



(86) Date de dépôt PCT/PCT Filing Date: 2016/03/24
(87) Date publication PCT/PCT Publication Date: 2016/10/06
(85) Entrée phase nationale/National Entry: 2017/08/14
(86) N° demande PCT/PCT Application No.: US 2016/023902
(87) N° publication PCT/PCT Publication No.: 2016/160476
(30) Priorité/Priority: 2015/04/03 (US62/142,706)

(51) Cl.Int./Int.Cl. *C12N 15/13* (2006.01),
A61K 39/00 (2006.01), *A61P 37/00* (2006.01),
C07K 16/24 (2006.01), *C07K 16/28* (2006.01),
C07K 16/46 (2006.01), *C12N 5/10* (2006.01),
C12P 21/08 (2006.01)

(71) Demandeur/Applicant:
ELI LILLY AND COMPANY, US

(72) Inventeurs/Inventors:
ALLAN, BARRETT, US;
KIKLY, KRISTINE KAY, US;
WITCHER, DERRICK RYAN, US

(74) Agent: GOWLING WLG (CANADA) LLP

(54) Titre : ANTICORPS BISPECIFIQUES ANTI-CD20 / ANTI-BAFF
(54) Title: ANTI-CD20- / ANTI-BAFF BISPECIFIC ANTIBODIES

(57) **Abrégé/Abstract:**

Bispecific antibodies are provided that bind CD20 and B-cell Activating Factor of the TNF Family (BAFF) and are characterized as having high affinity and strong simultaneous neutralizing properties to both CD20 and BAFF. The bispecific antibodies of the invention are useful for treating autoimmune diseases including Systemic Lupus Erythematosus, Lupus Nephritis, and primary Sjgren's Syndrome.



(51) **International Patent Classification:**
C07K 16/28 (2006.01) *A61P 37/00* (2006.01)
C07K 16/46 (2006.01) *A61K 39/00* (2006.01)

(21) **International Application Number:**
PCT/US2016/023902

(22) **International Filing Date:**
24 March 2016 (24.03.2016)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
62/142,706 3 April 2015 (03.04.2015) US

(71) **Applicant:** ELI LILLY AND COMPANY [US/US];
Lilly Corporate Center, Indianapolis, Indiana 46285 (US).

(72) **Inventors:** ALLAN, Barrett; c/o Eli Lilly and Company,
P. O. Box 6288, Indianapolis, Indiana 46206-6288 (US).
KIKLY, Kristine Kay; c/o Eli Lilly and Company, P. O.
Box 6288, Indianapolis, Indiana 46206-6288 (US).
WITCHER, Derrick Ryan; c/o Eli Lilly and Company, P.
O. Box 6288, Indianapolis, Indiana 46206-6288 (US).

(74) **Agents:** MARKS, Duane et al.; Eli Lilly and Company, P.
O. Box 6288, Indianapolis, Indiana 46206-6288 (US).

(81) **Designated States** (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,

BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Declarations under Rule 4.17:

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

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

Published:

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

— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title:** ANTI-CD20- / ANTI-BAFF BISPECIFIC ANTIBODIES

(57) **Abstract:** Bispecific antibodies are provided that bind CD20 and B-cell Activating Factor of the TNF Family (BAFF) and are characterized as having high affinity and strong simultaneous neutralizing properties to both CD20 and BAFF. The bispecific anti-bodies of the invention are useful for treating autoimmune diseases including Systemic Lupus Erythematosus, Lupus Nephritis, and primary Sjgren's Syndrome.

WO 2016/160476 A1

-1-

ANTI-CD20- / ANTI-BAFF BISPECIFIC ANTIBODIES

The present invention is in the field of medicine, particularly in the novel field of bispecific antibodies directed against CD20 and B-cell Activating Factor of the TNF Family (BAFF). The bispecific antibodies of the present invention are expected to be
5 useful in treating one or more autoimmune diseases having B-cell dysregulation such as Systemic Lupus Erythematosus (SLE), Lupus Nephritis (LN), primary Sjögren's Syndrome (pSS), and/or Grave's Disease (GD).

SLE, LN, and pSS are systemic autoimmune diseases characterized by the development of auto-antibodies. An estimated 30-60% of patients with SLE develop
10 renal involvement (a histological reference indicating progression into LN) at some stage during the course of their disease. LN is a complex, multi-factorial autoimmune disease that, if not controlled, can lead to kidney failure. pSS is characterized by chronic inflammation of the exocrine glands (e.g., salivary and lacrimal glands) and may impact respiratory, vascular, neurological, hepatic, and/or renal functions. There is currently no
15 disease-modifying treatment approved for pSS. Likewise, response to currently available treatments of SLE and LN is slow and often incomplete (with approximately only 25-50% of patients reaching remission).

CD20 is a B-lymphocyte-restricted differentiation antigen expressed by B-cells during early pre-B-cell development. Multiple studies demonstrate CD20 acts as a
20 regulator of B-cell activation, a step required for differentiation of B-cells into plasma cells. Because differentiated B-cells are critical to adaptive immunity, B cell depletion therapy (BCDT) has been explored as a treatment for multiple autoimmune diseases, including SLE and LN. The role played by CD20 in B-cell differentiation lends to the appeal of CD20 as a target for BCDT. However, despite multiple efforts, to date no
25 BCDT targeting CD20 has been approved for the treatment of SLE, LN, or pSS.

BAFF is a cytokine which stimulates B-cell survival, proliferation, and differentiation. Over expression of BAFF has been reported in murine models of autoimmune diseases mimicking SLE, LN, and pSS. Multiple studies suggest that BAFF overexpression associated with autoimmune diseases acts to "rescue" autoreactive B-cells
30 from the deletion pathway. However, anti-BAFF antibody therapies have proven only moderately effective.

-2-

Accordingly, there remains a need for alternative therapies for the treatment of SLE, LN, and pSS. Preferably such treatment will be capable of demonstrating efficacy in a large number of patients non-responsive to, or inadequately benefited by, currently available options. One such alternative therapy may include the co-administration of two different bioproducts (e.g., antibodies). Co-administration requires either injections of two separate products or a single injection of a co-formulation of two different antibodies. While two injections permit flexibility of dose amounts and timing, it is inconvenient to patients for compliance, pain, and cost. Additionally, it is often quite challenging or impossible to find co-formulation conditions that permit chemical and physical stability of two different antibodies. Thus, there remains a need for alternative therapies for the treatment of SLE, LN, and pSS, and preferably such alternative therapies will comprise a bispecific antibody.

WO199509917 discloses a method of recombinantly producing bispecific, tetravalent antibodies having dual specificity by producing a single chain variable fragment (scFv) polypeptide fused to a complete antibody (Ab) having a different specificity. U.S. Patent Number 8,153,125 discloses antibodies directed against human CD20. U.S. Patent Number 7,317,089 discloses antibodies directed against both soluble and membrane bound forms of human BAFF. However, when following the teachings in WO199509917 to create a starting bispecific antibody comprising the CD20 antibodies of U.S. Patent Number 8,153,125 and the BAFF antibodies of U.S. Patent Number 7,317,089, the present inventors discovered significant problems associated with chemical and physical stability. Many modifications were required in the starting bispecific antibody to sufficiently overcome these problems. Neither the need for nor the actual changes are suggested in the art. Further, the several changes are not routine or derived from common general knowledge. Likewise, the single antibodies themselves did not have these problems, suggesting that the local environment around these amino acid domains differed in the context of bispecific antibodies. Thus, pharmacological intervention with a bispecific antibody that neutralizes both CD20 and BAFF is needed.

The present invention provides a bispecific antibody having four polypeptide chains, two first polypeptide chains and two second polypeptide chains, wherein each first polypeptide chain comprises a single chain variable fragment (scFv) independently linked at the N-terminus of a mAb IgG heavy chain (HC) via a polypeptide linker (L1) and each

-3-

of the second polypeptide chains comprises a mAb light chain (LC). According to bispecific antibodies of the present invention, each HC comprises a heavy chain variable region (HCVR1) with heavy chain complementarity determining regions (HCDRs) 1-3 and each LC comprises a light chain variable region (LCVR1) with light chain complementarity determining regions (LCDRs) 1-3. Additionally, according to bispecific antibodies of the present invention, each scFv comprises a light chain variable region (LCVR2) with LCDRs 4-6 and a heavy chain variable region (HCVR2) with HCDRs 4-6. Also, according to bispecific antibodies of the present invention, LCVR2 is linked at its C-terminus to L1 and linked at its N-terminus to a polypeptide linker (L2) which is linked to the C-terminus of HCVR2.

According to particular embodiments of bispecific antibodies of the present invention, the amino acid sequence of HCDR1 is given by SEQ ID NO:9, the amino acid sequence of HCDR2 is given by SEQ ID NO:10, the amino acid sequence of HCDR3 is given by SEQ ID NO:11, the amino acid sequence of LCDR1 is given by SEQ ID NO:15, the amino acid sequence of LCDR2 is given by SEQ ID NO:16, the amino acid sequence of LCDR3 is given by SEQ ID NO:17, the amino acid sequence of HCDR4 is given by SEQ ID NO:12, the amino acid sequence of HCDR5 is given by SEQ ID NO:13, the amino acid sequence of HCDR6 is given by SEQ ID NO:14, the amino acid sequence of LCDR4 is given by SEQ ID NO:18, the amino acid sequence of LCDR5 is given by SEQ ID NO:19, and the amino acid sequence of LCDR6 is given by SEQ ID NO:20.

According to particular embodiments, the HC comprises a mAb IgG1 isotype. In some embodiments, bispecific antibodies of the present invention include one or more modifications in the constant region of each HC that enhance effector function. Even more particular embodiments include the amino acid glutamic acid at residue 601 and glutamine at residue 608 (residue numbering based on the first polypeptide of the exemplified bispecific antibody of Table 1).

According to further particular embodiments, each LC comprises a mAb kappa light chain. In some such embodiments, the amino acid sequence of LCVR1 is given by SEQ ID NO:7, and the amino acid sequence of LCVR2 is given by SEQ ID NO:8. According to further particular embodiments the amino acid sequence of HCVR1 is given by SEQ ID NO:5 and the amino acid sequence of HCVR2 is given by SEQ ID NO:6. According to even further embodiments, the amino acid sequence of L1 is given by SEQ

-4-

ID NO:21 and the amino acid sequence of L2 is given by SEQ ID NO:22. In an even more particular embodiment of a bispecific antibody of the present invention, the amino acid sequence of each of the first polypeptides is given by SEQ ID NO:1 and the amino acid sequence of each of the second polypeptides is given by SEQ ID NO:2.

5 The present invention also relates to nucleic acid molecules and expression vectors encoding bispecific antibodies of the present invention. In an embodiment, the present invention provides a DNA molecule comprising a polynucleotide sequence encoding the first polypeptide, wherein the amino acid sequence of the first polypeptide is SEQ ID NO:1. In an embodiment, the present invention also provides a DNA molecule
10 comprising a polynucleotide sequence encoding the second polypeptide, wherein the amino acid sequence of the second polypeptide is SEQ ID NO:2. In a further embodiment, the present invention provides a DNA molecule comprising a polynucleotide sequence encoding the first polypeptide having the amino acid sequence of SEQ ID NO:1, and comprising a polynucleotide sequence encoding the second
15 polypeptide having the amino acid sequence of SEQ ID NO:2. In a particular embodiment the polynucleotide sequence encoding the first polypeptide having the amino acid sequence of SEQ ID NO:1 is given by SEQ ID NO:3 and the polynucleotide sequence encoding the second polypeptide having the amino acid sequence of SEQ ID NO:2 is given by SEQ ID NO:4.

20 The present invention also provides a mammalian cell transformed with DNA molecule(s) which cell is capable of expressing a bispecific antibody comprising the first polypeptide and the second polypeptide of the present invention. Also, the present invention provides a process for producing a bispecific antibody comprising the first polypeptide and the second polypeptide, comprising cultivating the mammalian cell under
25 conditions such that a bispecific antibody of the present invention is expressed. The present invention also provides a bispecific antibody produced by said process.

