(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 November 2016 (17.11.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/183104 A1

(51) International Patent Classification:
C07K 16/28 (2006.01)

(21) International Application Number:
PCT/US2016/031683

(22) International Filing Date:
10 May 2016 (10.05.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/159,876 11 May 2015 (11.05.2015) US
62/300,052 25 February 2016 (25.02.2016) US

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(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).

[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS OF TREATING LUPUS NEPHRITIS

(57) Abstract: The invention provides methods for treating or delaying progression of lupus nephritis in an individual that has lupus. In some embodiments, the methods comprise administering to the individual an effective amount of a type II anti-CD20 antibody. The invention also provides methods for treating or delaying progression of rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) in an individual. In some embodiments, the methods comprise administering an effective amount of an anti-CD20 antibody.

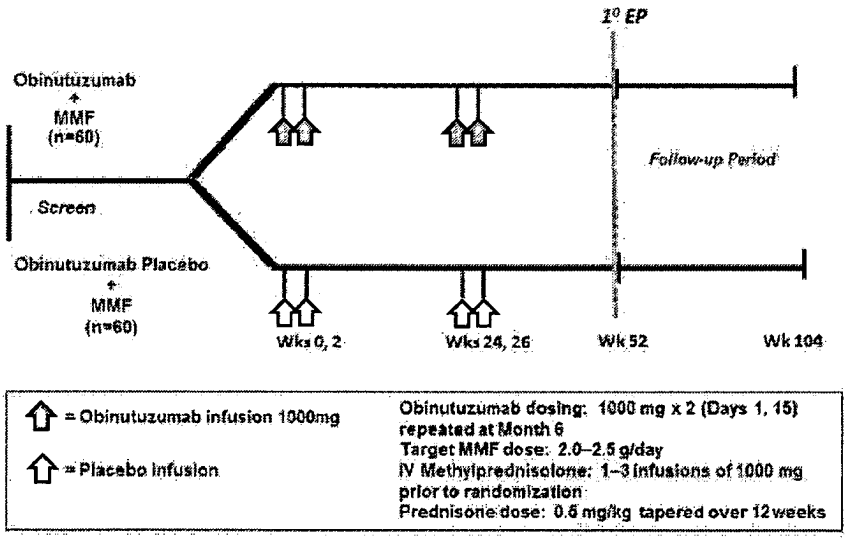

FIG. 1

WO 2016/183104 A1

Published:

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

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

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

COMPOSITIONS AND METHODS OF TREATING LUPUS NEPHRITIS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application Serial Nos. 62/159,876, filed May 11, 2015; and 62/300,052, filed February 25, 2016; each of which is incorporated herein by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 146392032240SeqList.txt, date recorded: May 5, 2016, size: 37 KB).

FIELD OF THE INVENTION

[0003] Provided herein are methods for treating or delaying progression of lupus nephritis in an individual that has lupus by administering a type II anti-CD20 antibody. Also provided herein are methods for treating or delaying progression of rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) in an individual by administering an anti-CD20 antibody.

BACKGROUND

[0004] Lupus is an autoimmune disease involving antibodies that attack connective tissue. The disease is estimated to affect nearly 1 million Americans, primarily women between the ages of 20-40. The principal form of lupus is a systemic one (systemic lupus erythematosus; SLE). SLE has an incidence of about 1 in 700 women between the ages of 20 and 60. SLE can affect any organ system and can cause severe tissue damage. Untreated lupus can be fatal as it progresses from attack of skin and joints to internal organs, including lung, heart, and kidneys, with renal disease, termed lupus nephritis (LN), being the primary concern. Lupus mainly appears as a series of flare-ups, with intervening periods of little or no disease manifestation.

[0005] LN is one of the most acute areas of damage associated with pathogenicity in SLE, and accounts for at least 50% of the mortality and morbidity of the disease. Currently, there are no really curative treatments for patients who have been diagnosed with SLE or LN. From a practical standpoint, physicians generally employ a number of powerful immunosuppressive drugs such as high-dose corticosteroids, e.g., prednisone, or azathioprine

or cyclophosphamide, which are given during periods of flare-ups, but may also be given persistently for those who have experienced frequent flare-ups. Even with effective treatment, which reduces symptoms and prolongs life, many of these drugs have potentially harmful side effects to the patients being treated. As such, there remains a need for more effective treatments against LN with fewer harmful side effects.

[0006] Two anti-CD20 antibodies have been tested in clinical studies for efficacy in treating lupus nephritis. Rituximab, a type I anti-CD20 antibody, failed to meet its primary endpoint of overall response (weighted toward complete renal response, or CRR) but resulted in a 15.3% increase in partial renal response (PRR) (Rovin, B.H. *et al.* (2012) *Arthritis Rheum.* 64:1215-1226). Ocrelizumab, another type I anti-CD20 antibody, was terminated, in part, because of an imbalance of serious infectious events (Mysler, E.F. *et al.* (2013) *Arthritis Rheum.* 65:2368-2379).

[0007] Obinutuzumab, a type II anti-CD20 antibody, has been shown to produce superior B cell depletion, as compared to rituximab. Significantly greater B cell depletion was observed with obinutuzumab treatment, compared to rituximab treatment, in cynomolgous monkeys (Mössner, E. *et al.* (2010) *Blood* 115:4393-4402). Therefore, there remains a need for testing the efficacy of type II anti-CD20 antibodies in treating or preventing LN in patients with lupus.

[0008] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

SUMMARY

[0009] In certain aspects, provided herein are methods for treating or delaying progression of lupus nephritis in an individual, comprising administering to the individual at least a first antibody exposure to a type II anti-CD20 antibody and a second antibody exposure to the type II anti-CD20 antibody. In some embodiments, the individual has lupus. In some embodiments, the second antibody exposure is not provided until from about 18 weeks to about 26 weeks after the first antibody exposure. In some embodiments, the second antibody exposure is not provided until from about 4.5 months to about 6.5 months after the first antibody exposure. In some embodiments, the first antibody exposure comprises one or two doses of the type II anti-CD20 antibody, the first antibody exposure comprising a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody. In some embodiments, the second antibody exposure comprises one or two doses of the type II anti-CD20 antibody, the second antibody exposure comprising a total exposure of between

about 1800mg and about 2200mg of the type II anti-CD20 antibody. In some embodiments, the type II anti-CD20 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:1, HVR-H2 sequence of SEQ ID NO:2, and HVR-H3 sequence of SEQ ID NO:3, and a light chain comprising HVR-L1 sequence of SEQ ID NO:4, HVR-L2 sequence of SEQ ID NO:5, and HVR-L3 sequence of SEQ ID NO:6. In some embodiments, the individual is at risk for developing class III or class IV lupus nephritis. In some embodiments, the methods are for preventing lupus nephritis in an individual that has lupus. In some embodiments, the methods are for preventing lupus nephritis in an individual that has SLE. In some embodiments, the methods are for treating or delaying progression of lupus nephritis in an individual that has SLE.

[0010] In some embodiments, the first antibody exposure comprises a first dose of the type II anti-CD20 antibody and a second dose of the type II anti-CD20 antibody, and the second dose of the first antibody exposure is not provided until from about 1.5 weeks to about 2.5 weeks after the first dose of the first antibody exposure. In some embodiments, the first antibody exposure comprises a first dose of the type II anti-CD20 antibody and a second dose of the type II anti-CD20 antibody, and the second dose of the first antibody exposure is not provided until about 2 weeks after the first dose of the first antibody exposure. In some embodiments, the first antibody exposure comprises a first dose of the type II anti-CD20 antibody and a second dose of the type II anti-CD20 antibody, and the second dose of the first antibody exposure is not provided until from about 10 days to about 17 days after the first dose of the first antibody exposure. In some embodiments, the first antibody exposure comprises a first dose of the type II anti-CD20 antibody and a second dose of the type II anti-CD20 antibody, and the second dose of the first antibody exposure is not provided until about 14 after the first dose of the first antibody exposure. In some embodiments, the first dose of the first antibody exposure is about 1000mg of the type II anti-CD20 antibody. In some embodiments, the second dose of the first antibody exposure is about 1000mg of the type II anti-CD20 antibody. In some embodiments, the second antibody exposure comprises a first dose of between about 900mg and about 1100mg of the type II anti-CD20 antibody and a second dose of between about 900mg and about 1100mg of the type II anti-CD20 antibody. In some embodiments, the second antibody exposure comprises a first dose of the type II anti-CD20 antibody and a second dose of the type II anti-CD20 antibody, and the second dose of the second antibody exposure is not provided until from about 1.5 weeks to about 2.5 weeks after the first dose of the second antibody exposure. In some embodiments, the second antibody exposure comprises a first dose of the type II anti-CD20 antibody and a second dose

of the type II anti-CD20 antibody, and the second dose of the second antibody exposure is not provided until about 2 weeks after the first dose of the second antibody exposure. In some embodiments, the second antibody exposure comprises a first dose of the type II anti-CD20 antibody and a second dose of the type II anti-CD20 antibody, and the second dose of the second antibody exposure is not provided until from about 10 days to about 17 days after the first dose of the second antibody exposure. In some embodiments, the second antibody exposure comprises a first dose of the type II anti-CD20 antibody and a second dose of the type II anti-CD20 antibody, and the second dose of the second antibody exposure is not provided until about 14 days after the first dose of the second antibody exposure. In some embodiments, the first dose of the second antibody exposure is about 1000mg of the type II anti-CD20 antibody. In some embodiments, the second dose of the second antibody exposure is about 1000mg of the type II anti-CD20 antibody. In some embodiments, the first antibody exposure and the second antibody exposure are administered intravenously. In some embodiments, the individual has class III or class IV lupus nephritis. In some embodiments, the individual is at risk for developing class III or class IV lupus nephritis.

[0011] In certain aspects, provided herein are methods for treating or delaying progression of lupus nephritis in an individual that has lupus, comprising administering to the individual an effective amount of a type II anti-CD20 antibody; wherein the type II anti-CD20 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:1, HVR-H2 sequence of SEQ ID NO:2, and HVR-H3 sequence of SEQ ID NO:3, and a light chain comprising HVR-L1 sequence of SEQ ID NO:4, HVR-L2 sequence of SEQ ID NO:5, and HVR-L3 sequence of SEQ ID NO:6; and wherein the individual has class III or class IV lupus nephritis. In some embodiments, the individual is at risk for developing class III or class IV lupus nephritis. In some embodiments, the methods are for preventing lupus nephritis in an individual that has lupus. In some embodiments, the methods are for preventing lupus nephritis in an individual that has SLE. In some embodiments, the methods are for treating or delaying progression of lupus nephritis in an individual that has SLE.

[0012] In certain aspects, provided herein are methods for treating or delaying progression of lupus nephritis in an individual that has lupus, comprising administering to the individual a dose of about 1000mg of a type II anti-CD20 antibody, wherein the type II anti-CD20 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:1, HVR-H2 sequence of SEQ ID NO:2, and HVR-H3 sequence of SEQ ID NO:3, and a light chain comprising HVR-L1 sequence of SEQ ID NO:4, HVR-L2 sequence of SEQ ID NO:5, and HVR-L3 sequence of SEQ ID NO:6, and wherein the dose is administered to the individual

once on days 1, 15, 168, and 182. In certain aspects, provided herein are methods for treating or delaying progression of lupus nephritis in an individual that has lupus, comprising administering to the individual a dose of about 1000mg of a type II anti-CD20 antibody, wherein the type II anti-CD20 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:1, HVR-H2 sequence of SEQ ID NO:2, and HVR-H3 sequence of SEQ ID NO:3, and a light chain comprising HVR-L1 sequence of SEQ ID NO:4, HVR-L2 sequence of SEQ ID NO:5, and HVR-L3 sequence of SEQ ID NO:6, and wherein the dose is administered to the individual once on weeks 0, 2, 24, and 26. In some embodiments, week 0 corresponds to day 1. In some embodiments, the individual has class III or class IV lupus nephritis. In some embodiments, the type II anti-CD20 antibody is obinutuzumab.

[0013] In some embodiments of any of the above embodiments, the type II anti-CD20 antibody is administered intravenously. In some embodiments of any of the above embodiments, the individual does not have class III (C) or class IV (C) lupus nephritis. In some embodiments of any of the above embodiments, the individual has class V lupus nephritis. In some embodiments of any of the above embodiments, the methods further include administering to the individual an effective amount of an immunosuppressive agent. In some embodiments, the immunosuppressive agent comprises mycophenolic acid, a derivative thereof, or a salt thereof. In some embodiments, the immunosuppressive agent comprises mycophenolate mofetil. In some embodiments of any of the above embodiments, the methods further include administering to the individual an effective amount of a glucocorticoid or corticosteroid. In some embodiments, the glucocorticoid or corticosteroid comprises methylprednisolone. In some embodiments, the glucocorticoid or corticosteroid comprises prednisone. In some embodiments of any of the above embodiments, the methods further include administering to the individual an effective amount of an antihistamine. In some embodiments, the antihistamine comprises diphenhydramine. In some embodiments of any of the above embodiments, the methods further include administering to the individual an effective amount of a non-steroidal anti-inflammatory drug (NSAID). In some embodiments, the NSAID comprises acetaminophen. In some embodiments of any of the above embodiments, the methods further include administering to the individual a standard of care treatment. In some embodiments, the standard of care treatment comprises treatment with one or more of an angiotensin-converting enzyme (ACE) inhibitor, an angiotensin-receptor blocker, cyclophosphamide, mycophenolate mofetil, azathioprine, and a glucocorticoid or corticosteroid. In some embodiments, the standard of care treatment is administered after the first antibody exposure to the type II anti-CD20 antibody and/or after the second antibody

exposure to the type II anti-CD20 antibody. In some embodiments of any of the above embodiments, the methods further include administering to the individual an effective amount of an antihypertensive agent. In some embodiments, the antihypertensive agent is an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin-receptor blocker. In some embodiments of any of the above embodiments, the method results in a complete renal response (CRR) in the individual. In some embodiments of any of the above embodiments, the method results in a depletion of circulating peripheral B cells in the individual. In some embodiments, the circulating peripheral B cells are CD19+ B cells. In some embodiments of any of the above embodiments, the type II anti-CD20 antibody is a humanized or human antibody. In some embodiments of any of the above embodiments, the type II anti-CD20 antibody is afucosylated. In some embodiments of any of the above embodiments, the type II anti-CD20 antibody is nonfucosylated (*e.g.*, as described in U.S. Patent No. 8,883,980). In some embodiments of any of the above embodiments, the heavy chain of the type II anti-CD20 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:7. In some embodiments of any of the above embodiments, the light chain of the type II anti-CD20 antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:8. In some embodiments of any of the above embodiments, the type II anti-CD20 antibody is obinutuzumab. In some embodiments of any of the above embodiments, the individual or patient is a human.

[0014] In certain aspects, provided herein are kits or articles of manufacture for treating or delaying progression of lupus nephritis in an individual that has lupus, comprising (a) a container comprising a type II anti-CD20 antibody, wherein the type II anti-CD20 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:1, HVR-H2 sequence of SEQ ID NO:2, and HVR-H3 sequence of SEQ ID NO:3, and a light chain comprising HVR-L1 sequence of SEQ ID NO:4, HVR-L2 sequence of SEQ ID NO:5, and HVR-L3 sequence of SEQ ID NO:6; and (b) a package insert with instructions for treating or delaying progression of lupus nephritis in an individual, wherein the instructions indicate that at least a first antibody exposure to a type II anti-CD20 antibody and a second antibody exposure to the type II anti-CD20 antibody are administered to the individual, the second antibody exposure not being provided until from about 18 weeks to about 26 weeks after the first antibody exposure; wherein the first antibody exposure comprises one or two doses of the type II anti-CD20 antibody, the first antibody exposure comprising a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody; and wherein the second antibody exposure comprises one or two doses of the type II anti-CD20 antibody, the second

antibody exposure comprising a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody. In some embodiments, the kits or articles of manufacture further include (c) a second medicament, wherein the type II anti-CD20 antibody is a first medicament; and (d) instructions on the package insert for administering the second medicament to the subject. In some embodiments, the second medicament is an immunosuppressive agent, a glucocorticoid, a corticosteroid, an anti-malarial agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone. In some embodiments, the heavy chain of the type II anti-CD20 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:7. In some embodiments, the light chain of the type II anti-CD20 antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:8. In some embodiments, the type II anti-CD20 antibody is obinutuzumab. In some embodiments, the kits or articles of manufacture are for preventing lupus nephritis in an individual that has SLE. In some embodiments, the kits or articles of manufacture are for treating or delaying progression of lupus nephritis in an individual that has SLE.

