL	4.42	2.37	289	87.2	4940	1080
AUC_{all}/Dose	hr*kg*µg/mL/mg	442	237	2890	872	4940	1080

[0243] Following a single intravenous dose of 0.01, 0.10 or 1.0 mg/kg of BS3/20-001 in

cynomolgus monkeys, mean peak concentrations (C_{max}) of 0.261, 2.32 and 33.4 µg/mL, respectively, were observed at the first sampling time point (0.083 hr). Mean AUC_{all} values of 4.42, 289 and 4940 µg*hr/mL were observed at doses of 0.01, 0.1 and 1.0 mg/kg. Dose-normalized AUC values (AUC_{all}/Dose) of 442, 2890 and 4940 µg*hr/mL per mg/kg indicate that plasma exposure (AUC_{all}) increases with increasing dose in a non-linear fashion. Greater than proportional increase in plasma drug exposure was observed with increased antibody dose, suggesting that BS3/20-001 may be undergoing some target-mediated clearance. The overall pharmacokinetic profile of BS3/20-001 is typical of monoclonal antibodies dosed in cynomolgus monkey.

[0244] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

CLAIMS

1. A fully human bispecific antibody comprising a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that specifically binds human CD20; wherein the first antigen-binding domain comprises heavy chain complementarity determining regions (A1-HCDR1 , A1-HCDR2 and A1-HCDR3) comprising the amino acid sequences of SEQ ID NOs: 1252, 1254 and 1256, respectively, 1268, 1270 and 1272, respectively, or 1284, 1286 and 1288, respectively, and light chain complementarity determining regions (A1-LCDR1 , A1-LCDR2 and A1-LCDR3) comprising the amino acid sequences of SEQ ID NO: 1260, 1262 and 1264, respectively, 1276, 1278 and 1280, respectively, or 1292, 1294 and 1296, respectively; and wherein the second antigen-binding domain comprises heavy chain complementarity determining regions (A2-HCDR1 , A2-HCDR2 and A2-HCDR3) comprising the amino acid sequences of SEQ ID NO: 1244, 1246 and 1248, respectively, and light chain complementarity determining regions (A2-LCDR1 , A2-LCDR2 and A2-LCDR3) comprising the amino acid sequences of SEQ ID NO: 1260, 1262 and 1264, respectively, or 1276, 1278 and 1280, respectively, wherein the bispecific antibody induces human T-cell proliferation and monkey T-cell proliferation *in vitro*.