 The present invention also provides a method of treating an autoimmune disease having B-cell dysregulation, such as SLE, LN, pSS, or GD comprising administering to a patient in need thereof an effective amount of a bispecific antibody of the present
30 invention.

-5-

The present invention also provides a bispecific antibody of the present invention for use in therapy. More particularly, the present invention provides a bispecific antibody of the present invention for use in the treatment of SLE, LN, pSS, or GD.

5 The present invention also provides a pharmaceutical composition comprising a bispecific antibody of the present invention and one or more pharmaceutically acceptable carriers, diluents, or excipients. Pharmaceutical compositions of the present invention can be used in the treatment of SLE, LN, pSS, or GD, whereby such treatment comprises administering to a patient in need thereof pharmaceutical compositions of the present invention.

10 In an embodiment, the present invention also provides the use of a bispecific antibody of the present invention in the manufacture of a medicament for the treatment of SLE, LN, pSS, or GD.

As referred to herein, the term “bispecific antibody” refers to an engineered polypeptide comprising four antigen binding sites. Two of the four antigen binding sites
15 bind CD20 and the other two antigen binding sites bind BAFF. A bispecific antibody of the present invention is capable of binding CD20 and BAFF alone or simultaneously, and neutralizes at least one human CD20 bioactivity and at least one human BAFF bioactivity in vitro and/or in vivo. The bispecific antibodies of the present invention are also potent inhibitors of CD20 in the presence or absence of BAFF in vitro.

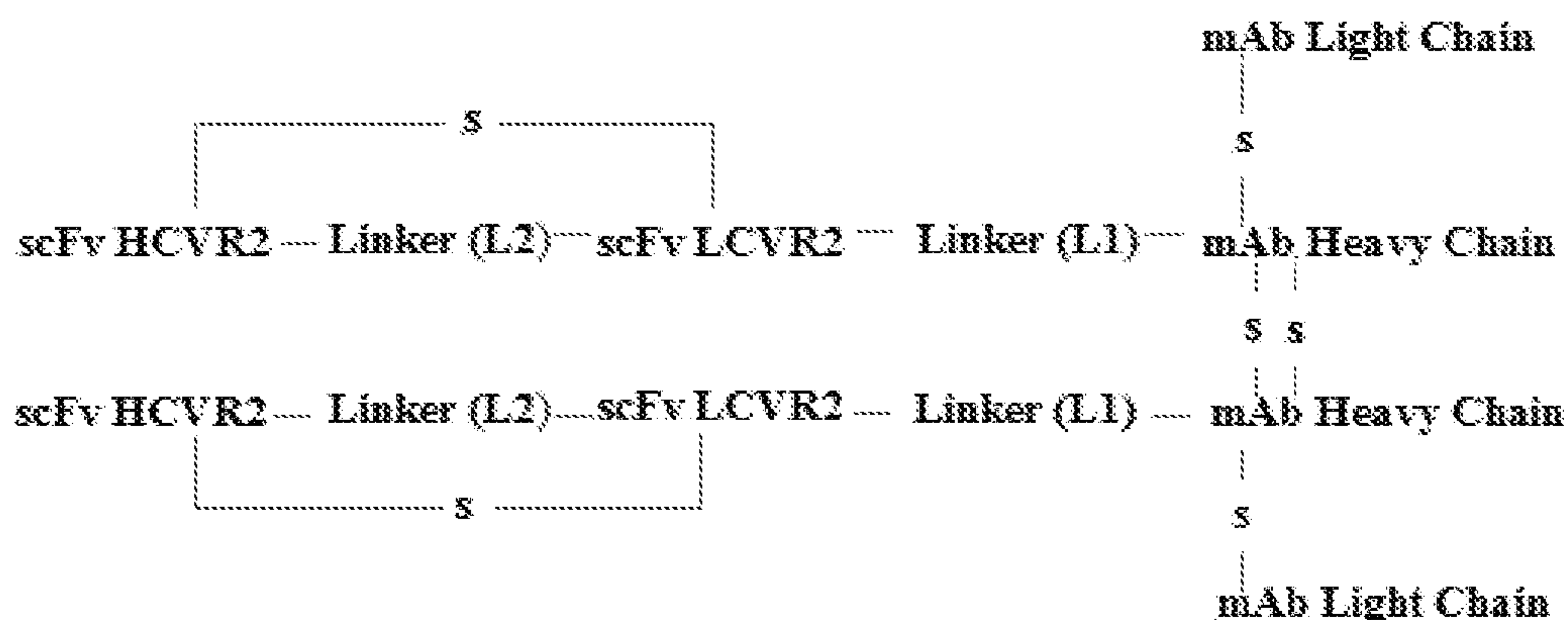
20 Also, a bispecific antibody, as referred to herein, comprises four polypeptide chains, two first polypeptides and two second polypeptides. Each of the first polypeptides is engineered to comprise a single chain variable fragment (scFv) linked at the N-terminus of a mAb heavy chain (HC) by a polypeptide linker (L1). Each of the second polypeptides is engineered to comprise a mAb light chain (LC) and form inter-
25 chain disulfide bonds with one of the first polypeptides, specifically within the HC of a first polypeptide. Each first polypeptide is engineered to form inter-chain disulfide bonds with the other first polypeptide, specifically between the HC of each of the first polypeptides. Each first polypeptide is further engineered to form intra-chain disulfide bonds, specifically within the scFv of each respective first polypeptide.

30 As referred to herein, a “single chain variable fragment” (scFv) of a first polypeptide, refers to a polypeptide chain comprising a heavy chain variable region (HCVR2) and a light chain variable region (LCVR2) linked via a polypeptide linker (L2).

-6-

Additionally, as referred to herein (and as represented in the following schematic), the LCVR2 of each scFv is: a.) linked, at its C-terminus, to the N-terminus of one HC (of a first polypeptide) by a polypeptide linker (L1); and b.) linked, at its N-terminus, to the C-terminus of the HCVR2 of the same scFv via a second polypeptide linker (L2).

5



According to bispecific antibodies of the present invention, the HC of each first
 10 polypeptide is classified as gamma, which defines the isotype (e.g., as an IgG). The
 isotype may be further divided into subclasses (e.g., IgG₁, IgG₂, IgG₃, and IgG₄). In
 particular embodiments, bispecific antibodies of the present invention are IgG1. The C-
 terminal portion of each HC defines a constant region (CH) primarily responsible for
 effector function. In particular embodiments, bispecific antibodies of the present
 15 invention have one or more modifications in the CH of each HC that enhances effector
 function. In more particular embodiments, bispecific antibodies of the present invention
 are IgG1 and have modifications in the CH of both HCs that enhance effector function.
 In even more particular embodiments, such modifications include the amino acid
 glutamic acid at residue 601 and glutamine at residue 608 in the CH of both HCs (residue
 20 numbering based on the first polypeptide of the exemplified bispecific antibody of Table
 1). The N-terminal portion of each HC of each first polypeptide includes a variable
 region (HCVR1).

Additionally, according to bispecific antibodies of the present invention each mAb
 light chain (LC), comprising the second polypeptide, is classified as kappa or lambda and
 25 characterized by a particular constant region as known in the art. In particular
 embodiments the bispecific antibodies of the present invention comprise kappa LCs. The

-7-

C-terminal portion of each LC defines a light chain constant region, whereas the N-terminal portion of each LC of each second polypeptide includes a light chain variable region (LCVR1).

The HCVR1 and LCVR1, of each HC and LC respectively, and HCVR2 and LCVR2, of each scFv, can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Preferably, the framework regions of the bispecific antibodies of the invention are of human origin or substantially of human origin. Each HCVR1, HCVR2, LCVR1 and LCVR2 of bispecific antibodies according to the present invention are 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. Herein the 3 CDRs of each HCVR1 are referred to as "HCDR1, HCDR2 and HCDR3;" the 3 CDRs of each HCVR2 are referred to as "HCDR4, HCDR5 and HCDR6;" the 3 CDRs of each LCVR1 are referred to as "LCDR1, LCDR2 and LCDR3;" and the 3 CDRs of each LCVR2 are referred to as "LCDR4, LCDR5 and LCDR6." The CDRs contain most of the residues which form specific interactions with the antigen. The functional ability of a bispecific antibody of the present invention to bind a particular antigen is largely influenced by the six CDRs.

As used interchangeably herein, "antigen-binding portion," "antigen-binding site," and "antigen-binding region" refers to those portions of bispecific antibodies of the present invention which contain the amino acid residues that interact with an antigen and confer to the bispecific antibody specificity and affinity for a respective antigen. According to bispecific antibodies of the present invention, antigen-binding sites are formed by HCVR1 / LCVR1 pairs (of a LC and HC bound by inter-chain disulfide bonds) and by a scFv HCVR2 / LCVR2 pair. Additionally, according to bispecific antibodies of the present invention, antigen-binding sites formed by each HCVR1 / LCVR1 pair are the same (e.g., comprises specificity and affinity for a same antigen), and antigen-binding sites formed by each scFv HCVR2 / LCVR2 pair are the same (e.g., comprises specificity and affinity for a same antigen). However, according to bispecific antibodies of the instant invention, antigen-binding sites formed by each HCVR1 / LCVR1 pair are different (e.g., comprises specificity and affinity for a different antigen) from antigen-binding sites formed by each scFv HCVR2 / LCVR2 pair.

-8-

A “parent antibody” or “parental antibody,” as used interchangeably herein, is an antibody encoded by an amino acid sequence which is used for preparation of one of the mAb (e.g., both HCs and LCs) and scFv of the bispecific antibody, for example through amino acid substitutions and structural alteration. The parent antibody may be a murine,
5 chimeric, humanized, or human antibody. Initial attempts in constructing a bispecific antibody according to the present invention included constructs in which a parental BAFF antibody (such as described in U.S. Patent No. 7,317,089) was engineered to comprise a scFv portion in various configurations and orientations, and a parental CD20 antibody (such as described in U.S. Patent Number 8,153,125) was engineered to comprise both a
10 scFV as well as mAb portion of the bispecific antibody.

The terms “Kabat numbering” or “Kabat labeling” are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (i.e., hypervariable) than other amino acid residues in the heavy chain and light chain variable regions of an antibody (Kabat, et al.,
15 *Ann. NY Acad. Sci.* 190:382-93 (1971); Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 (1991)).

The terms “North numbering” or “North labeling” are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering
20 amino acid residues which are more variable (i.e., hypervariable) than other amino acid residues in the heavy and light chains variable regions of an antibody and is based, at least in part, on affinity propagation clustering with a large number of crystal structures, as described in (North et al., *A New Clustering of Antibody CDR Loop Conformations*, *Journal of Molecular Biology*, 406:228-256 (2011)).

25 The relationship of the various regions and linkers are exemplified, in an exemplified bispecific antibody of the present invention, in Table 1 (numbering of amino acids applies linear numbering; assignment of amino acids to variable domains is based on the International Immunogenetics Information System[®] available at www.imgt.org; assignment of amino acids to CDR domains is based on the well-known Kabat and North
30 numbering conventions and is reflected at the end of Table 1):

Table 1: Amino acid regions of an exemplified bispecific antibody of the present invention.