[0015] In certain aspects, provided herein are methods for treating or delaying progression of rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) in an individual, comprising administering to the individual an effective amount of an anti-CD20 antibody, wherein the antibody comprises a heavy chain variable region comprising an HVR-H1 sequence of SEQ ID NO:1, an HVR-H2 sequence of SEQ ID NO:2, and an HVR-H3 sequence of SEQ ID NO:3, and a light chain variable region comprising an HVR-L1 sequence of SEQ ID NO:4, an HVR-L2 sequence of SEQ ID NO:5, and an HVR-L3 sequence of SEQ ID NO:6. In some embodiments, the antibody is administered intravenously. In some embodiments, the method results in a depletion of circulating peripheral B cells in the individual. In some embodiments, the circulating peripheral B cells are CD19+ B cells. In some embodiments, the antibody is a humanized or human antibody. In some embodiments, the antibody is afucosylated. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:7. In some embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO:8. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:7 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:8.In some embodiments, the antibody is obinutuzumab. In some embodiments, the antibody comprises a modified Fc region. In some embodiments, the Fc region comprises a modification for attenuating effector function. In some embodiments, the Fc region is a

human IgG1 Fc region. In some embodiments, the human IgG1 Fc region comprises L234A, L235A and P329G amino acid substitutions, numbering according to EU index. In some embodiments of any of the above embodiments, the individual or patient is a human.

[0016] In certain aspects, provided herein are compositions for use in treating or delaying progression of rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) in an individual, the compositions comprising an anti-CD20 antibody, wherein the antibody comprises a heavy chain variable region comprising an HVR-H1 sequence of SEQ ID NO:1, an HVR-H2 sequence of SEQ ID NO:2, and an HVR-H3 sequence of SEQ ID NO:3, and a light chain variable region comprising an HVR-L1 sequence of SEQ ID NO:4, an HVR-L2 sequence of SEQ ID NO:5, and an HVR-L3 sequence of SEQ ID NO:6. In some embodiments, the composition is administered intravenously. In some embodiments, administering the composition results in a depletion of circulating peripheral B cells in the individual. In some embodiments, the circulating peripheral B cells are CD19+ B cells. In some embodiments, the antibody is a humanized or human antibody. In some embodiments, the antibody is afucosylated. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:7. In some embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO:8. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:7 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:8. In some embodiments, the antibody is obinutuzumab. In some embodiments, the antibody comprises a modified Fc region. In some embodiments, the Fc region comprises a modification for attenuating effector function. In some embodiments, the Fc region is a human IgG1 Fc region. In some embodiments, the human IgG1 Fc region comprises L234A, L235A and P329G amino acid substitutions, numbering according to EU index.

[0017] In certain aspects, provided herein is use of an anti-CD20 antibody for the manufacture of a medicament for use in treatment of rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) in an individual, wherein the antibody comprises a heavy chain variable region comprising an HVR-H1 sequence of SEQ ID NO:1, an HVR-H2 sequence of SEQ ID NO:2, and an HVR-H3 sequence of SEQ ID NO:3, and a light chain variable region comprising an HVR-L1 sequence of SEQ ID NO:4, an HVR-L2 sequence of SEQ ID NO:5, and an HVR-L3 sequence of SEQ ID NO:6.

[0018] In certain aspects, provided herein are kits or articles of manufacture for treating or delaying progression of rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) in an individual, comprising (a) a container comprising an anti-CD20 antibody, wherein the

antibody comprises a heavy chain variable region comprising an HVR-H1 sequence of SEQ ID NO:1, an HVR-H2 sequence of SEQ ID NO:2, and an HVR-H3 sequence of SEQ ID NO:3, and a light chain variable region comprising an HVR-L1 sequence of SEQ ID NO:4, an HVR-L2 sequence of SEQ ID NO:5, and an HVR-L3 sequence of SEQ ID NO:6; and (b) a package insert with instructions for administering an effective amount of anti-CD20 antibody to treat or delay progression of rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) in an individual. In some embodiments, the package insert includes instructions for administering the antibody intravenously. In some embodiments, the antibody is a humanized or human antibody. In some embodiments, the antibody is afucosylated. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:7. In some embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO:8. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:7 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:8. In some embodiments, the antibody is obinutuzumab. In some embodiments, the antibody comprises a modified Fc region. In some embodiments, the Fc region comprises a modification for attenuating effector function. In some embodiments, the Fc region is a human IgG1 Fc region. In some embodiments, the human IgG1 Fc region comprises L234A, L235A and P329G amino acid substitutions, numbering according to EU index.

[0019] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art. These and other embodiments of the invention are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE FIGURES

[0020] **FIG. 1** shows the study design for a Phase II study examining obinutuzumab + mycophenolate mofetil vs. placebo + mycophenolate mofetil. EP = endpoint; MMF = mycophenolate mofetil.

[0021] **FIG. 2A-2D** show whole blood B-cell-depletion, internalization and complement-dependent cellular cytotoxicity elicited by Obinutuzumab or Rituximab in RA and SLE patient samples. **FIG. 2A** shows whole blood samples from patients with RA (n=31) and SLE (n=34). Samples were incubated with or without anti-CD20 mAbs, RTX, OBZ_{Gly} and OBZ for 24 hours before flow cytometry to analyze B cell death. Values are the mean of triplicate

wells. The horizontal line in the box represents the median, the box represents the interquartile range and the whiskers represent the range. **Fig. 2B** shows the frequency of surface accessible mAbs. The frequency was assessed by flow cytometry after six hours of incubation with isolated B cells from patients with RA (n=5) and SLE (n=8) with or without prior incubation with anti-FcγRII blocking mAb, AT10. The horizontal line represents the median. **FIG. 2C** shows CDC induced by RTX and OBZ. Isolated B cells (Healthy control (HC), n=2; RA, n=2 and SLE, n=3) were incubated with RTX or OBZ for 30 minutes with normal healthy serum (NHS) or heat inactivated serum (HIS) before analyzing for the frequency of lysed CD19+Av+PI+ B cells. **FIG. 2D** shows the fold increase in CD19+Av+PI+ cells in samples incubated with NHS vs HIS representing the efficiency of CDC by mAbs. RTX, rituximab; OBZ, Obinutuzumab; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus. HC, Healthy control * $p<0.05$; **, $p<0.005$; ***, $p<0.0001$ and ns, not significant.

[0022] **FIG. 3A-3G** show the flow cytometry-gating strategy to assess NK cell degranulation, describing the relationship between NK cell expression of CD107a and CD16. Whole blood samples were incubated with or without mAbs for 24 hours before analyzing by flow cytometry. NK cells were identified based on forward- and side-scatter properties and expression of CD56 but not CD3. The frequency of CD3-CD56+CD107a+ cells represented activated/degranulated NK cells. FSC, forward-scatter; SSC, side-scatter. **FIG. 3A** shows flow cytometry gating of forward scatter vs. side scatter. **FIG. 3B** shows flow cytometry gating of CD56 vs. CD3. **FIG. 3C** shows flow cytometry gating of forward scatter vs. CD107a. **FIG. 3D** shows flow cytometry gating of forward scatter vs. CD16. Three subpopulations of CD3-CD56+ NK cells were identified based on the relative expression of CD16 (boxed as high, medium, and low). The relative frequency of activated CD107a+ NK cells differed in these 3 subpopulations based on CD16 expression in a hierarchical manner CD16++ < CD16+ < CD16-. **FIG. 3E** shows flow cytometry gating of forward scatter vs. CD107a for the high box. **FIG. 3F** shows flow cytometry gating of forward scatter vs. CD107a for the medium box. **FIG. 3G** shows flow cytometry gating of forward scatter vs. CD107a for the low box.

[0023] **FIG. 4A-4D** show that OBZ is more efficient than RTX at activating NK cells in RA and SLE patient samples. NK cell activation was assessed in whole blood samples from patients with RA (n=18) and SLE (n=23) incubated for 24 hours in the presence or absence of mAbs. *, $p<0.05$; **, $p<0.005$; ***, $p<0.0001$; ns, not significant and Spearman correlation coefficient, r^2 was considered significant when p was at least <0.05. **FIG. 4A** shows the

frequency of CD3-CD56+ NK cells in the lymphocyte gate, CD3-CD56+CD107a+ NK cells and CD3-CD56-CD16+ NK cells as a percentage of total NK cells or CD19+ cells. Horizontal lines represent the median. **FIG. 4B** shows the frequency of CD3-CD56+CD107a+ NK cells and the fold increase in the frequency of CD3-CD56+CD107a+ NK cells and the frequency of CD3-CD56+16+ NK cells in samples incubated with RTX and OBZ, from patients with RA and SLE. Horizontal lines represent the median. **FIG. 4C** shows the relationship between the frequency of CD3-CD56+CD107a+ NK cells in samples incubated with or without RTX and OBZ in samples from patients with RA (n=18). **FIG. 4D** shows the relationship between the frequency of CD3-CD56+CD107a+ NK cells in samples incubated with or without RTX and OBZ in samples from patients with SLE (n=23).

[0024] **FIG.5A-5D** show that Obinutuzumab is more efficient than Rituximab at evoking NK cell-mediated cellular cytotoxicity in RA and SLE patient samples. **FIG. 5A** shows a whole blood B-cell depletion assay showing the percentage B-cell depletion by RTX, OBZ_{Gly} and OBZ in samples from patients with RA (n=18) and SLE (n=23). Box and whiskers represent the interquartile range and the range, the horizontal line in the box represents the median. **FIG. 5B** shows the frequency of CD3-CD56+CD107a+ NK cells in whole blood samples from patients with RA and SLE after 24-hour incubation with or without mAbs, analyzed by flow cytometry. **FIG. 5C** shows the relative increase in the frequency of CD3-CD56+CD107a+ NK cells in whole blood samples incubated with or without mAbs, analyzed by flow cytometry. **FIG. 5D** shows the frequency of CD3-CD56+CD16+ NK cells in whole blood samples from patients with RA (n=18) and SLE (n=23) after 24 hour incubation with or without mAbs, analyzed by flow cytometry. For the bar graphs, the error bars represent the median and interquartile ranges. * $p<0.05$; **, $p<0.005$; ***, $p<0.0001$; and ns, not significant.

[0025] **FIG. 6A-6D** show that Obinutuzumab is more efficient than Rituximab at activating neutrophils in RA and SLE patient samples. **FIG. 6A** shows the mean fluorescence intensity (MFI) of CD11b on CD15+ neutrophils after 24 hour incubation of whole blood samples from patients with RA (n=10) and SLE (n=22) incubated with or without mAbs (1μg/ml). The Median and interquartile ranges are represented by the error bars. **FIG. 6B** shows the relationship between the MFI of CD11b on CD15+neutrophils in samples incubated with or without mAbs in RA and SLE samples. **FIG. 6C** shows the MFI of CD62L on CD15+neutrophils in samples incubated with or without mAbs in RA and SLE samples. **FIG. 6D** shows the relationship between the MFI of CD62L on CD15+ neutrophils, in samples incubated with or without mAbs in RA (n=10) and SLE (n=22) samples. * $p<0.05$; **,

$p<0.005$; ***, $p<0.0001$. Spearman correlation coefficient, r^2 was considered significant when p was at least <0.05.

[0026] **FIG. 7A-7D** show assessment of direct cell death, internalization and expression of CD20 and FcγRIIb in B-cell subpopulations from RA and SLE samples. **FIG. 7A** shows the frequency of Annexin V+ cells as a proportion of all CD19+ B cells and also B-cell subpopulations based on the relative expression of IgD and CD27: (IgD+CD27- naïve cells; IgD+CD27+ unswitched memory cells; IgD-CD27+ switched memory cells; and IgD-CD27- double negative cells); in samples from patients with RA (n=5) and SLE (n=4) incubated with or without mAbs. **FIG. 7B** shows the mean fluorescence intensity (MFI) of CD20 on all CD19+ cells and B-cell subpopulations in samples from patients with SLE (n= 9). **FIG. 7C** shows a surface fluorescence-quenching assay. The frequency of surface accessible mAbs after 6 hours of incubation with isolated B-cells, with or without prior incubation with anti-FcγRII mAb, AT10 from patients with SLE (n=9), in all CD19+ B-cells and B-cell subpopulations. **FIG. 7D** shows the MFI of FcγRIIb on all CD19+ B-cells and B-cell subpopulations in samples from patients with SLE (n=9). For the bar graphs, the error bars represent the median and interquartile ranges. Box and whiskers represent the interquartile range and the horizontal line in the box represents the median. * $p<0.05$; **, $p<0.005$; ***, $p<0.0001$.

[0027] **FIG. 8** shows the gating strategy for the complement-dependent cytotoxicity assay, and CDC by RTX and OBZ. Isolated B cells were incubated with mAbs either with NHS or HIS for 30 minutes at room temperature before analyzing by flow cytometry. The frequency of An V+ PI+ cells represented cell death. HIS, heat inactivated serum; NHS, normal healthy serum; RTX, rituximab; OBZ, Obinutuzumab; An V, Annexin V and PI, propidium iodide.

[0028] **FIG. 9** shows the flow cytometry-gating strategy to assess neutrophil activation. After 24 hours of incubation, whole blood samples were analysed by flow cytometry. Neutrophils were identified by forward- and side-scatter and CD15 positivity. The mean fluorescence intensity of CD11b and CD62L was analyzed on gated neutrophils positive for CD15.

[0029] **FIG. 10** shows the flow cytometry-gating strategy to assess direct cell death. After 6 hours of incubation with or without mAbs at 37°c and 5% CO_2, isolated B-cells were analyzed by flow cytometry. CD19+ B-cells were categorized into naïve (IgD+CD27-), unswitched memory cells (IgD+CD27+), switched memory cells (IgD-CD27+) and double

negative cells (IgD-CD27-). The frequency of Annexin V + cells represented direct cell death.

[0030] **FIG. 11** shows the inherent susceptibility to spontaneous cell death in B-cell subpopulations. Isolated B-cells incubated in RPMI supplemented with 10% foetal calf serum for 6 hours at at 37°c and 5% CO_2 were analyzed by flow cytometry. The frequency of Annexin V + cells represented direct cell death in CD19+ cells as a whole and also in B-cell subpopulations categorized into naïve (IgD+CD27-), unswitched memory cells (IgD+CD27+), switched memory cells (IgD-CD27+) and double negative cells (IgD-CD27-). Av, Annexin V; * $p<0.05$; ***, $p<0.0001$; ns, not significant.

DETAILED DESCRIPTION

[0031] In one aspect, provided herein are methods for treating or delaying progression of lupus nephritis in an individual, including administering to the individual at least a first antibody exposure to a type II anti-CD20 antibody and a second antibody exposure to the type II anti-CD20 antibody. In some embodiments, the individual has lupus. In some embodiments, the second antibody exposure is not provided until from about 18 weeks to about 26 weeks after the first antibody exposure. In some embodiments, the first antibody exposure includes one or two doses of the type II anti-CD20 antibody, the first antibody exposure containing a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody. In some embodiments, the second antibody exposure includes one or two doses of the type II anti-CD20 antibody, the second antibody exposure containing a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody. In some embodiments, the antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:1, HVR-H2 sequence of SEQ ID NO:2, and HVR-H3 sequence of SEQ ID NO:3, and a light chain comprising HVR-L1 sequence of SEQ ID NO:4, HVR-L2 sequence of SEQ ID NO:5, and HVR-L3 sequence of SEQ ID NO:6.

[0032] In another aspect, provided herein are methods for treating or delaying progression of lupus nephritis in an individual that has lupus, including administering to the individual an effective amount of a type II anti-CD20 antibody. In some embodiments, the antibody includes a heavy chain containing HVR-H1 sequence of SEQ ID NO:1, HVR-H2 sequence of SEQ ID NO:2, and HVR-H3 sequence of SEQ ID NO:3, and a light chain containing HVR-L1 sequence of SEQ ID NO:4, HVR-L2 sequence of SEQ ID NO:5, and HVR-L3 sequence of SEQ ID NO:6. In some embodiments, the individual has class III or class IV lupus nephritis.

[0033] In another aspect, provided herein are methods for treating or delaying progression of rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) in an individual, comprising administering to the individual an effective amount of an anti-CD20 antibody. In some embodiments, the antibody comprises a heavy chain variable region comprising an HVR-H1 sequence of SEQ ID NO:1, an HVR-H2 sequence of SEQ ID NO:2, and an HVR-H3 sequence of SEQ ID NO:3, and a light chain variable region comprising an HVR-L1 sequence of SEQ ID NO:4, an HVR-L2 sequence of SEQ ID NO:5, and an HVR-L3 sequence of SEQ ID NO:6.

I. General Techniques

[0034] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 3d edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology* (F.M. Ausubel, et al. eds., (2003)); the series *Methods in Enzymology* (Academic Press, Inc.): *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *Antibodies, A Laboratory Manual*, and *Animal Cell Culture* (R.I. Freshney, ed. (1987)); *Oligonucleotide Synthesis* (M.J. Gait, ed., 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J.E. Cellis, ed., 1998) Academic Press; *Animal Cell Culture* (R.I. Freshney), ed., 1987); *Introduction to Cell and Tissue Culture* (J.P. Mather and P.E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; *Handbook of Experimental Immunology* (D.M. Weir and C.C. Blackwell, eds.); *Gene Transfer Vectors for Mammalian Cells* (J.M. Miller and M.P. Calos, eds., 1987); *PCR: The Polymerase Chain Reaction*, (Mullis et al., eds., 1994); *Current Protocols in Immunology* (J.E. Coligan et al., eds., 1991); *Short Protocols in Molecular Biology* (Wiley and Sons, 1999); *Immunobiology* (C.A. Janeway and P. Travers, 1997); *Antibodies* (P. Finch, 1997); *Antibodies: A Practical Approach* (D. Catty., ed., IRL Press, 1988-1989); *Monoclonal Antibodies: A Practical Approach* (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); *Using Antibodies: A Laboratory Manual* (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); *The Antibodies* (M. Zanetti and J. D. Capra, eds., Harwood

Academic Publishers, 1995); and *Cancer: Principles and Practice of Oncology* (V.T. DeVita et al., eds., J.B. Lippincott Company, 1993).

II. Definitions

[0035] The term "lupus nephritis (LN)" refers to a manifestation of lupus (*e.g.,* systemic lupus erythematosus, drug-induced lupus, neonatal lupus, or discoid lupus) in the kidney(s).

[0036] The term "antibody" includes monoclonal antibodies (including full length antibodies which have an immunoglobulin Fc region), antibody compositions with polyepitopic specificity, multispecific antibodies (*e.g.*, bispecific antibodies, diabodies, and single-chain molecules, as well as antibody fragments (*e.g.*, Fab, $F(ab')_2$, and Fv). The term "immunoglobulin" (Ig) is used interchangeably with "antibody" herein.

[0037] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. An IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called a J chain, and contains 10 antigen binding sites, while IgA antibodies comprise from 2-5 of the basic 4-chain units which can polymerize to form polyvalent assemblages in combination with the J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (V_H) followed by three constant domains (C_H) for each of the α and γ chains and four C_H domains for μ and ε isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_H1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see *e.g.*, *Basic and Clinical Immunology*, 8th Edition, Daniel P. Sties, Abba I. Terr and Tristram G. Parsolw (eds), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6. The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins:

IgA, IgD, IgE, IgG and IgM, having heavy chains designated α, δ, ε, γ and μ, respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in the CH sequence and function, *e.g.*, humans express the following subclasses: IgG1, IgG2A, IgG2B, IgG3, IgG4, IgA1 and IgA2.

[0038] The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as "*VH*" and "*VL*", respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites.

[0039] The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines the specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the entire span of the variable domains. Instead, it is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen binding site of antibodies (see Kabat *et al., Sequences of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant domains are not involved directly in the binding of antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[0040] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal"

indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method (*e.g.*, Kohler and Milstein., *Nature*, 256:495-97 (1975); Hongo *et al.*, *Hybridoma,* 14 (3): 253-260 (1995), Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567), phage-display technologies (see, *e.g.*, Clackson *et al.*, *Nature*, 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, *e.g.*, WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits *et al.*, *Nature* 362: 255-258 (1993); Bruggemann *et al.*, *Year in Immunol.* 7:33 (1993); U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks *et al.*, *Bio/Technology* 10: 779-783 (1992); Lonberg *et al.*, *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnol.* 14: 845-851 (1996); Neuberger, *Nature Biotechnol.* 14: 826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

[0041] The term "naked antibody" refers to an antibody that is not conjugated to a cytotoxic moiety or radiolabel.

[0042] The terms "full-length antibody," "intact antibody" or "whole antibody" are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment. Specifically whole antibodies include those with heavy and light chains including an Fc region. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

[0043] An "antibody fragment" comprises a portion of an intact antibody, preferably the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments; diabodies; linear antibodies (see U.S. Patent 5,641,870, Example 2; Zapata *et al.*, *Protein Eng.* 8(10): 1057-1062 [1995]); single-

chain antibody molecules and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produced two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V_H), and the first constant domain of one heavy chain (C_H1). Each Fab fragment is monovalent with respect to antigen binding, *i.e.*, it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large $F(ab')_2$ fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having a few additional residues at the carboxy terminus of the C_H1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0044] The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

[0045] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0046] "Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the V_H and V_L antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of the sFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0047] "Functional fragments" of the antibodies of the invention comprise a portion of an intact antibody, generally including the antigen binding or variable region of the intact antibody or the Fc region of an antibody which retains or has modified FcR binding capability. Examples of antibody fragments include linear antibody, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0048] The term "diabodies" refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10) residues) between the V_H and V_L domains such that inter-chain but not intra-chain pairing of the V domains is achieved, thereby resulting in a bivalent fragment, *i.e.*, a fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" sFv fragments in which the V_H and V_L domains of the two antibodies are present on different polypeptide chains. Diabodies are described in greater detail in, for example, EP 404,097; WO 93/11161; Hollinger *et al., Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993).

[0049] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is(are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison *et al., Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). Chimeric antibodies of interest herein include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with an antigen of interest. As used herein, "humanized antibody" is used a subset of "chimeric antibodies."

[0050] "Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an HVR (hereinafter defined) of the recipient are replaced by residues from an HVR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, framework ("FR") residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made

to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, *etc.* The number of these amino acid substitutions in the FR are typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.*, Jones *et al., Nature* 321:522-525 (1986); Riechmann *et al., Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See also, for example, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409.

[0051] A "human antibody" is an antibody that possesses an amino-acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.*, 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (see, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al., Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0052] The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1,

H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, e.g., Xu *et al., Immunity* 13:37-45 (2000); Johnson and Wu, in *Methods in Molecular Biology* 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, *e.g., Hamers-Casterman et al., Nature* 363:446-448 (1993); Sheriff *et al., Nature Struct. Biol.* 3:733-736 (1996).

[0053] A number of HVR delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat e*t al., Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B (Kabat numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35 (Chothia numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0054] HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat *et al., supra*, for each of these definitions.

[0055] The expression "variable-domain residue-numbering as in Kabat" or "amino-acid-position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat *et al., supra*. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue

52 of H2 and inserted residues (*e.g.* residues 82a, 82b, and 82c, *etc.* according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0056] "Framework" or "FR" residues are those variable-domain residues other than the HVR residues as herein defined.

[0057] A "human consensus framework" or "acceptor human framework" is a framework that represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). Examples include for the VL, the subgroup may be subgroup kappa I, kappa II, kappa III or kappa IV as in Kabat *et al., supra.* Additionally, for the VH, the subgroup may be subgroup I, subgroup II, or subgroup III as in Kabat *et al., supra.* Alternatively, a human consensus framework can be derived from the above in which particular residues, such as when a human framework residue is selected based on its homology to the donor framework by aligning the donor framework sequence with a collection of various human framework sequences. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less.

[0058] A "VH subgroup III consensus framework" comprises the consensus sequence obtained from the amino acid sequences in variable heavy subgroup III of Kabat *et al., supra.* In one embodiment, the VH subgroup III consensus framework amino acid sequence comprises at least a portion or all of each of the following sequences: EVQLVESGGGLVQPGGSLRLSCAAS (HC-FR1)(SEQ ID NO:35), WVRQAPGKGLEWV (HC-FR2), (SEQ ID NO:36), RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR (HC-FR3, SEQ ID NO:37), WGQGTLVTVSA (HC-FR4), (SEQ ID NO:38).

[0059] A "VL kappa I consensus framework" comprises the consensus sequence obtained from the amino acid sequences in variable light kappa subgroup I of Kabat *et al., supra.* In one embodiment, the VH subgroup I consensus framework amino acid sequence comprises at least a portion or all of each of the following sequences: DIQMTQSPSSLSASVGDRVTITC

(LC-FR1) (SEQ ID NO:39), WYQQKPGKAPKLLIY (LC-FR2) (SEQ ID NO:40), GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (LC-FR3)(SEQ ID NO:41), FGQGTKVEIKR (LC-FR4)(SEQ ID NO:42).

[0060] An "amino-acid modification" at a specified position, *e.g.* of the Fc region, refers to the substitution or deletion of the specified residue, or the insertion of at least one amino acid residue adjacent the specified residue. Insertion "adjacent" to a specified residue means insertion within one to two residues thereof. The insertion may be N-terminal or C-terminal to the specified residue. The preferred amino acid modification herein is a substitution.

[0061] An "affinity-matured" antibody is one with one or more alterations in one or more HVRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). In one embodiment, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks *et al., Bio/Technology* 10:779-783 (1992) describes affinity maturation by VH- and VL-domain shuffling. Random mutagenesis of HVR and/or framework residues is described by, for example: Barbas *et al. Proc Nat. Acad. Sci. USA* 91:3809-3813 (1994); Schier *et al. Gene* 169:147-155 (1995); Yelton *et al. J. Immunol.* 155:1994-2004 (1995); Jackson *et al., J. Immunol.* 154(7):3310-9 (1995); and Hawkins *et al, J. Mol. Biol.* 226:889-896 (1992).

[0062] As use herein, the term "specifically binds to" or is "specific for" refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody that specifically binds to a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets. In one embodiment, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, *e.g.,* by a radioimmunoassay (RIA). In certain embodiments, an antibody that specifically binds to a target has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, or $\leq$ 0.1 nM. In certain embodiments, an antibody specifically binds to an epitope on a protein that is conserved among the protein from different species. In another embodiment, specific binding can include, but does not require exclusive binding.

[0063] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions.

Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. Suitable native-sequence Fc regions for use in the antibodies of the invention include human IgG1, IgG2 (IgG2A, IgG2B), IgG3 and IgG4.

[0064] "Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors, FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see M. Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991); Capel *et al.*, *Immunomethods* 4: 25-34 (1994); and de Haas *et al., J. Lab. Clin. Med.* 126: 330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein.

[0065] The term "Fc receptor" or "FcR" also includes the neonatal receptor, *FcRn*, which is responsible for the transfer of maternal IgGs to the fetus. Guyer *et al., J. Immunol.* 117: 587 (1976) and Kim *et al., J. Immunol.* 24: 249 (1994). Methods of measuring binding to FcRn are known (see, *e.g.*, Ghetie and Ward, *Immunol. Today* 18: (12): 592-8 (1997); Ghetie *et al., Nature Biotechnology* 15 (7): 637-40 (1997); Hinton *et al., J. Biol. Chem.* 279 (8): 6213-6 (2004); WO 2004/92219 (Hinton *et al.*). Binding to FcRn in vivo and serum half-life of human FcRn high-affinity binding polypeptides can be assayed, *e.g.*, in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides having a variant Fc region are administered. WO 2004/42072 (Presta) describes

antibody variants which improved or diminished binding to FcRs. See also, *e.g.*, Shields *et al., J. Biol. Chem.* 9(2): 6591-6604 (2001).

[0066] The phrase "substantially reduced," or "substantially different," as used herein, denotes a sufficiently high degree of difference between two numeric values (generally one associated with a molecule and the other associated with a reference/comparator molecule) such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (*e.g.*, Kd values). The difference between said two values is, for example, greater than about 10%, greater than about 20%, greater than about 30%, greater than about 40%, and/or greater than about 50% as a function of the value for the reference/comparator molecule.

[0067] The term "substantially similar" or "substantially the same," as used herein, denotes a sufficiently high degree of similarity between two numeric values (for example, one associated with an antibody of the invention and the other associated with a reference/comparator antibody), such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (*e.g.*, Kd values). The difference between said two values is, for example, less than about 50%, less than about 40%, less than about 30%, less than about 20%, and/or less than about 10% as a function of the reference/comparator value.

[0068] "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0069] A "package insert" refers to instructions customarily included in commercial packages of medicaments that contain information about the indications customarily included in commercial packages of medicaments that contain information about the indications,

usage, dosage, administration, contraindications, other medicaments to be combined with the packaged product, and/or warnings concerning the use of such medicaments, *etc.*

[0070] As used herein, the term "treatment" refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. For example, an individual is successfully "treated" if one or more symptoms associated with lupus nephritis are mitigated or eliminated, including, but are not limited to, elevated serum creatinine, proteinuria, red cell casts, reduced renal function, nephrotic syndrome, granular casts, microhematuria, macrohematuria, hypertension, tubular abnormalities, hyperkalemia, rapidly progressive glomerulonephritis (RPGN), and acute renal failure (ARF).

[0071] As used herein, "delaying progression" of a disease (*e.g.*, lupus nephritis) means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual, *e.g.*, an individual at risk for developing the disease, does not develop the disease. For example, the progression of SLE in an individual before the onset of LN symptoms and/or pathology may be delayed such that the development of LN is postponed or prevented.

[0072] As used herein, "complete renal response (CRR)" refers to a response to treatment that includes a normalization of serum creatinine, inactive urinary sediment, and a urinary protein to creatinine ratio of less than 0.5.

[0073] As used herein, "partial renal response (PRR)" refers to a response to treatment that is less than a CRR but still includes mitigation of one or more symptoms including without limitation a reduction in serum creatinine, reduced urinary sediment, and a reduction in proteinuria.

[0074] An "effective amount" is at least the minimum concentration required to effect a measurable improvement or prevention of a particular disorder. An effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of the treatment are outweighed by the therapeutically beneficial effects. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the

disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. In the case of lupus nephritis, an effective amount of the drug may have the effect in and/or relieving to some extent one or more of the symptoms associated with the disorder. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0075] "CD20" as used herein refers to the human B-lymphocyte antigen CD20 (also known as CD20, B-lymphocyte surface antigen B1, Leu-16, Bp35, BM5, and LF5; the sequence is characterized by the SwissProt database entry P11836) is a hydrophobic transmembrane protein with a molecular weight of approximately 35 kD located on pre-B and mature B lymphocytes. (Valentine, M.A., et al., *J. Biol. Chem.* 264(19) (1989 11282-11287; Tedder, T.F., et al, P*roc. Natl. Acad. Sci. U.S.A.* 85 (1988) 208-12; Stamenkovic, I., et al., *J. Exp. Med.* 167 (1988) 1975-80; Einfeld, D.A., et al., *EMBO J.* 7 (1988) 711-7; Tedder, T.F., et al., *J. Immunol.* 142 (1989) 2560-8). The corresponding human gene is Membrane-spanning 4-domains, subfamily A, member 1, also known as MS4A1. This gene encodes a member of the membrane-spanning 4A gene family. Members of this nascent protein family are characterized by common structural features and similar intron/exon splice boundaries and display unique expression patterns among hematopoietic cells and nonlymphoid tissues. This gene encodes the B-lymphocyte surface molecule which plays a role in the development and differentiation of B-cells into plasma cells. This family member is localized to 11q12, among a cluster of family members. Alternative splicing of this gene results in two transcript variants which encode the same protein.

[0076] The terms "CD20" and "CD20 antigen" are used interchangeably herein, and include any variants, isoforms and species homologs of human CD20 which are naturally expressed by cells or are expressed on cells transfected with the CD20 gene. Binding of an antibody of the invention to the CD20 antigen mediate the killing of cells expressing CD20 (e.g., a tumor cell) by inactivating CD20. The killing of the cells expressing CD20 may occur by one or more of the following mechanisms: Cell death/apoptosis induction, ADCC and CDC.

[0077] Synonyms of CD20, as recognized in the art, include B-lymphocyte antigen CD20, B-lymphocyte surface antigen B1, Leu-16, Bp35, BM5, and LF5.

[0078] The term "anti-CD20 antibody" according to the invention is an antibody that binds specifically to CD20 antigen. Depending on binding properties and biological activities of anti-CD20 antibodies to the CD20 antigen, two types of anti-CD20 antibodies (type I and type II anti-CD20 antibodies) can be distinguished according to Cragg, M.S., et al., *Blood* 103 (2004) 2738-2743; and Cragg, M.S., et al., *Blood* 101 (2003) 1045-1052, see Table 1 below.