SEQ ID NO:1			SEQ ID NO:2		
Exemplified HCVR2 (BAFF)	Region	Positions			
	FRH1-1	1-22			
	HCDR4	23-35			
	FRH1-2	36-49			
	HCDR5	50-65			
	FRH1-3	66-95			
	HCDR6	96-112			
	FRH1-4	113-123			
Exemplified Linker	L2	124-143			
Exemplified LCVR2 (BAFF)	FRL1-1	144-166			
	LCDR4	167-177			
	FRL1-2	178-191			
	LCDR5	192-199			
	FRL1-3	200-231			
	LCDR6	232-240			
	FRL1-4	241-250			
Exemplified Linker	L1	251-265			
Exemplified HCVR1 (CD20)	FRH1	266-287	Exemplified LCVR1 (CD20)	Region	Positions
	HCDR1	288-300		FRL1	1-23
	FRH2	301-314		LCDR1	24-33
	HCDR2	315-331		FRL2	34-47
	FRH3	332-361		LCDR2	48-55
	HCDR3	362-375		FRL3	56-87
	FRH4	376-386		LCDR3	88-96
Exemplified Constant Region	CH	387-715	Exemplified Constant Region	CL	107-213
CDR	Starting Amino Acid Pos. Definition		Ending Amino Acid Pos. Definition		
HCDR1	North		Kabat		
HCDR2	Kabat		Kabat		
HCDR3	North		Kabat		
HCDR4	North		Kabat		

-10-

HCDR5	Kabat	Kabat
HCDR6	North	Kabat
LCDR1	Kabat	Kabat
LCDR2	North	Kabat
LCDR3	Kabat	Kabat
LCDR4	Kabat	Kabat
LCDR5	North	Kabat
LCDR6	Kabat	Kabat

The exemplified bispecific antibody of the present invention in Table 1 comprises two first polypeptides having amino acid sequences of SEQ ID NO:1 and two second polypeptides having amino acid sequences of SEQ ID NO:2. According to the exemplified bispecific antibody of the present invention presented in Table 1, each of the first polypeptides forms an inter-chain disulfide bond with each of the second polypeptides between cysteine residue 489 of SEQ ID NO:1 and cysteine residue 213 of SEQ ID NO:2; at least two inter-chain disulfide bonds with the other first polypeptide, the first inter-chain disulfide bond forming between cysteine residue 495 (of SEQ ID NO:1) of the first polypeptide and cysteine residue 495 (of SEQ ID NO:1) of the other first polypeptide, the second inter-chain disulfide bond forming between cysteine residue 498 (of SEQ ID NO:1) of the first polypeptide and cysteine residue 498 (of SEQ ID NO:1) of the other first polypeptide; and an intra-chain disulfide bond formed in the scFv of each first polypeptide between cysteine residue 44 (of SEQ ID NO:1) and cysteine residue 243 (of SEQ ID NO:1) of each respective first polypeptide. Further, the exemplified bispecific antibody of Table 1 is glycosylated at asparagine residue 566 of SEQ ID NO:1 of both first polypeptides.

Bispecific Antibody Engineering

Significant problems associated with chemical and physical stability were encountered when constructing a bispecific antibody of the present invention. Problems encountered included poor expression, splice variant formation, poor purification recovery, low thermostability, aggregation of expressed bispecific antibodies, misfolding of scFv, LC clipping, low binding affinity and low target neutralization activity, poor solubility, and rapid clearance in vivo.

-11-

For example, initial attempts in constructing a bispecific antibody according to the present invention included constructs in which a parental CD20 antibody (described in U.S. Patent No. 8,153,125) comprised the mAb antibody portion and a parental BAFF antibody (such as described in U.S. Patent No. 7,317,089) comprised the scFv portions of the bispecific antibody. Initial constructs included the scFv portion being conjugated to the mAb portion in various configurations, including at the amino-terminus or the carboxyl terminus for both HCs and LCs, respectively. Additionally, initial constructs included the scFv portion varying in arrangement of the HCVR2 and LCVR2 (e.g., mAb portion (C or N terminus) – linker 1 – LCVR2 or HCVR2 – linker 2 – the other of LCVR2 or HCVR2). Further, parental BAFF antibody constructs included combinations of heavy chain germline frameworks VH4-59 and VH4-34, and light chain germline framework L6. Parental CD20 antibody constructs also included combinations of heavy chain germline framework VH5-51 and light chain germline framework A27. Parental CD20 antibody constructs also included both HCs having an IgG1 subclass, as well as, modifications within the constant region (CH) of each HC (of IgG1 subclass), for example, as described in U.S. Patent No. 8,153,125. Initial constructs were cloned into a human IgG1-Fc mammalian expression vector and expressed in HEK293F cells. However, initially produced bispecific constructs as (described above) exhibited one or more of the chemical and/or physical problem(s) described above.

Structural modifications were therefore made to address the significant chemical and physical stability problems associated with attempting to construct a bispecific antibody of the present invention. For example, modifications, relative to the parental BAFF antibody, were engineered in HCDR4, HCDR5, LCDR4, LCDR5, and LCDR6. Additionally, constructed HCVR2 and LCVR2 were engineered into a BAFF scFv format according to the following formula: HCVR2 – L2 – LCVR2 – L1 – (N-term.) HC (CD20). A disulfide bond, for stabilizing BAFF scFv, was engineered between HCVR2 (G44C) and LCVR2 (G243C) (amino acid residue numbering based on exemplified bispecific antibody of Table 1). Additionally, the heavy chain germline framework was changed, relative to the parental BAFF antibody, from HC4-59 to HC4-34. The mAb portion of the bispecific antibody of the present invention was also engineered to address chemical and physical stability problems (described above), including binding affinity and neutralization of CD20. For example, modifications relative to the parental CD20

-12-

antibody were engineered in LCDR3 and the CH of the HC (specifically the CH2 region of the HC IgG1 backbone).

Further, electrostatic surface charge of the bispecific antibody was calculated and charged patches were identified and disrupted. Extensive stability studies were performed and the constructed bispecific antibodies were screened for thermostability and physical and chemical stability properties as well as BAFF and CD20 binding and neutralizing properties relative to the respective parental antibodies.

Nucleic acid sequence, encoding engineered BAFF scFv construct and CD20 mAb construct (comprising the extensive chemical and physical modifications and oriented in the manner described herein), was engineered into an IgG1 expression vector for expression by mammalian cells such as CHO and HEK293. Following initial expression of bispecific antibody of the present invention, splice variant sites were identified. Splice variant issues were eliminated through introduction of engineered silent modifications to the nucleic acid sequence, which was re-engineered into an IgG1 expression vector for expression by mammalian cells such as CHO and HEK293. Expressed bispecific antibodies of the present invention were extensively screened for chemical and physical stability properties, as well as functional properties including BAFF and CD20 binding affinity and neutralization properties.

A bispecific antibody engineered to include a CD20 mAb with a modified IgG1 subclass (relative to native IgG1: I601E, A608Q) and a CDR modification (relative to the parental CD20 antibody: LCDR3 at N93H), as well as BAFF scFv with seven CDR modifications (relative to the parental BAFF antibody: HCVR at Y32H, Y99A, Y100R, Y107L, F110Y, and Y112H, LCVR at W237H) and modified HC framework (relative to parental BAFF antibody: VH4-34 → VH4-39), when orientated as described herein, was identified as improving the expression, physical stability, chemical stability, thermostability, and affinity and neutralization issues (described above) demonstrated in initial constructs (amino acid residue numbering based on exemplified bispecific antibody of Table 1). Additionally, these engineered modifications resulted in an improved rate of clearance in cynomolgus monkeys. None of the above modifications are routine or common general knowledge suggested in the art and the need for these modifications was not identified in initial characterizations of the individual parental antibodies.

-13-

Bispecific Antibody Expression

Expression vectors capable of directing expression of genes to which they are operably linked are well known in the art. Expression vectors can encode a signal peptide that facilitates secretion of the polypeptide(s) from a host cell. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide. The first polypeptide chain and the second polypeptide chain may be expressed independently from different promoters to which they are operably linked in one vector or, alternatively, the first and second polypeptide chains may be expressed independently from different promoters to which they are operably linked in two vectors – one expressing the first polypeptide chain and one expressing the second polypeptide chain.

A host cell includes cells stably or transiently transfected, transformed, transduced, or infected with one or more expression vectors expressing a first polypeptide chain, a second polypeptide chain or both a first and a second polypeptide chain of the invention. Creation and isolation of host cell lines producing a bispecific antibody of the invention can be accomplished using standard techniques known in the art. Mammalian cells are preferred host cells for expression of bispecific antibodies. Particular mammalian cells are CHO and HEK 293. Preferably, the bispecific antibodies are secreted into the medium in which the host cells are cultured, from which the bispecific antibodies can be recovered or purified by conventional techniques. For example, the medium may be applied to and eluted from a Protein A or G column using conventional methods. Additionally, soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, or hydroxyapatite chromatography. The product may be immediately frozen, for example at -70°C, or may be lyophilized.

It is well known in the art that mammalian expression of antibodies results in glycosylation. Typically, glycosylation occurs in the Fc region of the antibody at a highly conserved N-glycosylation site. N-glycans typically attach to asparagine. By way of example, each HC of the exemplified bispecific antibody presented in Table 1 is glycosylated at asparagine residue 566 of SEQ ID NO:1.

30

-14-

Therapeutic Uses

As used herein, “treatment” and/or “treating” are intended to refer to all processes wherein there may be a slowing, interrupting, arresting, controlling, or stopping of the progression of the disorders described herein, but does not necessarily indicate a total
5 elimination of all disorder symptoms. Treatment includes administration of a bispecific antibody of the present invention for treatment of a disease or condition in a patient that would benefit from a decreased level of CD20 positive B cells and/or BAFF or decreased bioactivity of CD20 positive B cells and/or BAFF, and includes: (a) inhibiting further progression of the disease, *i.e.*, arresting its development; and (b) relieving the disease,
10 *i.e.*, causing regression of the disease or disorder or alleviating symptoms or complications thereof. The bispecific antibody of the present invention is expected to treat an autoimmune disease having B-cell dysregulation, including SLE, LN, pSS, and/or GD.

The terms “patient,” “subject,” and “individual,” used interchangeably herein,
15 refer to a human. In certain embodiments, the patient is further characterized with a disease, disorder, or condition (e.g., an autoimmune disorder) that would benefit from a decreased level or decreased bioactivity of both CD20 positive B cells and BAFF. In another embodiment the patient is further characterized as being at risk of developing a disorder, disease, or condition that would benefit from a decreased level or decreased
20 bioactivity of both CD20 positive B cells and BAFF.

Pharmaceutical Composition

A bispecific antibody of the invention can be incorporated into a pharmaceutical composition suitable for administration to a patient. A bispecific antibody of the
25 invention may be administered to a patient alone or with a pharmaceutically acceptable carrier and/or diluent in single or multiple doses. Such pharmaceutical compositions are designed to be appropriate for the selected mode of administration, and pharmaceutically acceptable diluents, carrier, and/or excipients such as dispersing agents, buffers, surfactants, preservatives, solubilizing agents, isotonicity agents, stabilizing agents and
30 the like are used as appropriate. Said compositions can be designed in accordance with conventional techniques disclosed in, *e.g.*, Remington, The Science and Practice of Pharmacy, 19th Edition, Gennaro, Ed., Mack Publishing Co., Easton, PA 1995 which

-15-

provides a compendium of formulation techniques as are generally known to practitioners. Suitable carriers for pharmaceutical compositions include any material which, when combined with a bispecific antibody of the invention, retains the molecule's activity and is non-reactive with the patient's immune system. A pharmaceutical composition of the present invention comprises a bispecific antibody and one or more pharmaceutically acceptable carriers, diluents, or excipients.

A pharmaceutical composition comprising a bispecific antibody of the present invention can be administered to a patient at risk for or exhibiting diseases or disorders as described herein using standard administration techniques.

A pharmaceutical composition of the invention contains an effective amount of a bispecific antibody of the invention. An effective amount refers to an amount necessary (at dosages and for periods of time and for the means of administration) to achieve the desired therapeutic result. An effective amount of the bispecific antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion(s) to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effect of the bispecific antibody is outweighed by the therapeutically beneficial effects.

Examples

Except as noted otherwise, the exemplified engineered bispecific antibody referred to throughout the Examples refers to the exemplified bispecific antibody of the present invention presented in Table 1 above.