Table 1. Properties of type I and type II anti-CD20 antibodies

Type I anti-CD20 antibodies	**type II anti-CD20 antibodies**
type I CD20 epitope	type II CD20 epitope
Localize CD20 to lipid rafts	Do not localize CD20 to lipid rafts
Increased CDC (if IgG1 isotype)	Decreased CDC (if IgG1 isotype)
ADCC activity (if IgG1 isotype)	ADCC activity (if IgG1 isotype)
Full binding capacity	Reduced binding capacity
Homotypic aggregation	Stronger homotypic aggregation
Apoptosis induction upon cross-linking	Strong cell death induction without cross-linking

[0079] Examples of type II anti-CD20 antibodies include e.g. humanized B-Ly1 antibody IgG1 (a chimeric humanized IgG1 antibody as disclosed in WO 2005/044859), 11B8 IgG1 (as disclosed in WO 2004/035607), and AT80 IgG1. Typically type II anti-CD20 antibodies of the IgG1 isotype show characteristic CDC properties. Type II anti-CD20 antibodies have a decreased CDC (if IgG1 isotype) compared to type I antibodies of the IgG1 isotype.

[0080] Examples of type I anti-CD20 antibodies include e.g. rituximab, HI47 IgG3 (ECACC, hybridoma), 2C6 IgG1 (as disclosed in WO 2005/103081), 2F2 IgG1 (as disclosed and WO 2004/035607 and WO 2005/103081) and 2H7 IgG1 (as disclosed in WO 2004/056312).

[0081] The afucosylated anti-CD20 antibodies according to the invention are preferably type II anti-CD20 antibodies, more preferably afucosylated humanized B-Ly1 antibodies as described in WO 2005/044859 and WO 2007/031875.

[0082] The "rituximab" antibody (reference antibody; example of a type I anti-CD20 antibody) is a genetically engineered chimeric human gamma 1 murine constant domain containing monoclonal antibody directed against the human CD20 antigen. However this antibody is not glycoengineered and not afocusylates and thus has an amount of fucose of at least 85 %. This chimeric antibody contains human gamma 1 constant domains and is identified by the name "C2B8" in US 5,736,137 (Andersen, et. al.) issued on April 17, 1998, assigned to IDEC Pharmaceuticals Corporation. Rituximab is approved for the treatment of patients with relapsed or refracting low-grade or follicular, CD20 positive, B cell non-Hodgkin's lymphoma. In vitro mechanism of action studies have shown that rituximab exhibits human complement-dependent cytotoxicity (CDC) (Reff, M.E., et. al, *Blood* 83(2) (1994) 435-445). Additionally, it exhibits activity in assays that measure antibody-dependent cellular cytotoxicity (ADCC).

[0083] The term "GA101 antibody" as used herein refers to any one of the following antibodies that bind human CD20: (1) an antibody comprising an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, an HVR-H3 comprising the amino acid sequence of SEQ ID NO:3, an HVR-L1 comprising the amino acid sequence of SEQ ID NO:4, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:6; (2) an antibody comprising a VH domain comprising the amino acid sequence of SEQ ID NO:7 and a VL domain comprising the amino acid sequence of SEQ ID NO:8, (3) an antibody comprising an amino acid sequence of SEQ ID NO:9 and an amino acid sequence of SEQ ID NO: 10; (4) an antibody known as obinutuzumab, or (5) an antibody that comprises an amino acid sequence that has at least 95%, 96%, 97%, 98% or 99% sequence identity with amino acid sequence of SEQ ID NO:9 and that comprises an amino acid sequence that has at least 95%, 96%, 97%, 98% or 99% sequence identity with an amino acid sequence of SEQ ID NO:10. In one embodiment, the GA101 antibody is an IgG1 isotype antibody. In some embodiments, the anti-CD20 antibody is a humanized B-Ly1 antibody.

[0084] The term "humanized B-Ly1 antibody" refers to humanized B-Ly1 antibody as disclosed in WO 2005/044859 and WO 2007/031875, which were obtained from the murine monoclonal anti-CD20 antibody B-Ly1 (variable region of the murine heavy chain (VH):

SEQ ID NO: 11; variable region of the murine light chain (VL): SEQ ID NO: 12- see Poppema, S. and Visser, L., *Biotest Bulletin* 3 (1987) 131-139) by chimerization with a human constant domain from IgG1 and following humanization (see WO 2005/044859 and WO 2007/031875). These "humanized B-Ly1 antibodies" are disclosed in detail in WO 2005/ 044859 and WO 2007/031875.

Variable region of the murine monoclonal anti-CD20 antibody B-Ly1 heavy chain (VH) (SEQ ID NO: 11)

```
Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys
Lys
 1               5                  10                  15
Ala Ser Gly Tyr Ala Phe Ser Tyr Ser Trp Met Asn Trp Val Lys
Leu
            20                  25                  30
Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Arg Ile Phe Pro Gly
Asp
        35                  40                  45
Gly Asp Thr Asp Tyr Asn Gly Lys Phe Lys Gly Lys Ala Thr Leu
Thr
    50                  55                  60
Ala Asp Lys Ser Ser Asn Thr Ala Tyr Met Gln Leu Thr Ser Leu
Thr
65                  70                  75                  80
Ser Val Asp Ser Ala Val Tyr Leu Cys Ala Arg Asn Val Phe Asp
Gly
                85                  90                  95
Tyr Trp Leu Val Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
Ala
            100                 105                 110
```

Variable region of the murine monoclonal anti-CD20 antibody B-Ly1 light chain (VL) (SEQ ID NO: 12)

```
Asn Pro Val Thr Leu Gly Thr Ser Ala Ser Ile Ser Cys Arg Ser
Ser
 1               5                  10                  15
Lys Ser Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr
Leu
            20                  25                  30
Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser
Asn
        35                  40                  45
Leu Val Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly
Thr
    50                  55                  60
Asp Phe Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val Gly
Val
65                  70                  75                  80
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Tyr Tyr Cys Ala Gln Asn Leu Glu Leu Pro Tyr Thr Phe Gly Gly
Gly
                85                  90                  95
Thr Lys Leu Glu Ile Lys Arg
            100
```

[0085] In one embodiment, the "humanized B-Ly1 antibody" has variable region of the heavy chain (VH) selected from group of SEQ ID NO:7, 8, and 13 to 33 (corresponding to, *inter alia*, B-HH2 to B-HH9 and B-HL8 to B-HL17 of WO 2005/044859 and WO 2007/031875). In one specific embodiment, such variable domain is selected from the group consisting of SEQ ID NOS:14, 15, 7, 19, 25, 27, and 29 (corresponding to B-HH2, BHH-3, B-HH6, B-HH8, B-HL8, B-HL11 and B-HL13 of WO 2005/044859 and WO 2007/031875). In one specific embodiment, the "humanized B-Ly1 antibody" has variable region of the light chain (VL) of SEQ ID NO:8 (corresponding to B-KV1 of WO 2005/044859 and WO 2007/031875). In one specific embodiment, the "humanized B-Ly1 antibody" has a variable region of the heavy chain (VH) of SEQ ID NO:7 (corresponding to B-HH6 of WO 2005/044859 and WO 2007/031875) and a variable region of the light chain (VL) of SEQ ID NO:8 (corresponding to B-KV1 of WO 2005/044859 and WO 2007/031875). Furthermore in one embodiment, the humanized B-Ly1 antibody is an IgG1 antibody. According to the invention such afocusylated humanized B-Ly1 antibodies are glycoengineered (GE) in the Fc region according to the procedures described in WO 2005/044859, WO 2004/065540, WO 2007/031875, Umana, P. et al., Nature Biotechnol. 17 (1999) 176-180 and WO 99/154342. In one embodiment, the afucosylated glyco-engineered humanized B-Ly1 is B-HH6-B-KV1 GE. In one embodiment, the anti-CD20 antibody is obinutuzumab (recommended INN, WHO Drug Information, Vol. 26, No. 4, 2012, p. 453). As used herein, obinutuzumab is synonymous for GA101 or RO5072759. This replaces all previous versions (e.g. Vol. 25, No. 1, 2011, p.75-76), and is formerly known as afutuzumab (recommended INN, WHO Drug Information, Vol. 23, No. 2, 2009, p. 176;Vol. 22, No. 2, 2008, p. 124). In some embodiments, the humanized B-Ly1 antibody is an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:9 and a light chain comprising the amino acid sequence of SEQ ID NO:10 or an antigen-binding fragment thereof. In some embodiments, the humanized B-Ly1 antibody comprises a heavy chain variable region comprising the three heavy chain CDRs of SEQ ID NO:9 and a light chain variable region comprising the three light chain CDRs of SEQ ID NO:10.

Heavy chain (SEQ ID NO:9)

```
QVQLVQSGAE VKKPGSSVKV SCKASGYAFS YSWINWVRQA PGQGLEWMGR 50
IFPGDGDTDY NGKFKGRVTI TADKSTSTAY MELSSLRSED TAVYYCARNV 100
FDGYWLVYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD 150
YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY 200
ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP SVFLFPPKPK 250
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS 300
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV 350
YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL 400
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPG   449
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Light chain (SEQ ID NO:10)

```
DIVMTQTPLS LPVTPGEPAS ISCRSSKSLL HSNGITYLYW YLQKPGQSPQ 50
LLIYQMSNLV SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP 100
YTFGGGTKVE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK 150
VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE 200
VTHQGLSSPV TKSFNRGEC                                   219
```

[0086] In some embodiments, the humanized B-Ly1 antibody is an afucosylated glyco-engineered humanized B-Ly1. Such glycoengineered humanized B-Ly1 antibodies have an altered pattern of glycosylation in the Fc region, preferably having a reduced level of fucose residues. Preferably the amount of fucose is 60 % or less of the total amount of oligosaccharides at Asn297 (in one embodiment the amount of fucose is between 40 % and 60 %, in another embodiment the amount of fucose is 50 % or less, and in still another embodiment the amount of fucose is 30 % or less). Furthermore the oligosaccharides of the Fc region are preferably bisected. These glycoengineered humanized B-Ly1 antibodies have an increased ADCC.

[0087] The "ratio of the binding capacities to CD20 on Raji cells (ATCC-No. CCL-86) of an anti-CD20 antibodies compared to rituximab" is determined by direct immunofluorescence measurement (the mean fluorescence intensities (MFI) is measured) using said anti-CD20 antibody conjugated with Cy5 and rituximab conjugated with Cy5 in a FACSArray (Becton Dickinson) with Raji cells (ATCC-No. CCL-86), as described in Example No. 2, and calculated as follows:

Ratio of the binding capacities to CD20 on Raji cells (ATCC-No. CCL-86) =

$$\frac{\text{MFI(Cy5-anti-CD20 antibody)}}{\text{MFI(Cy5-rituximab)}} \times \frac{\text{Cy5-labeling ratio(Cy5-rituximab)}}{\text{Cy5-labeling ratio(Cy5-anti-CD20 antibody)}}$$

[0088] MFI is the mean fluorescent intensity. The "Cy5-labeling ratio" as used herein means the number of Cy5-label molecules per molecule antibody.

[0089] Typically said type II anti-CD20 antibody has a ratio of the binding capacities to CD20 on Raji cells (ATCC-No. CCL-86) of said second anti-CD20 antibody compared to rituximab of 0.3 to 0.6, and in one embodiment, 0.35 to 0.55, and in yet another embodiment, 0.4 to 0.5.

[0090] In one embodiment said type II anti-CD20 antibody, e.g., a GA101 antibody, has increased antibody dependent cellular cytotoxicity (ADCC).

[0091] By "antibody having increased antibody dependent cellular cytotoxicity (ADCC)", it is meant an antibody, as that term is defined herein, having increased ADCC as determined by any suitable method known to those of ordinary skill in the art. One accepted in vitro ADCC assay is as follows:

1) the assay uses target cells that are known to express the target antigen recognized by the antigen-binding region of the antibody;

2) the assay uses human peripheral blood mononuclear cells (PBMCs), isolated from blood of a randomly chosen healthy donor, as effector cells;

3) the assay is carried out according to following protocol:

i) the PBMCs are isolated using standard density centrifugation procedures and are suspended at 5 x 10^6 cells/ml in RPMI cell culture medium;

ii) the target cells are grown by standard tissue culture methods, harvested from the exponential growth phase with a viability higher than 90%, washed in RPMI cell culture medium, labeled with 100 micro-Curies of ^{51}Cr, washed twice with cell culture medium, and resuspended in cell culture medium at a density of 10^5 cells/ml;

iii) 100 microliters of the final target cell suspension above are transferred to each well of a 96-well microtiter plate;

iv) the antibody is serially-diluted from 4000 ng/ml to 0.04 ng/ml in cell culture medium and 50 microliters of the resulting antibody solutions are added to the target cells in the 96-well microtiter plate, testing in triplicate various antibody concentrations covering the whole concentration range above;

v) for the maximum release (MR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of a 2% (VN) aqueous solution of non-ionic detergent (Nonidet, Sigma, St. Louis), instead of the antibody solution (point iv above);

vi) for the spontaneous release (SR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of RPMI cell culture medium instead of the antibody solution (point iv above);

vii) the 96-well microtiter plate is then centrifuged at 50 x g for 1 minute and incubated for 1 hour at 4°C;

viii) 50 microliters of the PBMC suspension (point i above) are added to each well to yield an effector:target cell ratio of 25:1 and the plates are placed in an incubator under 5% CO2 atmosphere at 37°C for 4 hours;

ix) the cell-free supernatant from each well is harvested and the experimentally released radioactivity (ER) is quantified using a gamma counter;

x) the percentage of specific lysis is calculated for each antibody concentration according to the formula (ER-MR)/(MR-SR) x 100, where ER is the average radioactivity quantified (see point ix above) for that antibody concentration, MR is the average radioactivity quantified (see point ix above) for the MR controls (see point V above), and SR is the average radioactivity quantified (see point ix above) for the SR controls (see point vi above);

4) "increased ADCC" is defined as either an increase in the maximum percentage of specific lysis observed within the antibody concentration range tested above, and/or a reduction in the concentration of antibody required to achieve one half of the maximum percentage of specific lysis observed within the antibody concentration range tested above. In one embodiment, the increase in ADCC is relative to the ADCC, measured with the above assay, mediated by the same antibody, produced by the same type of host cells, using the same standard production, purification, formulation and storage methods, which are known to those skilled in the art, except that the comparator antibody (lacking increased ADCC) has not been produced by host cells engineered to overexpress GnTIII and/or engineered to have reduced expression from the fucosyltransferase 8 (FUT8) gene (e.g., including, engineered for FUT8 knock out).

[0092] Said "increased ADCC" can be obtained by, for example, mutating and/or glycoengineering of said antibodies. In one embodiment, the antibody is glycoengineered to have a biantennary oligosaccharide attached to the Fc region of the antibody that is bisected by GlcNAc, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana

et al.); US 2005/0123546 (Umana et al.), Umana, P., et al., Nature Biotechnol. 17 (1999) 176-180). In another embodiment, the antibody is glycoengineered to lack fucose on the carbohydrate attached to the Fc region by expressing the antibody in a host cell that is deficient in protein fucosylation (e.g., Lec13 CHO cells or cells having an alpha-1,6-fucosyltransferase gene (FUT8) deleted or the FUT gene expression knocked down (see, e.g., Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004); Kanda, Y. et al., Biotechnol. Bioeng., 94(4):680-688 (2006); and WO2003/085107). In yet another embodiment, the antibody sequence has been engineered in its Fc region to enhance ADCC (e.g., in one embodiment, such engineered antibody variant comprises an Fc region with one or more amino acid substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues)).

[0093] The term "complement-dependent cytotoxicity (CDC)" refers to lysis of human tumor target cells by the antibody according to the invention in the presence of complement. CDC can be measured by the treatment of a preparation of CD20 expressing cells with an anti-CD20 antibody according to the invention in the presence of complement. CDC is found if the antibody induces at a concentration of 100 nM the lysis (cell death) of 20% or more of the tumor cells after 4 hours. In one embodiment, the assay is performed with ^{51}Cr or Eu labeled tumor cells and measurement of released ^{51}Cr or Eu. Controls include the incubation of the tumor target cells with complement but without the antibody.

[0094] The term "expression of the CD20" antigen is intended to indicate a significant level of expression of the CD20 antigen in a cell, e.g., a T- or B- Cell. In one embodiment, patients to be treated according to the methods of this invention express significant levels of CD20 on a B-cell. CD20 expression on a B-cell can be determined by standard assays known in the art. e.g., CD20 antigen expression is measured using immunohistochemical (IHC) detection, FACS or via PCR-based detection of the corresponding mRNA.

[0095] As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

[0096] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0097] It is understood that aspects and embodiments of the invention described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

III. Methods

[0098] In one aspect, provided herein are methods for treating or delaying progression of lupus nephritis in an individual that has lupus by administering an effective amount of a type II anti-CD20 antibody. In some embodiments, the individual has or is at risk for developing lupus nephritis. In some embodiments, the lupus nephritis is class III or class IV lupus nephritis. In some embodiments, the methods include administering to the individual at least a first antibody exposure to a type II anti-CD20 antibody and a second antibody exposure to the type II anti-CD20 antibody, the second antibody exposure not being provided until from about 18 weeks to about 26 weeks after the first antibody exposure; wherein the first antibody exposure comprises one or two doses of the type II anti-CD20 antibody, the first antibody exposure comprising a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody; and wherein the second antibody exposure comprises one or two doses of the type II anti-CD20 antibody, the second antibody exposure comprising a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody. As described below, in some embodiments, the antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:1, HVR-H2 sequence of SEQ ID NO:2, and HVR-H3 sequence of SEQ ID NO:3, and a light chain comprising HVR-L1 sequence of SEQ ID NO:4, HVR-L2 sequence of SEQ ID NO:5, and HVR-L3 sequence of SEQ ID NO:6. In some embodiments, the antibody comprises a VH domain comprising the amino acid sequence of SEQ ID NO:7 and a VL domain comprising the amino acid sequence of SEQ ID NO:8. In some embodiments, the antibody comprises an amino acid sequence of SEQ ID NO:9 and an amino acid sequence of SEQ ID NO:10. In some embodiments, the antibody comprises an antibody that comprises an amino acid sequence that has at least 95%, 96%, 97%, 98% or 99% sequence identity with amino acid sequence of SEQ ID NO:9 and that comprises an amino acid sequence that has at least 95%, 96%, 97%, 98% or 99% sequence identity with an amino acid sequence of SEQ ID NO:10.

Anti-CD20 antibodies

[0099] Certain aspects of the present disclosure relate to anti-CD20 antibodies, *e.g.*, for use in methods for treating or preventing progression of lupus nephritis. In some embodiments, the anti-CD20 antibody is a type II antibody. In some embodiments, the anti-CD20 antibody is human or humanized. In some embodiments, the anti-CD20 antibody is afucosylated. In some embodiments, the anti-CD20 antibody is a GA101 antibody.

[0100] Examples of type II anti-CD20 antibodies include e.g. humanized B-Ly1 antibody IgG1 (a chimeric humanized IgG1 antibody as disclosed in WO 2005/044859), 11B8 IgG1 (as disclosed in WO 2004/035607), and AT80 IgG1. Typically type II anti-CD20 antibodies of the IgG1 isotype show characteristic CDC properties. Type II anti-CD20 antibodies have a decreased CDC (if IgG1 isotype) compared to type I antibodies of the IgG1 isotype.

[0101] Examples of type I anti-CD20 antibodies include e.g. rituximab, HI47 IgG3 (ECACC, hybridoma), 2C6 IgG1 (as disclosed in WO 2005/103081), 2F2 IgG1 (as disclosed and WO 2004/035607 and WO 2005/103081) and 2H7 IgG1 (as disclosed in WO 2004/056312).

[0102] In some embodiments, the anti-CD20 antibody is a GA101 antibody described herein. In some embodiments, the anti-CD20 is any one of the following antibodies that bind human CD20: (1) an antibody comprising an HVR-H1 comprising the amino acid sequence of GYAFSY (SEQ ID NO:1), an HVR-H2 comprising the amino acid sequence of FPGDGDTD (SEQ ID NO:2), an HVR-H3 comprising the amino acid sequence of NVFDGYWLVY (SEQ ID NO:3), an HVR-L1 comprising the amino acid sequence of RSSKSLLHSNGITYLY (SEQ ID NO:4), an HVR-L2 comprising the amino acid sequence of QMSNLVS (SEQ ID NO:5), and an HVR-L3 comprising the amino acid sequence of AQNLELPYT (SEQ ID NO:6); (2) an antibody comprising a VH domain comprising the amino acid sequence of SEQ ID NO:7 and a VL domain comprising the amino acid sequence of SEQ ID NO:8, (3) an antibody comprising an amino acid sequence of SEQ ID NO:9 and an amino acid sequence of SEQ ID NO:10; (4) an antibody known as obinutuzumab, or (5) an antibody that comprises an amino acid sequence that has at least 95%, 96%, 97%, 98% or 99% sequence identity with amino acid sequence of SEQ ID NO:9 and that comprises an amino acid sequence that has at least 95%, 96%, 97%, 98% or 99% sequence identity with an amino acid sequence of SEQ ID NO:10. In one embodiment, the GA101 antibody is an IgG1 isotype antibody. In some embodiments, the anti-CD20 antibody comprises an HVR-H1, HVR-H2, HVR-H3, HVR-L1, HVR-L2, and HVR-L3 of any of the antibodies described herein, *e.g.*, 3 HVRs from SEQ ID NO:7 and 3 HVRs from SEQ ID NO:8, 3 HVRs from

SEQ ID NO:9 and 3 HVRs from SEQ ID NO:10, or any HVRs of the amino acid sequences provided in Table 2.

[0103] In some embodiments, the anti-CD20 antibody comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:7, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:8.

QVQLVQSGAEVKKPGSSVKVSCKAS**GYAFSY**SWINWVRQAPGQGLEWMGRI**FPGD GDTD**YNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCAR**NVFDGYWLVY**W GQGTLVTVSS (SEQ ID NO:7)

DIVMTQTPLSLPVTPGEPASISC**RSSKSLLHSNGITYLY**WYLQKPGQSPQLLIY**QMSN LVS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**AQNLELPYT**FGGGTKVEIKRTV (SEQ ID NO:8).

[0104] In some embodiments, the anti-CD20 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:9, and a light chain comprising the amino acid sequence of SEQ ID NO:10.

QVQLVQSGAEVKKPGSSVKVSCKAS***GYAFSY***SWINWVRQAPGQGLEWMGRI***FPGDGDTD***YNG KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCAR***NVFDGYWLVY***WGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:9)

DIVMTQTPLSLPVTPGEPASISC***RSSKSLLHSNGITYLY***WYLQKPGQSPQLLIY***QMSNLVS***G VPDRFSGSGSGTDFTLKISRVEAEDVGVYYC***AQNLELPYT***FGGGTKVEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:10)

[0105] In some embodiments, the anti-CD20 antibody is a humanized B-Ly1 antibody. In some embodiments, the humanized B-Ly1 antibody comprises a heavy chain variable region comprising the three heavy chain CDRs of SEQ ID NO:9 and a light chain variable region comprising the three light chain CDRs of SEQ ID NO:10. In some embodiments, the humanized B-Ly1 antibody comprises a heavy chain comprising the sequence of SEQ ID NO:9 and a light chain comprising the sequence of SEQ ID NO:10.

[0106] In some embodiments, the anti-CD20 antibody comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence listed in Table 2 below.

Table 2. Polypeptide sequences.

CONSTRUCT	POLYPEPTIDE SEQUENCE	SEQ ID NO
B-HH1	QVQLVQSGAEVKKPGSSVKVSCKASGYTFSYSWM SWVRQAPGQGLEWMGRIFPGDGDTDYAQKFQGRV TITADKSTSTAYMELSSLRSEDTAVYYCARNVFDG YWLVYWGQGTLVTVSS	13
B-HH2	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWM NWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGR VTITADKSTSTAYMELSSLRSEDTAVYYCARNVFD GYWLVYWGQGTLVTVSS	14
B-HH3	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWM NWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGR VTITADKSTSTAYMELSSLRSEDTAVYLCARNVFDG YWLVYWGQGTLVTVSS	15
B-HH4	QVQLVQSGAEVKKPGASVKVSCKVSGYAFSYSWM NWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGR VTITADKSTSTAYMELSSLRSEDTAVYYCARNVFD GYWLVYWGQGTLVTVSS	16
B-HH5	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWM SWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGRV TITADKSTSTAYMELSSLRSEDTAVYYCARNVFDG YWLVYWGQGTLVTVSS	17
B-HH6	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWIN WVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	7
B-HH7	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWIS WVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	18
B-HH8	QVQLVQSGAEVKKPGASVKVSCKASGYTFTYSWM NWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGR VTITADKSTSTAYMELSSLRSEDTAVYYCARNVFD GYWLVYWGQGTLVTVSS	19
B-HH9	QVQLVQSGAEVKKPGASVKVSCKASGYTFSYSWM NWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGR VTITADKSTSTAYMELSSLRSEDTAVYYCARNVFD GYWLVYWGQGTLVTVSS	20

CONSTRUCT	POLYPEPTIDE SEQUENCE	SEQ ID NO
B-HL1	QVQLVQSGAEVKKPGASVKVSCKASGYTFTYSWM HWVRQAPGQGLEWMGRIFPGDGDTDYAQKFQGR VTMTRDTSTSTVYMELSSLRSEDTAVYYCARNVFD GYWLVYWGQGTLVTVSS	21
B-HL2	EVQLVQSGAEVKKPGATVKISCKVSGYTFTYSWMH WVQQAPGKGLEWMGRIFPGDGDTDYAEKFQGRVT ITADTSTDTAYMELSSLRSEDTAVYYCATNVFDGY WLVYWGQGTLVTVSS	22
B-HL3	EVQLVQSGAEVKKPGATVKISCKVSGYTFTYSWMN WVQQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVT ITADTSTDTAYMELSSLRSEDTAVYYCATNVFDGY WLVYWGQGTLVTVSS	23
B-HL4	QMQLVQSGAEVKKTGSSVKVSCKASGYTFTYSWM SWVRQAPGQGLEWMGRIFPGDGDTDYAQKFQGRV TITADKSTSTAYMELSSLRSEDTAVYYCARNVFDG YWLVYWGQGTLVTVSS	24
B-HL8	EVQLVESGGGLVKPGGSLRLSCAASGFTFSYSWMN WVRQAPGKGLEWVGRIFPGDGDTDYNGKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	25
B-HL10	EVQLVESGGGLVKPGGSLRLSCAASGFAFSYSWMN WVRQAPGKGLEWVGRIFPGDGDTDYNGKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	26
B-HL11	QVQLVESGGGLVKPGGSLRLSCAASGFTFSYSWMN WVRQAPGKGLEWVGRIFPGDGDTDYNGKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	27
B-HL12	EVQLVESGAGLVKPGGSLRLSCAASGFTFSYSWMN WVRQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	28
B-HL13	EVQLVESGGGVVKPGGSLRLSCAASGFTFSYSWMN WVRQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	29
B-HL14	EVQLVESGGGLKKPGGSLRLSCAASGFTFSYSWMN WVRQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	30
B-HL15	EVQLVESGGGLVKPGSSLRLSCAASGFTFSYSWMN WVRQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	31

CONSTRUCT	POLYPEPTIDE SEQUENCE	SEQ ID NO
B-HL16	EVQLVESGGGLVKPGGSLRVSCAASGFTFSYSWMN WVRQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	32
B-HL17	EVQLVESGGGLVKPGGSLRLSCAASGFTFSYSWMN WVRQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	33
VH Signal Sequence	MDWTWRILFLVAAATGAHS	34
B-KV1	DIVMTQTPLSLPVTPGEPASISCRSSKSLLHSNGITYL YWYLQKPGQSPQLLIYQMSNLVSGVPDRFSGSGSG TDFTLKISRVEAEDVGVYYCAQNLELPYTFGGGTK VEIKRTV	8
VL Signal Sequence	MDMRVPAQLLGLLLLWFPGARC	43

[0107] In some embodiments, the anti-CD20 antibody (*e.g.*, a type II anti-CD20 antibody) is an afucosylated glyco-engineered antibody. Such glycoengineered antibodies have an altered pattern of glycosylation in the Fc region, preferably having a reduced level of fucose residues. Preferably the amount of fucose is 60 % or less of the total amount of oligosaccharides at Asn297 (in one embodiment the amount of fucose is between 40 % and 60 %, in another embodiment the amount of fucose is 50 % or less, and in still another embodiment the amount of fucose is 30 % or less). Furthermore the oligosaccharides of the Fc region are preferably bisected. These glycoengineered humanized anti-CD20 (e.g., B-Ly1) antibodies have an increased ADCC.

[0108] The oligosaccharide component can significantly affect properties relevant to the efficacy of a therapeutic glycoprotein, including physical stability, resistance to protease attack, interactions with the immune system, pharmacokinetics, and specific biological activity. Such properties may depend not only on the presence or absence, but also on the specific structures, of oligosaccharides. Some generalizations between oligosaccharide structure and glycoprotein function can be made. For example, certain oligosaccharide structures mediate rapid clearance of the glycoprotein from the bloodstream through interactions with specific carbohydrate binding proteins, while others can be bound by

antibodies and trigger undesired immune reactions. (Jenkins, N., et al., *Nature Biotechnol.* 14 (1996) 975-81).

[0109] Mammalian cells are the preferred hosts for production of therapeutic glycoproteins, due to their capability to glycosylate proteins in the most compatible form for human application. (Cumming, D.A., et al., *Glycobiology* 1 (1991) 115-30; Jenkins, N., et al., *Nature Biotechnol.* 14 (1996) 975-81). Bacteria very rarely glycosylate proteins, and like other types of common hosts, such as yeasts, filamentous fungi, insect and plant cells, yield glycosylation patterns associated with rapid clearance from the blood stream, undesirable immune interactions, and in some specific cases, reduced biological activity. Among mammalian cells, Chinese hamster ovary (CHO) cells have been most commonly used during the last two decades. In addition to giving suitable glycosylation patterns, these cells allow consistent generation of genetically stable, highly productive clonal cell lines. They can be cultured to high densities in simple bioreactors using serum free media, and permit the development of safe and reproducible bioprocesses. Other commonly used animal cells include baby hamster kidney (BHK) cells, NSO- and SP2/0-mouse myeloma cells. More recently, production from transgenic animals has also been tested. (Jenkins, N., et al., *Nature Biotechnol.* 14 (1996) 975-981).