Bispecific Antibody Expression and Purification

The exemplified bispecific antibody is expressed and purified essentially as follows. A glutamine synthetase (GS) expression vector containing the DNA of SEQ ID NO:3 (encoding the exemplified first polypeptide chain of SEQ ID NO:1) and SEQ ID NO:4 (encoding the exemplified second polypeptide chain of SEQ ID NO:2) is used to transfect a Chinese hamster cell line (CHO, GS knockout clone 1D3), by electroporation. The expression vector encodes a SV Early (Simian Virus 40E) promoter and the gene for GS. Expression of GS allows for the biochemical synthesis of glutamine, an amino acid required by the CHO cells. Post-transfection, cells undergo bulk selection with 50μM L-

-16-

methionine sulfoximine (MSX). The inhibition of GS by MSX is utilized to increase the stringency of selection. Cells with integration of the expression vector cDNA into transcriptionally active regions of the host cell genome can be selected against CHO wild type cells, which express an endogenous level of GS. Transfected pools are plated at low density to allow for close-to-clonal outgrowth of stable expressing cells. The masterwells are screened for bispecific antibody expression and then scaled up in serum-free, suspension cultures to be used for production. Clarified medium, into which the exemplified bispecific antibody has been secreted, is applied to a Protein A affinity column that has been equilibrated with a compatible buffer such as phosphate buffered saline (pH 7.4). The column is washed to remove nonspecific binding components. The bound bispecific antibody is eluted, for example, by pH gradient (such as 0.1 M sodium phosphate buffer pH 6.8 to 0.1 M sodium citrate buffer pH 2.5) and neutralized with Tris, pH 8 buffer. Bispecific antibody fractions are detected, such as by SDS-PAGE or analytical size-exclusion, and then are pooled. Soluble aggregate and multimers may be effectively removed by common techniques including size exclusion, hydrophobic interaction, ion exchange, or hydroxyapatite chromatography. The bispecific antibody is concentrated and/or sterile filtered using common techniques. The purity of the exemplified bispecific antibody after these chromatography steps is greater than 98% (monomer). The bispecific antibody may be immediately frozen at -70°C or stored at 4°C for several months.

Bispecific Antibody Binding Affinity to CD20

Binding affinity and stoichiometry of the exemplified bispecific antibody to human CD20 is measured by Meso Scale Discovery based solution equilibrium titration method (MSD-SET) using a MSD SI6000 instrument (Meso Scale Discovery, Rockville, MD). Multi-array 96-well assay plates (Meso Scale Discovery, p/n. L15XA-3) are coated with 0.5 µg/mL of a goat anti-human Fc capture antibody (Jackson ImmunoResearch, p/n. 109-005-098) in PBS and are incubated overnight at 4°C. Following incubation, the plates are washed with PBS and then blocked using 3% Blocker A solution (Meso Scale Discovery, p/n. R93AA-1) for 60 minutes at room temperature. The plates are then washed again with PBS.

-17-

Exemplified bispecific antibody samples are prepared in duplicate at one of 500 pM or 25 pM. Fixed WIL2-S cells (human B-lymphoma cells that express CD20, ATCC CRL-8885) are pelleted by centrifugation (500 x G for 4 minutes at 10°C), the supernatant is discarded and the pellet is resuspended in 3% Blocker A solution at a concentration of 400 million cells/mL. Cells are serially diluted (using 3-fold dilutions) in a V-bottom 96-well plate with 3% Blocker A solution down to 7000 cells/mL (creating a total of 11 cellular concentrations for each bispecific antibody concentration replicate). A blank (no cells/mL) sample is also created for inclusion in the titration curve (creating a 12th concentration for each replicate).

Exemplified bispecific antibody (diluted in 3% Blocker A solution to either 1 nM or 50 pM) is mixed, at a 1:1 ratio, with each of the 11 different cellular concentrations and blank of each replicate (final concentration for exemplified bispecific antibody is 500nM or 25pM; final concentration of WIL2-S cells is approx. 200 million cells/mL to approx. 3500 cells/mL). Mixtures are incubated, on a plate shaker, overnight at 37°C and then pelleted by centrifugation (500 x G for 4 minutes at 10°C). Following centrifugation, 50µL is added to wells of the MSD multi-array 96-well assay plates previously coated with goat anti-human Fc capture antibody and incubated for 60 minutes at room temperature on a plate shaker. Plates are then washed with PBS. Thereafter, 25 µL of 1 µg/mL SULFO-TAG conjugated anti-human/NHP kappa light chain secondary antibody (Meso Scale Discovery, p/n. D20TF-6) in 1% Blocker A solution is added to each well. Plates are then incubated, on a plate shaker, at room temperature for 60 minutes and are then washed with PBS three times. 1X Read Buffer T (Meso Scale Discovery, p/n. R92TC-1) is added to each well and plates are read on a MSDSI6000 plate reader (Meso Scale Discovery, Rockville MD). Data is exported to the KinExA Pro Software (Sapidyne Instruments, Inc., Boise ID) and results are fit to the “Affinity, Standard Constant Partner (Calculate TCM)” model to determine K_D.

The above assay is also performed in the presence of saturating amounts of human BAFF, where the concentration of exemplified bispecific antibody (after mixing with the 11 different cellular concentrations) is held at one of 100 pM or 2 nM and human BAFF is added in 10-fold molar excess (1 nM and 20 nM trimer concentrations, respectively).

The binding affinity of the exemplified bispecific antibody to human CD20 (not in presence of saturating human BAFF) (N=3), determined substantially as described herein,

-18-

is $K_D = 239 \pm 68 \text{ pM}$ (Avg. $\pm$ SD). The binding affinity of the exemplified bispecific antibody to human CD20 (in presence of saturating human BAFF) (N=3), determined substantially as described herein, is $K_D = 305 \pm 133 \text{ pM}$ (Avg. $\pm$ SD). These results demonstrate that the exemplified bispecific antibody of the present invention produces a binding response (dependent upon the concentration of bispecific antibody) with human CD20 in the presence or absence of saturating concentrations of recombinant human BAFF.

Bispecific Antibody Binding Affinity to BAFF

Binding affinity and stoichiometry of the exemplified bispecific antibody to human BAFF is measured by MSD-SET using purified recombinant human soluble BAFF (expressed from HEK293 cells). Purified recombinant BAFF is biotinylated using EZ-Link Sulfo-NHS-LC biotin reagent (Thermo Scientific, p/n. 21335). In vitro Biacore assays (data not shown), demonstrate that native and biotinylated recombinant BAFF display similar binding properties.

Multi-array 96-well assay plates (Meso Scale Discovery, p/n. L15XA-3) are coated with $3.5 \mu\text{g/mL}$ of bispecific antibody and incubated overnight at 4°C . Following incubation, the plates are washed three times with PBS, then blocked using 3% Blocker A solution for 45 minutes at room temperature. The plates are then washed again with PBS.

Exemplified bispecific antibody, starting at a concentration of 224 pM , is serially diluted (using 2-fold dilutions) in a V-bottom 96-well plate creating 12 different bispecific antibody concentrations. 7 pM of biotinylated recombinant BAFF is mixed with each dilution of exemplified bispecific antibody and then incubated at 37°C for 36-48 hours (to reach equilibrium). Equilibrated samples are added to a well of the MSD multi-array 96-well assay plate previously coated with exemplified bispecific antibody; samples are mixed for 2.5 minutes, and then washed three times with PBS. Thereafter, $25 \mu\text{L}$ of $1 \mu\text{g/mL}$ SULFO-TAG labeled streptavidin detection reagent (Meso Scale Discovery, p/n. R32AD-5) in 1% Blocker A solution is added to each well. Plates are then incubated, on a plate shaker, at room temperature for 3 minutes and then washed with PBS. 1X Read Buffer T (Meso Scale Discovery, p/n. R92TC-1) is added to each well and plates are read on a MSDSI6000 plate reader (Meso Scale Discovery, Rockville MD). Resulting binding signal reflects the amount of free antigen in solution. Data is

-19-

exported to the KinExA Pro Software and results are fit to a “Standard, Affinity” binding model to determine K_D .

The binding affinity of the exemplified bispecific antibody to recombinant human BAFF (N=3), determined substantially as described herein, is $K_D = 0.3 \pm 0.4 \mu\text{M}$ (Avg. $\pm$ SD). These results demonstrate that the exemplified bispecific antibody of the present invention produces a binding response (dependent upon the concentration of bispecific antibody) with human BAFF.

Simultaneous Binding of CD20 and BAFF

Flow cytometry is used to determine whether the exemplified bispecific antibody binds CD20 and BAFF simultaneously. Exemplified bispecific antibody and a control human IgG1 antibody are each fluorescently labeled with AlexaFluor488 (Life Technologies, p/n. A10468) according to manufacturer instructions. Recombinant human BAFF is biotinylated and serially diluted (using 5-fold dilutions) from 5000ng to 0.32 ng.

The JY cell line is an Epstein-Barr virus immortalized human B cell lymphoblastoid line that expresses CD20 on the cell surface (ATCC 77442). JY cells are incubated for 30 minutes at 4°C with each dilution of biotinylated BAFF and one of: a.) 1 μg of labeled exemplified bispecific antibody; or b.) 1 μg labeled control IgG1 antibody. Following incubation, cells are washed twice with FACS buffer (2% fetal bovine serum in PBS) and resuspended in FACS buffer for analysis. Both CD20 and BAFF binding is detected simultaneously via flow cytometry (Beckman Coulter FC500). CD20 binding is detected in the FL-1 (FITC) channel and BAFF binding is detected in the FL-2 (PE) channel. FACS data is analyzed by FCS Express 4 (De Novo Software). Results are presented in Table 2.

Table 2: Binding signal (Mean Fluorescence Intensity, MFI) of exemplified bispecific antibody to human CD20 and human BAFF in simultaneous binding assay.

Biotinylated BAFF concentration (ng)	Exemplified Bispecific Antibody		Control IgG1 Antibody	
	(MFI) BAFF	(MFI) CD20	(MFI) BAFF	(MFI) CD20
5000	443.73	501.58	1.26	8.5

-20-

1000	30.76	916.18	1.3	9.14
200	5.42	895	1.43	18.02
0.32	1.43	1363.73	1.31	9.8

The results of Table 2 demonstrate that the exemplified bispecific antibody of the present invention is able to bind human CD20 on a cell surface and soluble BAFF, even at saturating concentrations of BAFF.

5

Neutralization of Soluble and Membrane-Bound BAFF-Induced Proliferation of T1165.17 Cells *in Vitro*

T1165.17 cells are a BAFF-responsive murine cell line subcloned from T1165 murine plasmacytoma (derived from ascites tumors of BALB/c AnPt mice). Studies have shown
10 T1165.17 cells require low levels of IL-1 β , although in short-term proliferation assays other cytokines have been shown capable of substituting for IL-1 β .

Soluble human BAFF (5 ng/mL) or membrane-bound human BAFF (18.1 ng/mL) are added to wells of a 96-well plate in triplicate. One of: a.) exemplified bispecific antibody; b.) parental BAFF antibody (such as described in U.S. Pat. No. 7,317,089); and
15 c.) negative control antibody (IgG1 isotype antibody), is added to wells containing either soluble BAFF or membrane-bound BAFF at each of the following concentrations: 1.667, 0.556, 0.185, 0.062, 0.021, 0.007, and 0.002 (nM). T1165.17 cells are washed with serum free RPMI 1640 medium three times, then resuspended in assay medium containing RPMI 1640, 10% FBS, 1 mM sodium pyruvate, 5x10⁻⁵ M 2-mercaptoethanol
20 and an antibiotic/antimycotic (Gibco, p/n. 15240). The T1165.17 cells are added to each well at a concentration of 5000 cells/well and the plate is incubated for 44 hours at 5% CO₂ at 37°C. Following incubation, MTS Viability Substrate (Promega, p/n. G3580) is added to each well and the plate is incubated for an additional hour before an absorbance measurement at 490 nm on a microplate reader (Molecular Devices) is taken. Testing is
25 carried out in triplicate.

Table 3a: Neutralization of soluble BAFF *in vitro* (compared to parental BAFF antibody and negative control antibody).