[0110] All antibodies contain carbohydrate structures at conserved positions in the heavy chain constant regions, with each isotype possessing a distinct array of N-linked carbohydrate structures, which variably affect protein assembly, secretion or functional activity. (Wright, A., and Morrison, S.L., *Trends Biotech.* 15 (1997) 26-32). The structure of the attached N-linked carbohydrate varies considerably, depending on the degree of processing, and can include high-mannose, multiply-branched as well as biantennary complex oligosaccharides. (Wright, A., and Morrison, S.L., *Trends Biotech.* 15 (1997) 26-32). Typically, there is heterogeneous processing of the core oligosaccharide structures attached at a particular glycosylation site such that even monoclonal antibodies exist as multiple glycoforms. Likewise, it has been shown that major differences in antibody glycosylation occur between cell lines, and even minor differences are seen for a given cell line grown under different culture conditions. (Lifely, M.R., et al., *Glycobiology* 5(8) (1995) 813-22).

[0111] One way to obtain large increases in potency, while maintaining a simple production process and potentially avoiding significant, undesirable side effects, is to enhance the natural, cell-mediated effector functions of monoclonal antibodies by engineering their oligosaccharide component as described in Umana, P., et al., *Nature Biotechnol.* 17 (1999) 176-180 and US 6,602,684. IgG1 type antibodies, the most commonly

used antibodies in cancer immunotherapy, are glycoproteins that have a conserved N-linked glycosylation site at Asn297 in each CH2 domain. The two complex biantennary oligosaccharides attached to Asn297 are buried between the CH2 domains, forming extensive contacts with the polypeptide backbone, and their presence is essential for the antibody to mediate effector functions such as antibody dependent cellular cytotoxicity (ADCC) (Lifely, M.R., et al., *Glycobiology* 5 (1995) 813-822; Jefferis, R., et al., *Immunol. Rev.* 163 (1998) 59-76; Wright, A., and Morrison, S.L., *Trends Biotechnol.* 15 (1997) 26-32).

[0112] It was previously shown that overexpression in Chinese hamster ovary (CHO) cells of ß(1,4)-N-acetylglucosaminyltransferase I11 ("GnTIII17y), a glycosyltransferase catalyzing the formation of bisected oligosaccharides, significantly increases the in vitro ADCC activity of an antineuroblastoma chimeric monoclonal antibody (chCE7) produced by the engineered CHO cells. (See Umana, P., et al., *Nature Biotechnol.* 17 (1999) 176-180; and WO 99/154342, the entire contents of which are hereby incorporated by reference). The antibody chCE7 belongs to a large class of unconjugated monoclonal antibodies which have high tumor affinity and specificity, but have too little potency to be clinically useful when produced in standard industrial cell lines lacking the GnTIII enzyme (Umana, P., et al., *Nature Biotechnol.* 17 (1999) 176-180). That study was the first to show that large increases of ADCC activity could be obtained by engineering the antibody producing cells to express GnTIII, which also led to an increase in the proportion of constant region (Fc)-associated, bisected oligosaccharides, including bisected, non-fucosylated oligosaccharides, above the levels found in naturally-occurring antibodies.

[0113] In some embodiments, the anti-CD20 antibody (*e.g.,* a type II anti-CD20 antibody) comprises a human Fc region (*e.g.,* a human IgG1 Fc region). In some embodiments, the Fc region comprises an N-linked oligosaccharide that has been modified. In some embodiments, the N-linked oligosaccharides of the Fc region have reduced fucose residues as compared to an antibody with non-modified N-linked oligosaccharides. In some embodiments, the bisected oligosaccharide is a bisected complex oligosaccharide. In some embodiments, the N-linked oligosaccharides have been modified to have increased bisected, nonfucosylated oligosaccharides. In some embodiments, the bisected, nonfucosylated oligosaccharides are the hybrid type. In some embodiments, the bisected, nonfucosylated oligosaccharides are the complex type. For more detailed description, see, *e.g.,* WO 2003/011878 (Jean-Mairet *et al.*); US Patent No. 6,602,684 (Umana *et al.*); US 2005/0123546 (Umana *et al.*); and U.S. Patent No. 8,883,980 (Umana *et al.*).

[0114] In some embodiments, the anti-CD20 antibody (*e.g.*, a type II anti-CD20 antibody) is a multispecific antibody or a bispecific antibody.

Antibody Preparation

[0115] An antibody according to any of the above embodiments (*e.g.*, a type II anti-CD20 antibody of the present disclosure) may incorporate any of the features, singly or in combination, as described in Sections 1-7 below:

1. Antibody Affinity

[0116] In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M).

[0117] In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA). In one embodiment, an RIA is performed with the Fab version of an antibody of interest and its antigen. For example, solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0118] According to another embodiment, Kd is measured using a BIACORE® surface plasmon resonance assay. For example, an assay using a BIACORE®-2000 or a BIACORE ®-3000 (BIAcore, Inc., Piscataway, NJ) is performed at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). In one embodiment, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N'*- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE ® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 106 M-1 s-1 by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25oC of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO ™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

[0119] In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

[0120] Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

[0121] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; *see,* e.g., U.S. Patent No. 6,248,516 B1).

[0122] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

3. Chimeric and Humanized Antibodies

[0123] In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0124] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[0125] Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in

Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing specificity determining region (SDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[0126] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

[0127] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

[0128] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900,

describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

[0129] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[0130] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

[0131] Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

[0132] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0133] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

[0134] In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for CD20 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of CD20. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express CD20. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[0135] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., US

Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see,e.g. Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.* 147: 60 (1991).

[0136] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

[0137] The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to CD20 as well as another, different antigen (see, US 2008/0069820, for example).

7. Antibody Variants

[0138] In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

a) Substitution, Insertion, and Deletion Variants

[0139] In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table A under the heading of "preferred substitutions." More substantial changes are provided in Table A under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE A

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0140] Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[0141] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0142] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[0143] Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or residues that contact antigen, with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0144] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may, for example, be outside of antigen contacting residues in the HVRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0145] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0146] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

[0147] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0148] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0149] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[0150] Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc region variants

[0151] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

[0152] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc(RIII only, whereas monocytes express Fc(RI, Fc(RII and Fc(RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g. Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in a animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed

using methods known in the art (see, e.g., Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[0153] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[0154] In certain embodiments, the Fc variants described herein further comprise one or more amino acid modifications for attenuating effector function (such as CDC and/or ADCC). In exemplary embodiments, the modification to attenuate effector function is a modification that does not alter the glycosylation pattern of the Fc region. In certain embodiments, the modification to attenuate effector function reduces or eliminates binding to human effector cells, binding to one or more Fc receptors, and/or binding to cells expressing an Fc receptor. In an exemplary embodiment, the Fc variants described herein comprise the following modifications: L234A, L235A and P329G in the Fc region of human IgG1, that result in attenuated effector function. Substitutions L234A, L235A, and P329G (the L234A/L235A/P329G triple variant is referred to as LALAPG) have previously been shown to reduce binding to Fc receptors and complement (see e.g., US Publication No. 2012/0251531).

[0155] In various embodiments, Fc variants having reduced effector function refer to Fc variants that reduce effector function (e.g., CDC, ADCC, and/or binding to FcR, etc. activities) by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or more as compared to the effector function achieved by a wild-type Fc region (e.g., an Fc region not having a mutation to reduce effector function, although it may have other mutations). In certain embodiments, Fc variants having reduced effector function refer to Fc variants that eliminate all detectable effector function as compared to a wild-type Fc region. Assays for measuring effector function are known in the art and described below.

[0156] *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity). The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-

limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g. Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)).

[0157] Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[0158] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[0159] In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

[0160] Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826).

[0161] See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

d) Cysteine engineered antibody variants

[0162] In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

e) Antibody Derivatives

[0163] In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the

antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

[0164] In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

A. Recombinant Methods and Compositions

[0165] Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-CD20 antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In one embodiment, a method of making an anti-CD20 antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

[0166] For recombinant production of an anti-CD20 antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[0167] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[0168] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

[0169] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

[0170] Plant cell cultures can also be utilized as hosts. *See,* e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

[0171] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR$^-$ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines

such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

B. Assays

[0172] Anti-CD20 antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

1. Binding assays and other assays

[0173] In one aspect, an antibody of the invention is tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blot, etc. CD20 binding may be determined using methods known in the art and exemplary methods are disclosed herein. In one embodiment, binding is measured using radioimmunoassay. An exemplary radioimmunoassay is provided below. CD20 antibody is iodinated, and competition reaction mixtures are prepared containing a fixed concentration of iodinated antibody and decreasing concentrations of serially diluted, unlabeled CD20 antibody. Cells expressing CD20 (e.g., BT474 cells stably transfected with human CD20) are added to the reaction mixture. Following an incubation, cells are washed to separate the free iodinated CD20 antibody from the CD20 antibody bound to the cells. Level of bound iodinated CD20 antibody is determined, e.g., by counting radioactivity associated with cells, and binding affinity determined using standard methods. In another embodiment, ability of CD20 antibody to bind to surface-expressed CD20 (e.g., on B cell subsets) is assessed using flow cytometry. Peripheral white blood cells are obtained (e.g., from human, cynomolgus monkey, rat or mouse) and cells are blocked with serum. Labeled CD20 antibody is added in serial dilutions, and T cells are also stained to identify T cell subsets (using methods known in the art). Following incubation of the samples and washing, the cells are sorted using flow cytometer, and data analyzed using methods well known in the art. In another embodiment, CD20 binding may be analyzed using surface plasmon resonance. An exemplary surface plasmon resonance method is exemplified in the Examples.

[0174] In another aspect, competition assays may be used to identify an antibody that competes with any of the anti-CD20 antibodies disclosed herein for binding to CD20. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by any of the anti-CD20 antibodies disclosed herein.

Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

[0175] In an exemplary competition assay, immobilized CD20 is incubated in a solution comprising a first labeled antibody that binds to CD20 (e.g., rituximab, a GA101 antibody, etc.) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to CD20. The second antibody may be present in a hybridoma supernatant. As a control, immobilized CD20 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to CD20, excess unbound antibody is removed, and the amount of label associated with immobilized CD20 is measured. If the amount of label associated with immobilized CD20 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to CD20. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

2. Activity assays

[0176] Anti-CD20 antibodies of the present disclosure (*e.g.,* a type II antibody) may be identified and/or characterized by one or more activity assays known in the art. For example, a complement-dependent cytotoxicity (CDC) and/or antibody-dependent cellular cytotoxicity (ADCC) may be used, as described herein.

[0177] It is understood that any of the above assays may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-CD20 antibody.

[0178] It is understood that any of the above assays may be carried out using anti-CD20 antibody and an additional therapeutic agent.

C. Immunoconjugates

[0179] The invention also provides immunoconjugates comprising an anti-CD20 antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

[0180] In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235

B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

[0181] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[0182] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

[0183] Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives

(such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

[0184] The immunuoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

Methods for Treating or Delaying Progression of Lupus Nephritis

[0185] Certain aspects of the present disclosure relate to methods for treating or delaying progression of lupus nephritis (LN) in an individual that has lupus. In some embodiments, the individual or patient is a human.

[0186] LN is known in the art as a manifestation of lupus (*e.g.,* systemic lupus erythematosus, drug-induced lupus, neonatal lupus, or discoid lupus) in the kidney(s). The most common type of lupus that manifests in the kidneys is systemic lupus erythematosus (SLE). It is thought that 25-50% of SLE patients have abnormalities in the urine and/or renal function early in the course of their disease, with up to 60% of adults and 80% of children eventually developing LN (for more details, see Cameron, J.S. (1999) *J. Am. Soc. Nephrol.* 10:413-424). LN is thought to account for at least 50% of the morbidity and mortality associated with SLE.

[0187] In addition, renal manifestations have also been noted in other types of lupus, such as discoid (Roujeau, J.C. *et al.* (1984) *Acta Derm. Venereol.* 64:160-163) and drug-induced lupus (Smith, P.R. *et al.* (1999) *Rheumatology (Oxford)* 38:1017-1018). In some embodiments, the individual has SLE, discoid lupus, or drug-induced lupus.

[0188] Diagnosis of SLE may be according to current American College of Rheumatology (ACR) criteria. Active disease may be defined by one British Isles Lupus Activity Group's (BILAG) "A" criteria or two BILAG "B" criteria; SLE Disease Activity Index (SLEDAI); or systemic lupus erythematosus (SLE) responder index (SRI) as noted in the Examples below and descrived in Furie et al., Arthritis Rheum. 61(9):1143-51 (2009). Some signs, symptoms, or other indicators used to diagnose SLE adapted from: Tan et al. "The Revised Criteria for the Classification of SLE" Arth Rheum 25 (1982) may be malar rash such as rash over the cheeks, discoid rash, or red raised patches, photosensitivity such as reaction to sunlight, resulting in the development of or increase in skin rash, oral ulcers such as ulcers in the nose or mouth, usually painless, arthritis, such as non-erosive arthritis involving two or more peripheral joints (arthritis in which the bones around the joints do not become destroyed), serositis, pleuritis or pericarditis, renal disorder such as excessive protein in the urine (greater than 0.5 gm/day or 3+ on test sticks) and/or cellular casts (abnormal elements derived from the urine and/or white cells and/or kidney tubule cells), neurologic signs, symptoms, or other indicators, seizures (convulsions), and/or psychosis in the absence of drugs or metabolic disturbances that are known to cause such effects, and hematologic signs, symptoms, or other indicators such as hemolytic anemia or leukopenia (white blood count below 4,000 cells per cubic millimeter) or lymphopenia (less than 1,500 lymphocytes per cubic millimeter) or thrombocytopenia (less than 100,000 platelets per cubic millimeter). The leukopenia and lymphopenia must be detected on two or more occasions. The thrombocytopenia must be detected in the absence of drugs known to induce it. The invention is not limited to these signs, symptoms, or other indicators of lupus.

[0189] The presence of autoantibodies may be tested as an indication for lupus. Autoantibodies may include without limitation anti-dsDNA antibodies, anti-complement antibodies, and antinuclear antibodies (*e.g.*, an ENA panel). ENA refers to Extractable Nuclear Antigens, i.e., a group of nuclear antigens including, e.g., RNP, Ro/SS-A, La/ SS-B, Sm, SCL-70, Jo-1, as described in McNeilage et al., J., Clin. Lab. Immunol. 15:1-17 (1984); Whittingham, Ann. Acad. Med. 17(2):195-200 (1988); Wallace and Hahn, DUBOIS' LUPUS ERYTHEMATOSUS, 7TH ED. LIPPINCOTT (2007); Tang et al., Medicine 89(1): 62-67 (2010). Antibodies to ENA have been correlated to lupus. McNeilage et al., 1984; Whittingham 1988; Asherson et al., Medicine 68(6): 366-374 (1989); and Tang et al., 2010. Reduced complement activity may also be associated with lupus, *e.g.*, as measured by C3 levels, C4 levels, and/or a CH50 assay.

[0190] As described above in reference to SLE, it is known in the art that LN often manifests progressively in patients with lupus (*e.g.*, systemic lupus erythematosus, drug-induced lupus, neonatal lupus, or discoid lupus). That is to say, a patient may be diagnosed with lupus without a clinical or pathological manifestation of one or more LN symptoms. Nonetheless, the patient may still be considered to be at risk for developing LN due to the high frequency of lupus patients that eventually develop LN. Therefore, in some embodiments, the methods of the present disclosure may find use in delaying progression of LN, or preventing LN, in a patient with lupus. In some embodiments, the methods of the present disclosure may find use in postponing or preventing the onset of LN in a patient with lupus (*e.g.*, a form of lupus that lacks a manifestation in the kidney(s)).

[0191] LN pathology may be classified according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification system, as shown in the table below (see Markowitz GS, D'Agati VD (2007) *Kidney Int* 71:491–495 and Weening, JJ (2004) *Kidney Int* 65:521-530 for further descriptions and definitions of terms).

Table 3. ISN/RPS 2003 Classification of Lupus Nephritis.

Class I	Minimal mesangial LN (Normal glomeruli by light microscopy, but mesangial immune deposits by immunofluorescence)
Class II	Mesangial proliferative LN (Purely mesangial hypercellularity of any degree or mesangial matrix expansion by light microscopy, with mesangial immune deposits. A few isolated subepithelial or subendothelial deposits may be visible by immunofluorescence or electron microscopy, but not by light microscopy)
Class III	Focal LN (Active or inactive focal, segmental or global endo- or extracapillary glomerulonephritis involving < 50% of all glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations) III (A): active lesions (focal proliferative LN) III (A/C): active and chronic lesions (focal proliferative and sclerosing LN) III (C): chronic inactive lesions with glomerular scars (focal sclerosing LN)

Class IV	Diffuse LN (Active or inactive diffuse, segmental or global endo- or extracapillary glomerulonephritis involving ≥ 50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is divided into diffuse segmental (IV-S) LN when ≥ 50% of the involved glomeruli have segmental lesions, and diffuse global (IV-G) LN when ≥ 50% of the involved glomeruli have global lesions. Segmental is defined as a glomerular lesion that involves less than half of the glomerular tuft. This class includes cases with diffuse wire loop deposits but with little or no glomerular proliferation.) IV-S (A): active lesions (diffuse segmental proliferative LN) IV-G (A): active lesions (diffuse global proliferative LN) IV-S (A/C): active and chronic lesions (diffuse segmental proliferative and sclerosing LN) IV-G (A/C): active and chronic lesions (diffuse global proliferative and sclerosing LN) IV-S (C): chronic inactive lesions with scars (diffuse segmental sclerosing LN) IV-G (C): chronic inactive lesions with scars (diffuse global sclerosing LN)
Class V	Membranous LN (Global or segmental subepithelial immune deposits or their morphologic sequelae by light microscopy and by immunofluorescence or electron microscopy, with or without mesangial alterations.)
Class VI	Advanced sclerotic LN (≥ 90% of glomeruli globally sclerosed without residual activity)

LN = lupus nephritis; A = active; C = chronic; G = global; S = segmental.

Note: Class V may occur in combination with Class III or IV, in which case both will be diagnosed. Class V LN may show advanced sclerosis.

[0192] In some embodiments, the patient has class III or class IV LN. In some embodiments, the patient has class III LN. For example, in some embodiments, the patient has class III(A) or class III(A/C) LN. In some embodiments, the patient has class IV LN. For example, in some embodiments, the patient has class IV-S(A), IV-G(A), IV-S(A/C), or IV-G(A/C) LN. As shown in Table 3 above, class V LN may also occur concomitantly with class III or class IV LN. In some embodiments, the methods of the present disclosure are used to treat a patient with class III or class IV LN and concomitant class V LN.

[0193] As discussed above, a high frequency of patients with lupus (*e.g.*, SLE) eventually develop LN. In some embodiments, the patient is at risk for developing LN. In some embodiments, the patient is at risk for developing class III or class IV LN. In some

embodiments, the patient is at risk for developing class III or class IV LN with concomitant class V LN.

[0194] In some embodiments, the patient does not have class III(C) LN (*e.g.*, as described in Table 3 above). In some embodiments, the patient does not have class IV(C) LN, such as class IV-S(C) or IV-G(C) LN (*e.g.*, as described in Table 3 above).

[0195] Several lab tests known in the art may be used to diagnose and/or monitor the presence, progression, and/or response to treatment in lupus nephritis. In some embodiments, serum creatinine may be measured. In some embodiments, the normal range for serum creatinine may be from about 0.6 to about 1.3 mg/dL, with some variation seen by age, between men and women, and from lab to lab. In some embodiments, the presence of urinary sediment and/or casts may be measured, *e.g.*, by microscopic examination of urine. For example, the number of red blood cells in a urine sample may be assayed by microscopic examination. In some embodiments, a normal value for urinary sediment may be about 4 red blood cells (RBC) or less per high power field (HPF). Urinary casts may include without limitation red blood cell casts, white blood cell casts, renal tubular epithelial cell casts, waxy casts, hyaline casts, granular casts, and fatty casts. In some embodiments, a urinary protein to creatinine ratio (UPCR) may be measured. The presence of protein in the urine (proteinuria) may also be assayed by tests including without limitation a urine albumin to creatinine ratio (UACR) and dipstick urinalysis. Other tests and/or measures that may be useful for examining renal function include without limitation a renal panel, creatinine clearance, sodium, potassium, chloride, bicarbonate, phosphorus, calcium, albumin, blood urea nitrogen (BUN), creatinine, glucose, estimated glomerular filtration rate (eGFR), BUN/creatinine ratio, and anion gap, and may include a measurement of the above parameters in the blood and/or urine, where appropriate. For more detailed description, see, *e.g.*, the American College of Rheumatology Guidelines for Screening, Case Definition, Treatment and Management of Lupus Nephritis (Hahn, B. *et al.* (2012) *Arthritis Care Res.* 64:797-808).

[0196] In some embodiments, the methods of the present disclosure include administering to the individual at least a first antibody exposure to a type II anti-CD20 antibody of the present disclosure and a second antibody exposure to the type II anti-CD20 antibody. Any of the type II anti-CD20 antibodies described herein may be used, *e.g.*, a GA101 antibody such as obinutuzumab. In some embodiments, the second antibody exposure is not provided until from about 18 weeks to about 26 weeks after the first antibody exposure. In some embodiments, the second antibody exposure is not provided until about 18 weeks after the first antibody exposure, about 19 weeks after the first antibody exposure, about 20 weeks

after the first antibody exposure, about 21 weeks after the first antibody exposure, about 22 weeks after the first antibody exposure, about 23 weeks after the first antibody exposure, about 24 weeks after the first antibody exposure, about 25 weeks after the first antibody exposure, or about 26 weeks after the first antibody exposure. In some embodiments, the second antibody exposure is not provided until less than about any of the following weeks after the first antibody exposure: 26, 25, 24, 23, 22, 21, 20, or 19. In some embodiments, the second antibody exposure is not provided until greater than about any of the following weeks after the first antibody exposure: 18, 19, 20, 21, 22, 23, 24, or 25. That is, the second antibody exposure is not provided until any of a range of weeks having an upper limit of 26, 25, 24, 23, 22, 21, 20, or 19 and an independently selected lower limit of 18, 19, 20, 21, 22, 23, 24, or 25, wherein the lower limit is less than the upper limit.

[0197] The dosing regimens described herein use a consistent system for tracking time between doses whereby the first dose is administered to the patient on Day 1. As described herein, an antibody exposure of the present disclosure may include one or two doses. In cases where the antibody exposures contain one dose, references to a second antibody exposure not provided until a period of time has elapsed after a first antibody exposure (as described herein) refer to the amount of time elapsed between the dose of the first antibody exposure (*e.g.,* Day 1) and the dose of the second antibody exposure. If the first antibody exposure includes two doses, the first dose of the first antibody exposure is provided on Day 1. In cases where the antibody exposures contain two doses, references to a second antibody exposure not provided until a period of time has elapsed after a first antibody exposure (as described herein) refer to the amount of time elapsed between the first of the two doses of the first antibody exposure (*e.g.,* Day 1) and the first dose of the two doses of the second antibody exposure. For example, if a method of the present disclosure includes a first antibody exposure with two doses and a second antibody exposure with two doses, and the second antibody exposure is not provided until about 22 weeks after the first antibody exposure, then the interval between the first dose of the first antibody exposure and the first dose of the second antibody exposure is about 22 weeks.

[0198] In some embodiments, a first antibody exposure of the present disclosure includes one or two doses of a type II anti-CD20 antibody of the present disclosure. In some embodiments, the first antibody exposure contains a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody. In some embodiments, the first antibody exposure contains a total exposure of about 1800mg, about 1900mg, about 2000mg, about 2100mg, or about 2200mg of the type II anti-CD20 antibody.

[0199] In some embodiments, the first antibody exposure includes two doses. In some embodiments, the first antibody exposure includes a first dose of between about 900mg and about 1100mg of the type II anti-CD20 antibody and a second dose of between about 900mg and about 1100mg of the type II anti-CD20 antibody. In some embodiments, the first dose of the first antibody exposure contains about 1000mg of the type II anti-CD20 antibody. In some embodiments, the second dose of the first antibody exposure contains about 1000mg of the type II anti-CD20 antibody. In some embodiments, the second dose of the first antibody exposure is not provided until about 1.5 weeks to about 2.5 weeks after the first dose of the first antibody exposure. In some embodiments, the second dose of the first antibody exposure is not provided until about 2 weeks after the first dose of the first antibody exposure.

[0200] In some embodiments, a second antibody exposure of the present disclosure includes one or two doses of a type II anti-CD20 antibody of the present disclosure. In some embodiments, the second antibody exposure contains a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody. In some embodiments, the second antibody exposure contains a total exposure of about 1800mg, about 1900mg, about 2000mg, about 2100mg, or about 2200mg of the type II anti-CD20 antibody.

[0201] In some embodiments, the second antibody exposure includes two doses. In some embodiments, the second antibody exposure includes a first dose of between about 900mg and about 1100mg of the type II anti-CD20 antibody and a second dose of between about 900mg and about 1100mg of the type II anti-CD20 antibody. In some embodiments, the first dose of the second antibody exposure contains about 1000mg of the type II anti-CD20 antibody. In some embodiments, the second dose of the second antibody exposure contains about 1000mg of the type II anti-CD20 antibody. In some embodiments, the second dose of the second antibody exposure is not provided until about 1.5 weeks to about 2.5 weeks after the first dose of the second antibody exposure. In some embodiments, the second dose of the second antibody exposure is not provided until about 2 weeks after the first dose of the second antibody exposure.

[0202] In some embodiments, a type II anti-CD20 antibody of the present disclosure is administered intravenously (*e.g.*, by IV infusion).

[0203] In some embodiments, the methods of the present disclosure further include administering an effective amount of an immunosuppressive agent (*e.g.*, in conjunction with a type II anti-CD20 antibody as described herein). Several classes of immunosuppressive agents are known in the art, including without limitation cytostatics (*e.g.*, cytotoxic agents

such as antibiotics, alkylating agents (*e.g.*, cyclophosphamide, also known as cytophosphane), inosine monophosphate dehydrogenase inhibitors, antimetabolites such as protein synthesis inhibitors, folic acid analogs, purine analogs, pyrimidine analogs, and the like), immunosuppressive antibodies, glucocorticoids, drugs targeting immunophilins (*e.g.*, tacrolimus, sirolimus, rapamycin and analogs thereof, ciclosporin, and the like), mTOR active site inhibitors, mycophenolic acid and derivatives or salts thereof, TNF binding proteins, interferons, opiods, and other small molecules (*e.g.*, fingolimod). In some embodiments, the immunosuppressive agent includes mycophenolic acid, a derivative of mycophenolic acid, or a salt of mycophenolic acid. In some embodiments, the immunosuppressive agent includes mycophenolate mofetil. In some embodiments, the immunosuppressive agent includes CellCept® (Roche). In some embodiments, the immunosuppressive agent includes Myfortic® (Novartis). Effective amounts of the immunosuppressive agents of the present disclosure are known in the art and readily ascertainable by standard assays. For example, mycophenolate mofetil may be administered at 2.0-2.5g/day as illustrated in **FIG. 1**. In some embodiments, mycophenolate mofetil may be administered starting at 1000mg/day in divided doses (2 times/day) and titrating up to 2.0-2.5g/day in divided doses (2 times/day) by week 4.

[0204] In some embodiments, an immunosuppressive agent may be administered before, during, or after administration of a type II anti-CD20 antibody of the present disclosure, *e.g.*, as a treatment for lupus. In some embodiments, an immunosuppressive agent may be administered throughout the period of treatment with a type II anti-CD20 antibody of the present disclosure. In some embodiments, mycophenolate mofetil may be administered as described above throughout the period of treatment with the type II anti-CD20 antibody.

[0205] In some embodiments, the methods of the present disclosure further include administering an effective amount of a glucocorticoid or corticosteroid (*e.g.*, in conjunction with a type II anti-CD20 antibody as described herein). A variety of naturally occurring and synthetic glucocorticoids/corticosteroids are known in the art, including without limitation beclometasone, triamcinolone, dexamethasone, betamethasone, prednisone, methylprednisolone, prednisolone, cortisone, and cortisol. In some embodiments, the glucocorticoids/corticosteroid includes methylprednisolone. In some embodiments, the glucocorticoids/corticosteroid includes prednisone. Effective amounts of the glucocorticoids/corticosteroids of the present disclosure are known in the art and readily ascertainable by standard assays. For example, methylprednisolone may be administered at 750-1000mg doses once daily by IV. As another example, prednisone may be administered orally at 0.5mg/kg and optionally tapered to 7.5mg/day.

[0206] In some embodiments, a glucocorticoid may be administered before, during, or after administration of a type II anti-CD20 antibody of the present disclosure, *e.g.,* to treat LN clinical activity. In some embodiments, a glucocorticoid may be administered prior to administration of a type II anti-CD20 antibody of the present disclosure, *e.g.,* 30-60 minutes before the type II anti-CD20 antibody. In some embodiments, 80mg methylprednisolone may be administered by IV 30-60 minutes before administration of a type II anti-CD20 antibody of the present disclosure. In some embodiments, prednisone (*e.g.,* orally administered) and/or methyl prednisolone (*e.g.,* IV administered) may be administered with treatment, followed by a maintenance treatment (*e.g.,* mycophenolate mofetil or cyclophosphamide).

[0207] In some embodiments, the methods of the present disclosure further include administering an effective amount of an antihistamine (*e.g.,* in conjunction with a type II anti-CD20 antibody as described herein). Antihistamines known in the art and currently in clinical use include histamine H_1-receptor and histamine H_2-receptor antagonists or inverse agonists. In some embodiments, the antihistamine includes diphenhydramine. Effective amounts of the antihistamines of the present disclosure are known in the art and readily ascertainable by standard assays. For example, diphenhydramine may be administered in 50mg oral doses.

[0208] In some embodiments, an antihistamine may be administered before, during, or after administration of a type II anti-CD20 antibody of the present disclosure, *e.g.,* as a prophylactic treatment. In some embodiments, an antihistamine may be administered prior to administration of a type II anti-CD20 antibody of the present disclosure, *e.g.,* 30-60 minutes before the type II anti-CD20 antibody. In some embodiments, 50mg diphenhydramine may be administered orally 30-60 minutes before administration of a type II anti-CD20 antibody of the present disclosure.

[0209] In some embodiments, the methods of the present disclosure further include administering an effective amount of a non-steroidal anti-inflammatory drug or NSAID (*e.g.,* in conjunction with a type II anti-CD20 antibody as described herein). NSAIDs known in the art include acetic acid derivatives, propionic acid derivatives, salicylates, enolic acid derivatives, anthranilic acid derivatives, selective COX-2 inhibitors, sulfonanilides, and the like. In some embodiments, the NSAID includes acetaminophen. Effective amounts of the NSAIDs of the present disclosure are known in the art and readily ascertainable by standard assays. For example, acetaminophen may be administered in 650-1000mg oral doses.

[0210] In some embodiments, an NSAID may be administered before, during, or after administration of a type II anti-CD20 antibody of the present disclosure, *e.g.,* as a

prophylactic treatment. In some embodiments, an NSAID may be administered prior to administration of a type II anti-CD20 antibody of the present disclosure, *e.g.*, 30-60 minutes before the type II anti-CD20 antibody. In some embodiments, 650-1000mg acetaminophen may be administered orally 30-60 minutes before administration of a type II anti-CD20 antibody of the present disclosure.

[0211] In some embodiments, the methods of the present disclosure further include administering an effective amount of an anti-malarial agent (*e.g.*, in conjunction with a type II anti-CD20 antibody as described herein). Examples of anti-malarial agents that may be used include without limitation hydroxychloroquine, chloroquine, and quinacrine. In some embodiments, an anti-malarial agent may be administered before, during, or after administration of a type II anti-CD20 antibody of the present disclosure, *e.g.*, as a treatment for one or more symptoms of lupus.

[0212] In some embodiments, the methods of the present disclosure further include administering an effective amount of an integrin antagonist (*e.g.*, in conjunction with a type II anti-CD20 antibody as described herein). Examples of integrin antagonists that may be used include without limitation an LFA-1 antibody, such as efalizumab (RAPTIVA®) commercially available from Genentech, or an alpha 4 integrin antibody such as natalizumab (ANTEGREN®) available from Biogen, or diazacyclic phenylalanine derivatives, phenylalanine derivatives, phenylpropionic acid derivatives, enamine derivatives, propanoic acid derivatives, alkanoic acid derivatives, substituted phenyl derivatives, aromatic amine derivatives, ADAM disintegrin domain polypeptides, antibodies to alphavbeta3 integrin, aza-bridged bicyclic amino acid derivatives, etc. In some embodiments, an integrin antagonist may be administered before, during, or after administration of a type II anti-CD20 antibody of the present disclosure, *e.g.*, as a treatment for one or more symptoms of lupus.

[0213] In some embodiments, the methods of the present disclosure further include administering an effective amount of a cytokine antagonist (*e.g.*, in conjunction with a type II anti-CD20 antibody as described herein). Examples of cytokine antagonists that may be used include without limitation an antagonist (*e.g.*, an antagonist antibody) against IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-15; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). In some embodiments, a cytokine antagonist may be administered before, during, or after administration of a type II anti-CD20 antibody of the present disclosure, *e.g.*, as a treatment for one or more symptoms of lupus.