	% Inhibition (Mean $\pm$ Std. Dev; N=3)		
Ab Concentration	Exemplified	Parental BAFF	Negative Control

-21-

(nM)	Bispecific Ab	Ab	Ab
1.667	97.96 ± 1.00	97.88 ± 1.99	-0.03 ± 3.40
0.556	99.20 ± 0.40	97.70 ± 4.96	-0.42 ± 2.94
0.185	99.08 ± 0.99	96.86 ± 1.73	-0.23 ± 5.86
0.062	96.97 ± 1.38	53.14 ± 10.58	1.83 ± 3.05
0.021	28.05 ± 1.87	-1.36 ± 5.14	2.08 ± 0.76
0.007	9.50 ± 2.10	-0.85 ± 3.53	3.50 ± 0.34
0.002	2.95 ± 1.86	1.52 ± 5.61	4.19 ± 1.77

Table 3b: Neutralization of membrane-bound BAFF *in vitro* (compared to parental BAFF antibody and negative control antibody).

	% Inhibition (Mean ± Std. Dev; N=3)		
Ab Concentration (nM)	Exemplified Bispecific Ab	Parental BAFF Ab	Negative Control Ab
1.667	100.94 ± 1.21	103.71 ± 0.61	0.87 ± 1.31
0.556	101.49 ± 0.44	103.93 ± 0.85	7.68 ± 4.26
0.185	98.49 ± 0.74	84.08 ± 3.07	1.12 ± 2.03
0.062	49.67 ± 1.74	18.06 ± 4.46	1.04 ± 2.16
0.021	11.48 ± 2.00	8.88 ± 3.67	1.98 ± 3.85
0.007	2.68 ± 3.21	7.99 ± 3.14	6.30 ± 9.33
0.002	1.98 ± 4.00	6.50 ± 4.41	4.13 ± 0.96

5 The results provided in Tables 3a and 3b demonstrate that the exemplified bispecific antibody of the present invention is able to completely neutralize (soluble and membrane-bound) BAFF-induced proliferation of T1165.17 cells. This neutralization is improved over the positive control antibody (parental BAFF antibody). Negative control IgG1 antibody does not demonstrate inhibition of either soluble or membrane-bound

10 BAFF-induced proliferation of the T1165.17 cells.

In Vitro Analysis of Complement Dependent Cellular Cytotoxicity (CDC) Activity

Exemplified bispecific antibody and parental CD20 antibody (such as described in U.S. Pat. No. 8,153,125) are each respectively serially diluted (using 2-fold dilutions)

15 from 10 µg/mL to 0.01 µg/mL. In order to assess if saturating amount of BAFF, when

-22-

bound to the scFv portion of the exemplified bispecific antibody, impacts the CDC activity of the exemplified bispecific antibody each dilution is replicated in the presence and absence of saturating amounts (100 ng/mL) of soluble human BAFF trimer. Each dilution of both the exemplified bispecific antibody and parental CD20 antibody (with and without soluble human BAFF trimer) are added to WILS-2 B lymphoma cells (which express transmembrane CD20) plated on 96 well plates at a concentration of 40,000 cells/well and plates are incubated for 30 minutes at 37°C. Thereafter, purified human serum complement (Quidel, p/n. A133) at a dilution of 1:15 is added to each well and plates are incubated for 2 hours at 37°C. Following incubation, Alamar Blue (Biosource, p/n. DAL1100) is added to each well and plates are incubated overnight at 37°C. Relative Fluorescence Units (reflecting quantity of viable cells per well) is recorded at 540 nm excitation and 590 nm emission using a Molecular Devices Spectra Max Gemini EM Reader. Collected data is modeled to a 4-parameter fit sigmoidal dose response curve to calculate EC₅₀ values. Resulting EC₅₀ values are presented in Table 4.

Table 4: In Vitro Analysis of Complement Dependent Cellular Cytotoxicity in the Presence or Absence of Saturating BAFF.

Measurement Model	EC ₅₀ (nM)	
	Exemplified Bispecific Ab	Parental CD20 Ab
WIL2-S	0.94 ± 0.04 (N=2)	0.83 ± 0.05 (N=2)
WIL2-S + human BAFF	1.17 ± 0.06 (N=2)	0.83 ± 0.07 (N=2)

The results provided in Table 4 demonstrate that the exemplified bispecific antibody of the present invention possesses CDC activity in the presence or absence of saturating amounts of soluble human BAFF, reducing the population of viable WIL2-S cells per well in a concentration dependent manner. The observed CDC activity of exemplified bispecific antibody is comparable to the parental CD20 antibody. The bispecific antibody of the present invention demonstrates effective CDC activity even with simultaneous engagement of BAFF.

-23-

In Vitro Analysis of Antibody Dependent Cellular Cytotoxicity (ADCC) Activity

WIL2-S B lymphoma cells (which express human CD20), and human Jurkat T cells (ATCC #TIB-152) transduced with retroviral vectors to co-express NFAT-Luciferase Report Gene, gamma chain, and either a FcγIIIa (F158) receptor or a FcγIIIa (V158) receptor, are used to analyze ADCC activity of the bispecific antibody of the present invention. WIL2-S cells, at a concentration of 40,000 cells/well, are incubated for 1 hour at 37°C with either exemplified bispecific antibody or parental CD20 antibody (such as described in U.S. Pat. No. 8,153,125), both of which have been serially diluted (using 3- fold dilutions) from 150 ng/mL to 0.07 ng/mL. Following incubation (1 hour at 37°C) the transfected Jurkat T cells are added to the wells at a concentration of 200,000 cells/well and the plates are incubated for 3 hours at 37°C.

Luciferase reporter signal is generated by adding 100 μl of Steady Glo Luciferase substrate (Promega, p/n. E2520) to each well and mixing for 15 minutes. Reporter luminescent signal, indicating increased FcγIIIa receptor engagement (translating to increased ADCC activity) is measured using a SPECTRAMax LMaxII (Molecular Devices) and modeled to a 4-parameter fit sigmoidal dose response curve to calculate EC₅₀ values. Resulting EC₅₀ values are presented in Table 5 below.

Table 5: In Vitro Analysis of ADCC Activity.

Measurement Model	<u>EC₅₀ (pM)</u>	
	Exemplified Bispecific Ab	Parental CD20 Ab
WIL2-S + Jurkat (V158)	4.61 ± 0.34 (N=2)	5.05 ± 1.27 (N=2)
WIL2-S + Jurkat (F158)	31.35 ± 3.74 (N=2)	55.12 ± 5.90 (N=2)

The results provided in Table 5 demonstrate exemplified bispecific antibody of the present invention engages FcγIIIa receptors in a concentration dependent manner. The observed ADCC activity of exemplified bispecific antibody is comparable to the

-24-

parental CD20 antibody. The exemplified bispecific antibody of the present invention demonstrates effective ADCC activity.

In order to assess if BAFF, when bound to the scFv portion of the bispecific antibody of the present invention, impacts the FcγIIIa receptor engagement (and ADCC) activity of the bispecific antibody of the present invention, the above experiment is reproduced (in duplicate) in the presence and absence of 1/3 serially diluted concentrations of soluble human BAFF trimer (from a starting concentration of 100 ng/mL). Parental CD20 antibody is tested in the absence of soluble human BAFF and at this highest starting concentration (100 ng/mL) of soluble human BAFF. Jurkat T cells transduced to co-express NFAT-Luciferase Report Gene and FcγIIIa (V158) receptor (as described above) are used. Resulting EC₅₀ data is presented in Table 6 below.

Table 6: In Vitro Analysis of ADCC Activity in the Presence of Saturating BAFF.

Measurement Model [WIL2-S + Jurkat (V158)]	Exemplified Bispecific Ab EC ₅₀	Parental CD20 Ab EC ₅₀
no BAFF	4.07 ± 0.64 (N=2)	5.91 ± 1.21 (N=2)
0.14 ng/mL BAFF	4.71 ± 0.40 (N=2)	N/A
0.41 ng/mL BAFF	5.15 ± 0.59 (N=2)	N/A
1.23 ng/mL BAFF	7.68 ± 1.09 (N=2)	N/A
3.7 ng/mL BAFF	11.61 ± 1.29 (N=2)	N/A
11.11 ng/mL BAFF	15.23 ± 2.19 (N=2)	N/A
33.33 ng/mL BAFF	16.08 ± 2.04 (N=2)	N/A
100 ng/mL BAFF	15.81 ± 1.16 (N=2)	6.83 ± 1.22 (N=2)

The results provided in Table 6 demonstrate exemplified bispecific antibody of the present invention engages FcγIIIa receptors, even in the presence of BAFF. As shown

-25-

in Table 6, exemplified bispecific antibody of the present invention experiences a slight reduction in FcγIIIa receptor engagement under saturating conditions of BAFF. The observed ADCC activity of exemplified bispecific antibody is slightly reduced compared to the parental CD20 antibody in the presence of saturating conditions of BAFF. The exemplified bispecific antibody of the present invention demonstrates effective ADCC activity during simultaneous engagement of BAFF.

In Vivo Analysis of BAFF Neutralization

Neutralization of human BAFF, in vivo, is assessed using transgenic FVB mice (FVB/N-TgN (LP40) 16989) which express human soluble BAFF. Human BAFF transgenic (Tg) mice are subcutaneously injected with 3.3 nmoles of: a.) exemplified bispecific antibody (N=5); b.) parental BAFF antibody (N=5); c.) human IgG4 negative control antibody (N=5); d.) parental CD20 antibody (N=5); or e.) untreated (naive mice, N=5). After eight days the mice are sacrificed and plasma and spleens are collected. Exposure of each antibody is confirmed by ELISA of plasma specific for human IgG.

A single cell suspension of spleen cells is prepared and the total number of leukocytes is determined following red blood cell lysis. The relative percentage of B lymphocytes is determined using cell surface marker B220 and flow cytometry, whereby the total number of B cells per spleen is able to be calculated by multiplying the percentage of B220 positive cells with the total number of leukocytes in the spleen. Results are presented in Table 7 below.

Table 7: B220 Positive Cells in Spleens of Treated Human BAFF Transgenic Mice (Mean + Std. Dev.).

	Naïve (untreated) mice	Pos. Control Ab (parental BAFF Ab)	Exemplified Bispecific Ab	Neg. Control (parental CD20 Ab)	Neg. Control (IgG4 Ab)
B220+ Cells/ spleen (x10 ⁶)	176.46 ± 19.73	76.09 ± 24.16	52.98 ± 11.57	179.18 ± 31.61	152.53 ± 9.93

The results provided in Table 7 reflect a reduction in the total number of B cells in spleens of human BAFF Tg mice treated with a single subcutaneous injection of exemplified bispecific antibody as compared to naïve, negative control, and parental

-26-

CD20 antibody treated transgenic mice. This reduction of B cells is equivalent to that observed with the parental BAFF antibody. The exemplified bispecific antibody of the present invention demonstrates effective in vivo neutralization of BAFF.

5 In Vivo Efficacy Models

The exemplified bispecific antibody of the present invention does not bind murine CD20 and has greatly reduced binding to murine BAFF (approximately a 250 fold reduction in binding to murine BAFF as compared to human BAFF). Additionally, a surrogate murine bispecific antibody was not able to be produced. Therefore, separate
 10 murine antibodies (one directed to murine CD20 and one directed to murine BAFF) are used for testing the therapeutic potential of dual targeting CD20 and BAFF in a rodent model.

Lupus-like autoimmunity is induced in host mice (female C57BL/6xDBA/2 F1 mice, Charles River Laboratories) via transfer of 14×10^6 CD4⁺ splenocytes from donor
 15 mice (7 to 8 week old female DBA/2 mice, Charles River Laboratories). CD4⁺ splenocytes from the donor mice are transferred into the tail vein of the host mice as described by Via CS and Shearer GM, Ann. NY Acad. Sci., 532: 44-50 (1988). Upon transfer, donor CD4⁺ splenocytes provide cognate help to host B cells, resulting in proliferation/expansion of host B cells, including autoreactive host B cells.

20 In order to explore B cell depletion in the context of an ongoing immune response, CD4⁺ splenocytes are transferred from donor mice to host mice. Seven days post-transfer host mice are tested via ELISA for anti-dsDNA autoantibodies (as described below) to confirm initiation of disease. On day 7 post-transfer, host mice are also treated subcutaneously according to one of four treatment groups:

- 25 (a) 65 μ g murine CD20 antibody + 65 μ g mIgG1 isotype control antibody (N=4);
 (b) 65 μ g murine BAFF antibody + 65 μ g mIgG1 isotype control antibody (N=4);
 (c) 65 μ g murine CD20 antibody + 65 μ g BAFF antibody (N=4); or
 (d) 130 μ g mIgG1 isotype control antibody (N=4).

Host mice are sacrificed 7 days post-antibody treatment and B220⁺ cell values in
 30 blood, lymph node, and spleen are determined by flow cytometry as described above (in relation to splenic B cell levels of treated transgenic BAFF mice). Results are presented in Table 8 below.

-27-

Table 8: B220+ Cells in Blood, Lymph Node, and Spleens of Treated Host Mice (Mean + Std. Dev.).

	Naïve Mice	(a) CD20 Ab + Control Ab	(b) BAFF Ab + Control Ab	(c) CD20 Ab + BAFF Ab	(d) Neg. Control (mIgG1 Ab)
B220+ Cells / μ l blood	5554 $\pm$ 1264	604 $\pm$ 220	3983 $\pm$ 1524	468 $\pm$ 124	3057 $\pm$ 1071
B220+ Cells ($\times 10^6$) / lymph node	0.59 $\pm$ 0.24	0.92 $\pm$ 0.39	1.91 $\pm$ 0.33	0.26 $\pm$ 0.04	4.40 $\pm$ 0.90
B220+ Cells ($\times 10^6$) / spleen	21.91 $\pm$ 8.79	21.94 $\pm$ 10.89	32.93 $\pm$ 6.61	5.97 $\pm$ 4.65	52.12 $\pm$ 18.55

5

The results provided in Table 8 demonstrate the total number of B cells in blood, lymph node, and spleen of host mice treated with a single subcutaneous injection of the murine CD20 antibody and murine BAFF antibody is significantly reduced over negative control treated mice. The level of B cells in host mice treated with murine CD20 antibody and murine BAFF antibody is reduced to levels below that of naïve mice and mice treated with only one of murine CD20 antibody or murine BAFF antibody alone.

10

In a 7 week study, CD4+ splenocytes are transferred from donor mice to host mice, and on the day of splenocyte transfer host mice are treated subcutaneously according to one of four treatment groups:

15

- (a) 65 μ g murine CD20 antibody + 65 μ g mIgG1 isotype control antibody (N=10);
- (b) 65 μ g murine BAFF antibody + 65 μ g mIgG1 isotype control antibody (N=10);
- (c) 65 μ g murine CD20 antibody + 65 μ g murine BAFF antibody (N=10); or
- (d) 130 μ g mIgG1 isotype control antibody (N=10).

20

Host mice are sacrificed 7 weeks post treatment and anti-dsDNA autoantibody presence and urine albumin/creatinine values are measured as described in detail below.

25

Anti-dsDNA autoantibody presence in host mice plasma samples is determined by ELISA. Greiner 96-well microtiter plates (Belco Glass, p/n. 655 061) are coated with 10 μ g/mL of Trevigen (R&D Systems, p/n. 9600-5D) calf thymus DNA in Dulbecco's phosphate buffered saline (D-PBS, no Mg, no Ca) and incubated overnight at 4°C. Following incubation, plates are blocked with D-PBS containing 2% bovine serum

-28-

albumin (BSA) for 1 hour at room temperature (RT) then washed with 0.05 % Tween 20 in PBS.

Plasma from host mice is collected on the day of sacrifice and plasma samples are diluted with 0.05 % Tween 20 in PBS plus 2% BSA to a starting dilution of 1:100. Six 4-fold serial dilutions, from the starting 1:100 plasma dilution, are prepared for each plasma sample collected. Diluted plasma samples are added to individual wells of the plate and plates are incubated for 2 hours at room temperature, washed with 0.05 % Tween 20 in PBS, and then incubated for 90 minutes with goat anti-mouse IgG (H and L)-HRP (Jackson ImmunoResearch, p/n. 115-035-166) diluted to a 1:2000 dilution with 0.05 % Tween 20 in PBS plus 2% BSA. Thereafter, TMB substrate (Thermo Scientific, p/n. 34021) is added to each well. Color development is stopped with 0.5 M H₂SO₄. Colorimetric signal is read using a spectrophotometer at A450 (SpectraMax, Molecular Devices) and collected data is analyzed using a GraphPad Prism for determining anti-DNA titer (EC₅₀) values. Anti-dsDNA titer values are presented in Table 9.

15

Table 9: Anti-dsDNA Titer Values (Mean + Std. Dev.).

	Naïve Mice	(a) CD20 Ab + Control Ab	(b) BAFF Ab + Control Ab	(c) CD20 Ab + BAFF Ab	(d) mIgG1 Control Ab
Anti-dsDNA titer (EC₅₀)	90 ± 44	3633 ± 4622	3160 ± 3535	35 ± 58	2692 ± 1780

The results provided in Table 9 demonstrate upon treatment with both murine CD20 antibody and murine BAFF antibody, anti-DNA titers are significantly lower than treatments with CD20 antibody alone or BAFF antibody alone.

Albumin/Creatinine ratios are a useful metric for assessing renal involvement relating to the progression of SLE. Albumin/Creatinine ratio values are determined by measuring both albumin (µg/mL) and creatinine (mg/dL) values, individually, in host mice urine according to the equation: [(urine albumin (µg/mL) / urine creatinine (mg/dL)) x 100 = Albumin Creatinine Ratio (µg/mg)]. Urine creatinine levels are measured with a Cobas CREA Plus assay (Roche, p/n. 11775685) according to manufacturer instructions.

25

-29-

Urine albumin levels are determined by sandwich ELISA. Microtiter plates (Greiner Bio-One, p/n. 655061) are coated with 60 μ l of rabbit, anti-mouse albumin antibody (Acris Antibodies, p/n. AP09221PU-N) at a concentration of 0.75 μ g/mL (diluted in PBS), then washed with washing buffer (20 mM Tris, 0.15 M NaCl, 0.1% Tween 20, pH 7.4) and blocked using blocking buffer (casein in PBS, Pierce Protein Biology Products, p/n. 37528). Urine samples are prepared by 1:2000 dilution and 1:80,000 dilution with blocking buffer (Pierce, p/n. 37528). A standard curve is generated by serially diluting a 200 ng/mL concentration of mouse albumin (Innovative Research, p/n. IMSA) down to 0.78 ng/mL using 1:2 dilutions of blocking buffer (Pierce, p/n. 37528). Standard curve serial dilutions (50 μ L) and sample dilutions (50 μ L) are added to individual wells of the plate in duplicate and incubated at room temperature for 1.5 hours. Thereafter, plates are washed with washing buffer, and then incubated at room temperature for 1 hour with 50 μ L of a 1:3000 dilution of goat, anti-mouse albumin-HRP (Bethyl, p/n. A90-234P). Plates are washed with washing buffer, and then developed by addition of 50 μ l of OPD substrate (Sigma, p/n. P-6912) to each well. The colorimetric reaction is stopped by addition of 100 μ L of 1 N HCl. Colorimetric signal is measured at 490 nm using a plate reader (SpectraMax, Molecular Devices) and collected data is analyzed using SoftMax 3.1.2 software and a 4 parameter fit to back calculate sample concentrations. Albumin Creatinine Ratio values are presented in Table 10.

20

Table 10: Albumin Creatinine Ratio Values (Mean $\pm$ Std. Dev.).

	Naïve Mice	(a) CD20 Ab + Control Ab	(b) BAFF Ab + Control Ab	(c) CD20 Ab + BAFF Ab	(d) mIgG1 Control Ab
ACR (μg/mg)	9 $\pm$ 2	47211 $\pm$ 40805	26881 $\pm$ 36169	46 $\pm$ 113	14238 $\pm$ 12153

The results provided in Table 10 demonstrate a significant reduction in Albumin Creatinine Ratio of mice treated with both murine CD20 antibody and murine BAFF antibody, as opposed to mice treated with murine CD20 antibody alone or murine BAFF antibody alone.

30

-30-

Pharmacokinetic Assessment in Cynomolgus Monkey

The amino acid sequence of cynomolgus monkey shares 96.6% identify with the amino acid sequence of human CD20. Flow cytometry of cynomolgus monkey whole blood is used to confirm binding of the exemplified antibody to cynomolgus monkey

5 CD20.

Whole blood (50 μ L) collected from cynomolgus monkey is incubated at room temperature for 30 minutes with either: (a) exemplified bispecific antibody (1.5 μ g) (labeled with AlexaFluor647) and positive control antibody (1.5 μ g)(FITC labelled CD40 antibody reactive with cynomolgus monkey from BioLegend, p/n. 334306); or (b) human

10 IgG1 isotype negative control antibody (1.5 μ g)(labeled with AlexaFluor647) and positive control antibody (1.5 μ g)(FITC labelled CD40 antibody reactive with cynomolgus monkey from BioLegend, p/n. 334306). Following incubation, red blood cells are lysed by addition of FACS Lysing Solution according to the manufacturer's instructions (Becton Dickinson & Co., p/n. 349202). Cells are washed three times with FACS buffer

15 (PBS containing 2% fetal bovine serum), then resuspended in FACS buffer prior to flow cytometry using Beckman Coulter FC500. CD20 binding is detected in the FL-4 (647) channel and CD40 binding is detected in the FL-1 (FITC) channel. Results are presented in Table 11 below.

20 Table 11: Binding of Exemplified Antibody to CD40+ Cynomolgus Monkey B Cells.

	CD20 AlexaFluor647 (MFI)	CD40 FITC (MFI)
Exemplified Bispecific Ab + Pos. Control (CD40 Ab)	588.21	139.49
Neg. Control (human IgG1 Isotype Ab) + Pos. Control (CD40 Ab)	171.88	148.55

The results provided in Table 11 demonstrate the exemplified bispecific antibody of the present invention binds cynomolgus monkey CD20 of B cells, whereas the human

25 IgG1 negative control antibody does not bind the cynomolgus monkey B cells. These results support the use of cynomolgus monkey for in vivo testing of the bispecific antibody of the present invention.

-31-

In order to assess serum pharmacokinetics of the exemplified bispecific antibody, male cynomolgus monkeys are administered 13.3 mg/kg of exemplified bispecific antibody either intravenously (N=2) or subcutaneously (N=2). Exemplified bispecific antibody is prepared in solution of PBS, pH 7.4. Pharmacokinetic studies with a
5 bispecific antibody comprising parental CD20 antibody and parental BAFF were not possible due to the physical and chemical instability (described herein) preventing expression and recovery.

Prior to administration, approximately 1.5mL of blood is collected from each cynomolgus monkey. Post administration, blood samples (approximately 1.5mL) are
10 collected intravenously from a femoral vein into tubes serum separator tubes (e.g., containing no anticoagulant) at 1, 6, 12, 24, 48, 72, 96, 168, 240, 336, 432, 504, 600, and 672 hours post administration. Upon collection at the respective time points, samples are processed to serum.

Clearance of the exemplified bispecific antibody is determined by analysis of
15 serum samples by total human IgG ELISA utilizing AffiniPure F(ab')₂ Fragment Goat Anti-Human IgG, F(ab')₂ fragment specific (Jackson ImmunoResearch Laboratories, Inc., p/n. 109-006-097) coated on ELISA plates (Thermo Scientific™ Immulon® 4HBX). Serum samples (100μL), from each respective time point, are added to individual wells of ELISA plates and incubated at 25°C for 60 mins. Following incubation, 100μL (50,000
20 fold dilution) mouse anti-human IgG Fc-HRP (Southern Biotech, p/n. 9040-05) is added to wells of ELISA plate for detection of exemplified bispecific antibody. Unbound enzyme is removed via washing and 100μL TMB Microwell Peroxidase Substrate System (KPL, p/n.50-76-00) is added to individual wells of ELISA plate. Color development is stopped by addition of 100μL TMB Stop Solution (KPL, p/n.50-85-05) and optical
25 density of the wells is measured at 450 nm with wavelength correction set to 630 nm.

A standard curve of known amounts of exemplified bispecific antibody, in 100% cynomolgus monkey serum (BioreclamationIVT, p/n. CYN SRM), for use in
determination of clearance values is also generated. Exemplified bispecific antibody, in 100% cynomolgus monkey serum is diluted with 20-fold serial dilutions into blocker
30 casein in PBS (Thermo Scientific™ Blocker™, p/n. 37528). The standard curve range of exemplified bispecific antibody is 78.1 - 5000 ng/mL (with an upper and lower limit of quantification of 1900 ng/mL and 120 ng/mL, respectively).

-32-

Clearance values for the exemplified bispecific antibody are calculated using immunoreactivity versus time profile from time zero (administration of antibody) to 192 hours post administration and are determined via non-compartmental analysis using Phoenix software (WinNonLin 6.3, Connect 1.3). Exemplified bispecific antibody administered intravenously demonstrates a mean clearance of 0.83 mL/hr/kg and a mean half-life of 58 hours. When administered subcutaneously, exemplified bispecific antibody demonstrates a mean clearance of 1.22 mL/hr/kg, a mean half-life of 72 hours and bioavailability of 69%. The results demonstrate that the exemplified bispecific antibody possess sufficient half-life, clearance, and bioavailability to support subcutaneous dosing.

Stability of the exemplified bispecific antibody in cynomolgus circulation is determined based on a ratio of BAFF antigen capture values (of serum samples collected at the time points described above) to total human IgG ELISA values (calculated as described above). BAFF antigen capture utilizes BAFF antigen (Eli Lilly and Company), which is coated on ELISA plates (Thermo Scientific™ Immulon® 4HBX). Serum samples (100μL), from each respective time point described above, are added to individual wells of ELISA plates and incubated at 25°C for 60 mins. Following incubation, 100μL (10,000 fold dilution) mouse anti-human IgG Fc-HRP (Southern Biotech, p/n. 9040-05) is added to wells of ELISA plate for detection of exemplified bispecific antibody. Unbound enzyme is removed via washing, and 100μL TMB Microwell Peroxidase Substrate System (KPL, p/n. 50-76-00) is added to individual wells of ELISA plate. Color development is stopped by addition of 100μL TMB Stop Solution (KPL, p/n. 50-85-05) and optical density of the wells is measured at 450 nm with wavelength correction set to 630 nm.

Area under the curve (AUC) for both BAFF antigen capture and total human IgG ELISA are determined via non-compartmental analysis using Phoenix (WinNonLin 6.3, Connect 1.3). Mean percent area under the curve (%AUC) values are calculated as $(AUC_{0-tlast, \text{ BAFF antigen capture assay}} / AUC_{0-tlast, \text{ total human IgG assay}}) * 100\%$. The exemplified bispecific antibody demonstrates a %AUC, based on BAFF binding, of approximately 95% to 101%. The results reflect that the exemplified bispecific antibody possesses sufficient stability at both linkers L1 and L2.

-33-

Bispecific Antibody Physical and Chemical Property Analysis

Preliminary studies with a bispecific antibody comprising parental CD20 antibody and parental BAFF were not possible due to poor bispecific antibody expression, LC clipping, misfolding of scFv, poor purification recovery, bispecific antibody degradation / clipping, poor thermostability, and low affinity (relative to the parental antibodies). However, preliminary studies with the exemplified bispecific antibody of the present invention demonstrate unexpected beneficial properties, including expression of the bispecific antibody and unexpected beneficial chemical and physical stability and solubility properties. As demonstrated by the data provided herein, the bispecific antibody of the present invention exhibits low HMW aggregation, good solubility, good viscosity in solution, increased physical stability including low degradation levels and high photostability, low peptide bond cleavage, and both high and low concentration freeze thaw stability, while maintaining good affinity for both CD20 (as measured by FACS analysis described herein) and BAFF (as measured by ELISA analysis described herein), and maintaining neutralization activity of BAFF as well as ADCC activity (both analyzed as described herein).

Physical and Chemical Stability Analysis

For physical and chemical stability analysis, the exemplified bispecific antibody of Table 1 is formulated in 10 mM acetate pH 5.0, 0.02% Tween-80. Exemplified bispecific antibody samples are concentrated to 1 mg/mL, 50 mg/mL and 100 mg/mL (using Amicon U.C. filters, Millipore, p/n. UFC903024).

Sample stability, of samples concentrated to 1 mg/mL is assessed by incubating samples at one of 4, 25, and 40°C over a period of 4 weeks. Samples concentrated to 50 and 100 mg/mL are incubated in the presence and absence of 150 mM NaCl at one of 4 and 25°C over a period of 4 weeks.

Following the respective incubation periods, samples are analyzed for percent high molecular weight (%HMW) with size exclusion chromatography (SEC) using a TSK G3000SW (Tosoh Bioscience, p/n. 08541) column. 50 mM sodium phosphate + 350 mM NaCl, pH 7.0 is used as the mobile phase running at 0.5 mL/min for 35 mins. A volume of 10 μ L (10 μ g) of the 1 mg/mL antibody sample is injected into the column and the detection is measured at 214nm. For high concentration samples, a volume of 1 μ L (50 μ g

-34-

or 100 μ g) is injected into the column and the detection is measured at 280nm.

Chromatograms are analyzed using ChemStation and % high molecular weight (HMW) is calculated using the ratio of AUC of the peaks eluted before the monomer peak to total AUC. Results are summarized in Table 12.

5

Table 12: Change in % high molecular weight species from starting control over 4 weeks measured by SEC.

Sample Concentration	Incubation Temperature	Change in % of HMW
1 mg/mL	4°C	0.27
1 mg/mL	25°C	0.24
1 mg/mL	40°C	0.28
50 mg/mL	4°C	0.73
50 mg/mL	25°C	0.81
100 mg/mL	4°C	0.97
100 mg/mL	25°C	1.38

10 The results provided in Table 12 demonstrate the exemplified bispecific antibody of the present invention, formulated as described herein, achieves high protein concentrations and displays less than a 1.4% change in high molecule weight content following a four week degradation study of 25°C.

15 Sample degradation, of samples concentrated to 1 mg/mL, is also analyzed with capillary isoelectric focusing (cIEF) analysis using a Beckman-PA800 plus system according to manufacturer instructions. For samples incubated at 4°C, the percent sample degradation was measured as 0%; for samples incubated at 25°C the percent sample degradation was measured as 1.3%.

20 Samples concentrated to 1 mg/mL and stored at 40°C are also analyzed for identification of peptide bond cleavage with liquid chromatography-mass spectrometry (LC-MS) analysis using a Thermo Scientific Ion Trap LC-MS system according to manufacturer instructions. No region of the bispecific antibody demonstrated peptide bond cleavage of greater than 2% and the hinge region (of the first polypeptide chain, SEQ ID NO:1 of the exemplified bispecific antibody) demonstrated peptide bond
25 cleavage of only approx. 1%.

-35-

Solubility Analysis

Solubility of the exemplified bispecific antibody is analyzed at 4, -5 and -10°C following a one week incubation period at each temperature. Solubility is assessed with bispecific antibody samples concentrated to between 100 and 150 mg/mL (using Amicon U.C. filters, Millipore, catalog # UFC903024) and formulated in 10mM acetate at pH 5.0. The exemplified bispecific antibody exhibited solubility of at least 148 mg/mL, within acceptable values for therapeutic bispecific antibodies. The exemplified bispecific antibody also demonstrated only a 0.49% change in the level of HMW content at high concentration, and lacked phase separation following the incubation period.

Viscosity of the exemplified bispecific antibody is analyzed at room temperature. Viscosity is assessed with bispecific antibody concentrated to 100 mg/mL and formulated in 10mM acetate at pH 5.0. The exemplified bispecific antibody exhibited viscosity of 4.9cp at 100 mg/mL, an acceptable value for therapeutic bispecific antibodies.

Photostability Analysis

Photostability analysis of the exemplified bispecific antibody is assessed with bispecific antibody concentrated at 50 mg/mL and formulated in either: (a) 10 mM acetate, pH 5.0, 0.02% Tween-80; or (b) 10 mM citrate, pH 6.0, 0.02% Tween-80, 150 mM NaCl. Samples are exposed for 240,000 lux hour visible light plus 40 watt-hr/m² UV light. Control (“dark”) samples are not exposed to light. Samples are then analyzed on a size exclusion chromatography (SEC) using a TSKgel Super G3000SW column. Results are summarized in Table 13.

Table 13: Summary of change in percentage of high molecular weight species.

Sample Formulation (50 mg/mL)	Change in % of HMW compared to control samples
(a) 10 mM acetate, pH 5.0, 0.02% Tween-80	<3%
(b) 10 mM citrate, pH 6.0, 0.02% Tween-80, 150 mM NaCl	between 7 – 10%

The results provided in Table 13 demonstrate the exemplified bispecific antibody of the present invention, formulated in 10 mM acetate, pH 5.0, 0.02% Tween-80 at high

-36-

concentrations, possesses photostability comparable to other therapeutic bispecific antibodies.

High and Low Concentration Freeze/Thaw Analysis

5 Freeze thaw analysis of exemplified bispecific antibody of the present invention is assessed following three freeze/thaw cycles performed according to Table 14:

Table 14: One Cycle of a Freeze Thaw Analysis.

<u>Cycle Step</u>	1	2	3	4	5	6	7	8
Target Temp. (°C)	5	-1	-30	-70	-30	-1	0.5	15
Temp. Rate (°C/min.)	1	0.05	0.2	1	1	0.2	0.2	1
Hold (min.)	10	750	1	60	1	1000	1	1

10

Low concentration freeze/thaw analysis of the exemplified bispecific antibody is assessed with bispecific antibody concentrated at 1 mg/mL and formulated in 10 mM acetate, pH 5.0, with and without 0.02% Tween-80. Three freeze/thaw cycles (a single cycle represented in Table X) are performed and particle growth for each sample is
 15 assessed using a HIAC Particle Counter (Pacific Scientific, p/n. 9703). Particle counts for both formulations (with and without 0.02% Tween-80) were less than or equal to 100. Formulations with 0.02% Tween-80 demonstrated no difference in particle counts in comparison to control samples not subjected to freeze/thaw cycles, demonstrating the exemplified bispecific antibody of the present invention, under low concentration
 20 conditions, is stable following multiple freeze/thaw cycles.

High concentration freeze/thaw analysis of the exemplified bispecific antibody is assessed with bispecific antibody concentrated at 50 mg/mL and formulated in either: (a) 10 mM acetate, pH 5.0, 0.02% Tween-80; or (b) 10 mM citrate, pH 6.0, 0.02% Tween-80, with and without 150 mM NaCl. Three freeze/thaw cycles (a single cycle represented in
 25 Table X) are performed and particle growth for each sample is assessed using a HIAC Particle Counter. Results are provided in Table 15:

-37-

Table 15: Particle Count Following High Concentration Freeze/Thaw.

	(a) 10 mM acetate, pH 5.0, 0.02% Tween-80	(b) 10 mM citrate, pH 6.0, 0.02% Tween-80 + 150 mM NaCl - 150 mM NaCl	
0 Freeze/Thaw Cycles	118	66	90
3 Freeze Thaw Cycles	104	1728	1786

The results provided in Table 15 demonstrate the exemplified bispecific antibody
of the present invention at high concentrations and formulated in 10 mM acetate, pH 5.0,
0.02% Tween-80, is stabile following multiple freeze/thaw cycles.

Sequences

First Polypeptide of the exemplified bispecific antibody of Table 1 (SEQ ID NO:1)

10 QVQLQESGPGLVKPSSETLSLTCTVSGGSFSGHYWSWIRQPPGKCLEWIGEINHSGS
TNYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCARGARDILTGYLYYYD
HWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSC
RASQSVSRYLAWYQQKPGQAPRLLIYDASN RATGIPARFSGSGSGT DSTLTISSE
PEDFAVYYCQQR SNHPRTFGCGTKVEIKGGGGSGGGGSGGGGSEVQLVQSGAEV
15 KKPGE SLKISCKGSGRTFTSYNMHWVRQMPGKGLEWMGAIYPLTGDTSYNQKS
KLQVTISADKSISTAYLQWSSLKASDTAMY YCARSTYVGGDWQFDVWGKGTTV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
20 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
EEKTISKQKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS
LSLSPG

25 Second Polypeptide of the exemplified bispecific antibody of Table 1 (SEQ ID NO:2)

EIVLTQSPGTL SLSPGERATLSCRASSSVPIHWYQQKPGQAPRLLIYATSALASGI
PDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQWLSHPPTFGQGTKLEIKRTVAAPS

-38-

VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

DNA Seq. Encoding the First Polypeptide of the exemplified bispecific antibody of

Table 1 (SEQ ID NO:3)

5 cagggtgcagctgcaggagtcgggcccaggactggtgaagccttcggagaccctgtccctcacctgcactgtctctggaggctc
cttcagtggctcattactggagctggattcgccagcccccagggaagtgtctggagtggattggggaaatcaatcatagtgaagc
acaaactacaaccgctccctcaagagtcgagtcaccatatacagtagacacgtccaagaaccagttctccctgaaactgagctctg
tgaccgccgcggacacggctgtgtattactgtgcgagaggggctagagataatttgactggttatctatactactatgaccattggg
10 gccagggaaccctggtcaccgtctcctcagggggagggggcagcggaggaggcggatcgggcggaggagggaagtggag
gcggaggcagcgaaattgtgtgacgcagctctccagccaccctgtcttctctcctggggaaagagccaccctctcctgcaggg
ccagtcagagtgttagccgtacttagcctggtagcagaaacctggccaggctcccaggctcctcatctatgatgcaccaa
cagggccactggcatcccagccagggtcagtggcagtggtctgggacagactccactctcaccatcagcagcctagagcctg
aagattttgcagtttattactgtcagcagcgtagcaaccaccctcggacgttcggctgtgggaccaagggtggaaatcaaaggcgg
15 tgggtggcagcggcggaggcggatccggcggagggtggaagcgagggtgcagctgggtgcagctctggagcagagggtgaaaaagc
ccggggaggtctctgaagatctcctgtaagggtcttgccgtacatttacagttacaatatgcactgggtgcgccagatgccgg
gaaaggcctggagtggatgggggctatttatcccttgacgggtgatacttctacaatcagaagtcgaaactccaggtcaccatct
cagccgacaagtcacacagcaccgcctacctgcagtgaggcagcctgaaggcctcggacaccgccatgtattactgtgcgaga
tcgacttacgtgggcgggtgactggcagttcgatgtctggggcaaggggaccacgggtcaccgtctcctcagcctccaccaaggg
20 cccatcgggtcttcccgtagcaccctctccaagagcacctctggggggcacageggccctgggctgectgggtcaaggactactt
ccccgaaccgggtgacgggtgtcgtggaactcaggcgccctgaccagcggcgtgcacacctcccggtgtcctacagtcctcag
gactctactcctcagcagcgtgggtgaccgtgccctccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagc
ccagcaacaccaagggtggacaagaaagttgagcccaaatctgtgacaaaactcacacatgccaccgtgccagcacctgaa
ctcctgggggggaccgtcagttctctctccccccaaaacctaaaggacaccctcatgatctcccggaaccctgaggtcacatgcg
25 tgggtgggtggacgtgagccacgaagaccctgaggtcaagttcaactgggtacgtggacggcgtggagggtgcataatgccaagaca
aagccgcggggaggagcagtacaacagcacgtaccgtgtgggtcagcgtctcaccgtcctgcaccaggactgggtgaatggca
aggagtacaagtgcaaggctccaacaaagccctcccagcccccaggagaaaacctctccaaacagaaagggcagcccc
gagaaccacagggtgtacaccctgcccccatcccgggacgagctgaccaagaaccagggtcagcctgacctgctgtgcaagg
cttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgccecccggtgtg
30 gactccgacgggtccttctctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccg
tgatgcatgaggctctgcacaaccactacacacagaagagccctctccctgtctccgggt

-39-

DNA Seq. Encoding the Second Polypeptide of the exemplified bispecific antibody of Table 1 (SEQ ID NO:4)

gaaattgtgtgacgcagtcctcaggcaccctgtcttctccaggggaaagggccaccctctctgcagggccagctcaagtg
 taccgtacatccactggtaccagcagaaacctggccagggtcccagggtcctcatctatgccacatccgccctggcttctggcat
 5 cccagacaggttcagtggcagtggtctgggacagacttcactctcaccatcagcagactggagcctgaagatttgcagtgtatt
 actgtcagcagtggtgagtcacccacttttggccaggggaccaagctggagatcaaacgaactgtggctgcaccatctgt
 ctcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagagggc
 aaagtacagtggaaggtggataacgccctccaatcgggtaactccaggagagtgtcacagagcaggacagcaaggacagc
 acctacagcctcagcagcaccctgacgetgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatca
 10 gggcctgagctcgcctgcacaaagagcttcaacaggggagagtgc

HCVR1 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:5)

QVQLQESGPGLVKPSETLSLTCTVSGGSFSGHYWSWIRQPPGKCLEWIGEINHSGS
 TNYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCARGARDILTGYLYYYD
 15 HWGQGTLLTVSS

HCVR2 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:6)

EVQLVQSGAEVKKPGESLKISCKGSGRTFTSYNMHWVRQMPGKGLEWMGAIYP
 LTGDTSYNQKSKLQVTISADKSISTAYLQWSSLKASDTAMYYCARSTYVGGDWQ
 20 FDVWGKGTTVTSS

LCVR1 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:7)

EIVLTQSPGTLSPGERATLSCRASSVPYIHWYQQKPGQAPRLLIYATSALASGI
 PDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQWLSHPPTFGQGTKLEIK
 25

LCVR2 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:8)

EIVLTQSPATLSLSPGERATLSCRASQSVSRYLAWYQQKPGQAPRLLIYDASNRAT
 GIPARFSGSGSGTDSTLTSSLEPEDFAVYYCQQRSNHPRTFGCGTKVEIK

30 **HCDR1 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:9)**

KGSGRTFTSYNMH

-40-

HCDR2 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:10)

AIYPLTGDTSYNQKSKL

HCDR3 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:11)

5 ARSTYVGGDWQFDV

HCDR4 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:12)

TVSGGSFSGHYWS

10 HCDR5 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:13)

EINHSGSTNYNPSLKS

HCDR6 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:14)

ARGARDILTGYLYYYDH

15

LCDR1 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:15)

RASSVPYIH

LCDR2 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:16)

20 YATSALAS

LCDR3 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:17)

QQWLSHPPT

25 LCDR4 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:18)

RASQSVSRYLA

LCDR5 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:19)

YDASNRAT

30

LCDR6 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:20)

QQRSNHPRT

-41-

Polypeptide Linker 1 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:21)

GGGGSGGGGSGGGGSGGGGS

5

Polypeptide Linker 2 of the exemplified bispecific antibody of Table 1 (SEQ ID NO:22)

GGGGSGGGGSGGGGS

10

15

20

25

30

-42-

WE CLAIM:

1. A bispecific antibody comprising two first polypeptide chains and two second polypeptide chains wherein each of,
 - a.) said first polypeptide chain comprises a single chain variable fragment (scFv) and a mAb IgG heavy chain (HC), the HC having a heavy chain variable region (HCVR1) comprising heavy chain CDRs (HCDR) 1-3 wherein the amino acid sequence of HCDR1 is SEQ ID NO:9, the amino acid sequence of HCDR2 is SEQ ID NO:10, the amino acid sequence of HCDR3 is SEQ ID NO:11, and the scFv having a heavy chain variable region (HCVR2) and a light chain variable region (LCVR2), HCVR2 comprising HCDRs 4-6 and LCVR2 comprising LCDRs 4-6, wherein the amino acid sequence of HCDR4 is SEQ ID NO:12, the amino acid sequence of HCDR5 is SEQ ID NO:13, the amino acid sequence of HCDR6 is SEQ ID NO:14, the amino acid sequence of LCDR4 is SEQ ID NO:18, the amino acid sequence of LCDR5 is SEQ ID NO:19, and the amino acid sequence of LCDR6 is SEQ ID NO:20; and
 - b.) said second polypeptide comprises a mAb light chain (LC) comprising light chain CDRs (LCDR) 1-3, wherein the amino acid sequence of LCDR1 is SEQ ID NO:15, the amino acid sequence of LCDR2 is SEQ ID NO:16, the amino acid sequence of LCDR3 is SEQ ID NO:17,

wherein each scFv is independently linked to said HC via polypeptide linker (L1) covalently attached to the N-terminus of HC and the C-terminus of LCVR2, and LCVR2 is linked to HCVR2 of the same scFv via a second polypeptide linker (L2) covalently attached to the N-terminus of LCVR2 and the C-terminus of HCVR2.
2. The bispecific antibody of Claim 1, wherein the amino acid sequence of HCVR1 is SEQ ID NO:5, the amino acid sequence of LCVR1 is SEQ ID NO:7, the amino acid sequence of HCVR2 is SEQ ID NO:6, and the amino acid sequence of LCVR2 is SEQ ID NO:8.
3. The bispecific antibody of any of Claims 1 and 2, wherein the amino acid sequence of L1 is SEQ ID NO:21 and the amino acid sequence of L2 is SEQ ID NO:22.

-43-

4. A bispecific antibody comprising two first polypeptide chains and two second polypeptide chains wherein the amino acid sequence of each of the first polypeptides is SEQ ID NO:1 and the amino acid sequence of each of the second polypeptides is SEQ ID NO:2.
5. A DNA molecule comprising a polynucleotide sequence encoding a polypeptide chain having the amino acid sequence of SEQ ID NO:1.
6. A DNA molecule comprising a polynucleotide sequence encoding a polypeptide chain having the amino acid sequence of SEQ ID NO:2.
7. A DNA molecule comprising a polynucleotide sequence encoding a polypeptide chain having the amino acid sequence of SEQ ID NO:1 and comprising a polynucleotide sequence encoding a polypeptide chain having the amino acid sequence of SEQ ID NO:2.
8. A mammalian cell comprising the DNA molecule of Claim 5 and the DNA molecule of Claim 6, which cell is capable of expressing a polypeptide chain having the amino acid sequence of SEQ ID NO:1 and a polypeptide chain having the amino acid sequence of SEQ ID NO:2.
9. The mammalian cell of Claim 8, wherein the mammalian cell is CHO.
10. A mammalian cell comprising the DNA molecule of Claim 7, which cell is capable of expressing a polypeptide chain having the amino acid sequence of SEQ ID NO:1 and a polypeptide chain having the amino acid sequence of SEQ ID NO:2.
11. The mammalian cell of Claim 10, wherein the mammalian cell is CHO.
12. A process for producing a bispecific antibody comprising a polypeptide whose amino acid sequence is SEQ ID NO:1 and a polypeptide whose amino acid sequence is SEQ ID NO:2, comprising cultivating the mammalian cell of Claim 8 or Claim 10 under

-44-

conditions such that the bispecific antibody is expressed, and recovering the expressed bispecific antibody.

13. A bispecific antibody produced by the process of Claim 12.

5

14. A method of treating Systemic Lupus Erythematosus, Lupus Nephritis, or primary Sjögren's Syndrome comprising administering to a patient in need thereof a therapeutically effective amount of a bispecific antibody of any of Claims 1-4 and 13.

10 15. A bispecific antibody of any of Claims 1-4 and 13 for use in therapy.

16. A bispecific antibody of any of Claims 1-4 and 13 for use in the treatment of Systemic Lupus Erythematosus, Lupus Nephritis, or primary Sjögren's Syndrome.

15 17. A pharmaceutical composition comprising a bispecific antibody of any of Claims 1-4 and 13 and one or more pharmaceutically acceptable carriers, diluents, or excipients.

18. Use of the bispecific antibody of any of Claims 1-4 and 13 in the manufacture of a medicament for the treatment of Systemic Lupus Erythematosus, Lupus Nephritis, or
20 primary Sjögren's Syndrome.

25