[0214] In some embodiments, the methods of the present disclosure further include administering an effective amount of a hormone (*e.g.*, in conjunction with a type II anti-CD20 antibody as described herein). In some embodiments, a hormone (*e.g.*, for hormone replacement therapy) may be administered before, during, or after administration of a type II anti-CD20 antibody of the present disclosure, *e.g.*, for a medical treatment in a women with lupus.

[0215] In some embodiments, the methods of the present disclosure further include administering a standard of care treatment (*e.g.*, in conjunction with a type II anti-CD20 antibody as described herein). In some embodiments, a standard of care treatment may be administered before, during, or after administration of a type II anti-CD20 antibody of the present disclosure, *e.g.*, for treating or preventing one or more symptoms of lupus. In certain embodiments, a standard of care treatment may be administered after a second antibody exposure of the present disclosure. For example, a type II anti-CD20 antibody of the present disclosure may be administered as described herein to a patient as an induction therapy, then the patient may be treated according to standard of care as a maintenance therapy. Standard of care treatments for lupus are well known in the art and include without limitation an angiotensin-converting enzyme (ACE) inhibitor, an angiotensin-receptor blocker, cyclophosphamide, mycophenolate mofetil (*e.g.*, at a dose as described herein, such as 2.0-2.5 g/day), azathioprine, and a glucocorticoid or corticosteroid (*e.g.*, prednisone, such as a prednisone taper).

[0216] In some embodiments, the methods of the present disclosure further include administering an anti-hypertensive agent (*e.g.*, in conjunction with a type II anti-CD20 antibody as described herein). In some embodiments, an anti-hypertensive agent may be administered before, during, or after administration of a type II anti-CD20 antibody of the present disclosure, *e.g.*, for treating or preventing hypertension. In some embodiments, anti-hypertensive agents includes without limitation ACE inhibitors and angiotensin-receptor blockers. In some embodiments, an anti-hypertensive agent listed in Table 5 is administered, *e.g.*, at a dose within the ranges described in Table 5.

[0217] In some embodiments, the methods of the present disclosure result in a complete renal response (CRR) in an individual. In some embodiments, a CRR comprises all of the following: a normalization of serum creatinine, an inactive urinary sediment, and a urinary protein to creatinine ratio of < 0.5. In some embodiments, a normalization of serum creatinine is characterized by serum creatinine less than or equal to the upper limit of normal (ULN) range of central laboratory values, and/or serum creatinine $\leq 15\%$ above baseline and

less than or equal to the ULN range of central laboratory values if baseline (*e.g.*, Day 1) serum creatinine is within the normal range of the central laboratory values. In some embodiments, an inactive urinary sediment is characterized by < 10 RBCs/high-power field (HPF) and/or the absence of red cell casts. For more detailed discussion of CRR and partial renal response (PRR) in LN, see, *e.g.*, Chen, Y.E. *et al.* (2008) *Clin. J. Am. Soc. Nephrol.* 3:46-53.

[0218] In some embodiments, the methods of the present disclosure result in a complete renal response (CRR) or a partial renal response (PRR) in an individual. In some embodiments, a PRR comprises one or more of the following: a normalization of serum creatinine, an inactive urinary sediment, and a urinary protein to creatinine ratio of < 0.5. In some embodiments, a PRR comprises one or more of the following: mitigation of one or more symptoms including without limitation a reduction in serum creatinine, reduced urinary sediment, a reduction in proteinuria, and any other improvement in renal function. In some embodiments, a CRR or PRR comprises a reduction in one or more biomarkers of lupus activity, including without limitation anti-dsDNA antibodies, antinuclear antibodies/ENA, anti-complement antibodies, reduced levels of complement C3 and/or C4, and reduced complement activity (*e.g.*, as measured by CH50 assay).

[0219] In some embodiments, the methods of the present disclosure result in a depletion of circulating peripheral B cells in an individual. In some embodiments, after administration of a type II anti-CD20 antibody of the present disclosure (*e.g.*, according to any of the methods described herein), circulating peripheral B cells are present in peripheral blood at about 10 cells/μL or fewer, about 9 cells/μL or fewer, about 8 cells/μL or fewer, about 7 cells/μL or fewer, about 6 cells/μL or fewer, about 5 cells/μL or fewer, about 4 cells/μL or fewer, about 3 cells/μL or fewer, about 2 cells/μL or fewer, or about 1 cell/μL or fewer. In some embodiments, circulating peripheral B cells in the individual are depleted by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%. In some embodiments, depletion of circulating peripheral B cells refers to a measurement of circulating peripheral B cells taken after a first antibody exposure (*e.g.*, including 1 or 2 doses of an anti-CD20 antibody as described herein), after a second antibody exposure (*e.g.*, including 1 or 2 doses of an anti-CD20 antibody as described herein), 3 months after treatment (*e.g.*, after receiving a first and/or a second antibody exposure as described herein), 6 months after treatment (*e.g.*, after receiving a first and/or a second antibody exposure as

described herein), 9 months after treatment (*e.g.*, after receiving a first and/or a second antibody exposure as described herein), or 12 months after treatment (*e.g.*, after receiving a first and/or a second antibody exposure as described herein), *e.g.*, as compared to a corresponding measurement in the same individual before treatment, or as compared to a corresponding measurement in a control individual (*e.g.*, an individual that has not received treatment).

[0220] Methods for assaying depletion of circulating peripheral B cells in an individual are known in the art, *e.g.*, flow cytometry using one or more antibodies that recognize a B cell marker. In some embodiments, highly sensitive flow cytometry (HSFC) may be used to assay depletion of circulating peripheral B cells (see, *e.g.*, Vital, E.M. *et al.* (2011) *Arthritis Rheum.* 63:3038-3047). In some embodiments, the B cells are CD19+ B cells. In some embodiments, the B cells are naïve B cells (*e.g.*, CD19+ CD27- B cells), memory B cells (*e.g.*, CD19+ CD27+ B cells), or plasmablasts (*e.g.*, CD19+ CD27+ CD38++ B cells).

IV. Articles of Manufacture or Kits

[0221] In another aspect of the invention, an article of manufacture or kit containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture or kit comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody described herein (*e.g.*, a type II anti-CD20 antibody of the present disclosure). The label or package insert indicates that the composition is used for treating the condition of choice, *e.g.*, according to any of the methods described herein. Alternatively, or additionally, the article of manufacture or kit may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from

a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0222] In some embodiments, provided herein is a kit comprising a container comprising a type II anti-CD20 antibody of the present disclosure and an optional pharmaceutically acceptable carrier, and, optionally, a package insert comprising instructions for treating or delaying progression of lupus nephritis in an individual, *e.g.,* wherein the instructions indicate that at least a first antibody exposure to a type II anti-CD20 antibody and a second antibody exposure to the type II anti-CD20 antibody are administered to the individual, the second antibody exposure not being provided until from about 18 weeks to about 26 weeks after the first antibody exposure; wherein the first antibody exposure comprises one or two doses of the type II anti-CD20 antibody, the first antibody exposure comprising a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody; and wherein the second antibody exposure comprises one or two doses of the type II anti-CD20 antibody, the second antibody exposure comprising a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody. In some embodiments, provided herein is a kit comprising a container comprising a type II anti-CD20 antibody of the present disclosure and an optional pharmaceutically acceptable carrier, and, optionally, a package insert comprising instructions for treating or delaying progression of class III or class IV lupus nephritis in an individual. In some embodiments of any of the above embodiments, the type II anti-CD20 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:1, HVR-H2 sequence of SEQ ID NO:2, and HVR-H3 sequence of SEQ ID NO:3, and a light chain comprising HVR-L1 sequence of SEQ ID NO:4, HVR-L2 sequence of SEQ ID NO:5, and HVR-L3 sequence of SEQ ID NO:6. In some embodiments of any of the above embodiments, the type II anti-CD20 antibody is obinutuzumab.

[0223] The article of manufacture may still further comprise a second or third container comprising a second medicament, wherein the anti-CD20 antibody (*e.g.,* a type II anti-CD20 antibody of the present disclosure) is a first medicament, where the article further comprises instructions on the package insert for treating the subject with the second medicament. Exemplary second medicaments include a chemotherapeutic agent, an immunosuppressive agent, an anti-malarial agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, a hormone, and any of the treatments that may be used in conjunction with a type II anti-CD20 antibody as described herein. The article of manufacture in these embodiments may further comprise a package insert indicating that the compositions can be used to treat a particular condition.

[0224] It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-CD20 antibody.

[0225] The specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

EXAMPLES

[0226] The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: A Pharmacology Study of Obinutuzumab Administered with Mycophenolate Mofetil in Patients with Class III/IV Lupus Nephritis

Study Design

[0227] This Phase II study is designed to assess the safety and efficacy of obinutuzumab (*i.e.,* a type II anti-CD20 antibody) as an add-on therapy to mycophenolate mofetil (MMF) in patients with active ISN/RPS Class III/IV lupus nephritis (LN). The Phase II study is a parallel-group, double-blind, randomized, placebo-controlled study comparing the efficacy and safety of obinutuzumab plus MMF with placebo plus MMF in Class III and IV patients with proliferative LN (**FIG. 1**).

[0228] The study is also a prospective, multicenter study. Patients diagnosed with ISN/RPS Class III or IV LN, in some embodiments with a diagnosis of SLE according to current ACR criteria (at least 4 criteria must be present, one of which must be a positive anti-nuclear antibody), are enrolled in centers throughout the world. The study includes standard-of-care therapy with angiotensin-converting enzyme (ACE) inhibitors/angiotensin II receptor blockers, MMF (dosed at 2.0–2.5 g/day), and a prednisone taper.

[0229] As described in greater detail below, patients are 18–75 years of age and have ISN/RPS 2003 Class III or IV proliferative LN (see Weening, JJ (2004) *J. Am. Soc. Nephrol.* 15:241-250) as evidenced by renal biopsy performed within 6 months prior to screening and may have concomitant Class V disease (e.g., Class III/V or Class IV/V). Patients with Class III (C) or Class IV (C) disease are excluded because of the lower likelihood of response within these categories.

[0230] Inclusion criteria for the study include:

(a) Signed Informed Consent Form;

(b) Age 18-75 years;

(c) Ability to comply with study protocol;

(d) Diagnosis of systemic lupus erythematosus (SLE) according to current ACR criteria (at least 4 criteria must be present, one of which must be a positive anti-nuclear antibody);

(e) Diagnosis of ISN/RPS 2003 Class III or IV LN as evidenced by renal biopsy performed within 6 months prior to screening (patients may also co-exhibit Class V disease in addition to either Class III or Class IV disease);

(f) Demonstration of active urinary sediment as evidenced by ≥ 10 RBCs/HPF or the presence of red cell casts; and

(g) Proteinuria (urine protein to creatinine ratio > 1.0, based on a 24-hour urine collection).

[0231] Key exclusion criteria include:

(a) Retinitis, poorly controlled seizure disorder, acute confusional state, myelitis, stroke or stroke syndrome, cerebellar ataxia, or dementia that is currently active and resulting from SLE;

(b) Presence of rapidly progressive glomerulonephritis (defined by the presence of crescent formation in ≥ 50% of glomeruli assessed on renal biopsy or the doubling of serum creatinine within 12 weeks of screening);

(c) Severe renal impairment as defined by estimated GFR < 30 mL/min or the need for dialysis or renal transplant;

(d) Greater than 50% of glomeruli with sclerosis on renal biopsy;

(e) Treatment with cyclophosphamide or calcineurin inhibitors within the 3 months prior to randomization; and

(f) Unstable disease with thrombocytopenia or at high risk for developing clinically significant bleeding or organ dysfunction requiring therapies such as plasmapheresis or acute blood or platelet transfusions.

[0232] Patients receive an initial 1000 mg of methylprednisolone intravenously (IV) prior to or during screening, and may receive up to 3000 mg methylprednisolone IV prior to randomization for severe clinical activity according to guidelines of routine care for these patients. Patients receive 80 mg methylprednisolone (or methylprednisolone placebo) IV on the day of the obinutuzumab/placebo infusion to reduce infusion-related events. The oral prednisone taper is 0.5 mg/kg and is reduced over 12 weeks. This modified taper is initiated in recognition that prednisone doses above 10 mg/day are associated with significant adverse events, including increased risk of cardiovascular events (Bichile, T. and Petri, M. (2014) *Presse Med.* 43:e187-195). Prior experience with rituximab suggests that it can potentially enable complete and partial renal responses in the absence of oral prednisone or a prednisone taper, thus allowing the use of lower doses of corticosteroids (Condon, M.B. *et al.* (2013) *Ann. Rheum. Dis.* 72:1280-1286).

[0233] Patients are followed for 12 months until the primary endpoint evaluation, and an interim analysis at 6 months is performed to evaluate early differences in CRR. All patients have central reading of the renal biopsy histopathology and also receive repeat renal biopsy as available on the basis of clinical status and local practice. All patients are evaluated by high sensitivity flow cytometry (HSFC) to evaluate the ability of obinutuzumab to deplete circulating peripheral B cells, and an interim PD analysis is performed to assess whether patients do not fully deplete peripheral CD19+ B cells as anticipated.

Dosing and Non-Investigational Medicinal Products

[0234] The dosing regimen for the study is obinutuzumab administered by IV infusion at a dose of 1000 mg on Days 1, 15, 168, and 182 (test group); or obinutuzumab placebo (*e.g.*, saline IV, corresponding to the obinutuzumab1000-mg dose) administered by IV infusion on Days 1, 15, 168, and 182. The obinutuzumab/placebo is administered in a hospital or clinic environment where full resuscitation facilities are immediately available and under close supervision of the investigator or designee. After the end of the infusion, the IV line remains in place for at least 1 hour to enable administration of IV drugs if necessary. If no adverse events occur during this period of time, the IV line may be removed.

[0235] After screening, patients who are not already receiving MMF receive 1500 mg/day MMF in divided doses (2-3 times/day), and all patient doses are titrated up to a target dose of 2.0–2.5 g/day in divided doses (2-3 times/day) by Week 4, as tolerated. If reductions in dose are necessary, decreases are allowed in 250–500 mg decrements. During screening or at

randomization, if clinically indicated, patients may receive 750–1000 mg methylprednisolone IV once daily for up to three days to treat underlying LN clinical activity. Patients receive 0.5 mg/kg oral prednisone during screening or at randomization, tapering this prednisone dose, per protocol, starting on Day 16 and reducing the prednisone dosage to 7.5 mg/day by Week 12. These treatments are described in further detail below.

Concomitant Therapy and Clinical Practice

[0236] Patients who are not already taking vitamin D (400 IU/day) and calcium supplements (1200 mg/day of calcium citrate or 1500 mg/day of calcium carbonate) begin taking these supplements at randomization. All patients take either an angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker titrated to adequate blood pressure control as recommended by the National Kidney Foundation for chronic kidney disease. Other agents that affect proteinuria are not allowed to be initiated during the study, including but not limited to non-dihydropyridine calcium antagonists, dihydropyridine calcium antagonists, aldosterone antagonists, and direct renin antagonists.

Mycophenolate Mofetil (MMF)

[0237] All patients continue on or initiate use of MMF during screening or no later than Day 1. The initial dosage is 1500 mg/day by mouth, given in two or three divided doses and titrated upward to 2.0-2.5 g/day in divided doses by Week 4. MMF may be increased by 500 mg/week, as tolerated up to a maximum dosage of 2.5 g/day. Reductions are allowed because of adverse effects.

[0238] Newly diagnosed patients with LN with no prior exposure to MMF are recommended to initiate an induction agent (MMF or cyclophosphamide) and then be re-assessed for eligibility. A proportion of patients who are initially treated with MMF or cyclophosphamide achieve CRR and therefore have minimal need for added immunosuppression (Dall'Era, M. *et al.* (2011) *Arthritis Care Res.* 63:351-357).

[0239] For those patients who enter the study already receiving a dosage of MMF higher than 1500 mg/day, MMF will be titrated upward, as tolerated, to a goal of 2.5 g/day, given in divided doses, by Week 4. A patient's current dose of MMF is given in 2 or 3 divided doses and increased by 500 mg/week as tolerated.

Corticosteroid Administration

[0240] All patients receive a combination of IV and oral corticosteroids as part of their initial therapy for LN. Methylprednisolone (*e.g.*, Solu-Medrol®) is implemented for two purposes: as part of the usual care for patients with active Class III or IV LN and also to reduce infusion-related reactions (IRRs) on the days of obinutuzumab/placebo infusions. Up to three doses of IV methylprednisolone 1000 mg are given on the basis of investigator judgement and local practice. Up to three 1000 mg infusions may have been initiated prior to screening or during the screening interval.

[0241] On Days 1, 15, 168, and 186, patients receive 80 mg IV methylprednisolone or placebo 30-60 minutes prior to study drug infusion to prevent IRRs. Additionally, oral prednisone may be initiated before or during the screening interval, and a taper commences on Day 2. From Days 2 to 16, 0.50mg/kg/day oral prednisone is given (maximum dose 60 mg), except on the day of IV methylprednisolone/placebo infusions, and will continue until Day 16. From Day 16 onward, a prednisone taper commences.

[0242] All patients undergo a scheduled corticosteroid taper commencing on Day 16. Patients fractionally reduce their prednisone dose over 12 weeks until the dose is 7.5 mg/day by Week 12. After 14 weeks of tapering, patients continue on prednisone at 7.5mg/day or less. In patients whose disease is too clinically active for the patient to make the first step in their prednisone taper, as evidenced by active urinary sediment, rising serum creatinine, or moderate-to-severe extra-renal symptoms, these patients may continue to receive their initial prednisone dose for up to an additional 28 days.

[0243] To maintain consistency in the treatment of renal flares, retreatment with higher doses of corticosteroids is permitted if judged clinically appropriate by the investigator and if patients meet criteria for a renal flare. Patients may be treated with prednisone (up to 0.5mg/kg; not to exceed 60 mg/day) for 2 weeks. Prednisone is then tapered to achieve 10mg/day within 6 weeks after initial corticosteroid increase. Patients are allowed to receive corticosteroids for emergent illness (trauma, severe asthma) or surgery, if clinically warranted; the corticosteroid use is limited to a total of ≤ 7 days, if possible. Investigators are then allowed to increase the prednisone dose by ≤ 2.5mg/day to treat symptoms of adrenal insufficiency or corticosteroid withdrawal, after the patient's dosage has been tapered to 10 mg/day.

[0244] Patients who experience a severe extra-renal SLE flare may receive treatment with additional oral corticosteroids, if judged clinically appropriate by the investigator. These

patients may be retreated with prednisone (up to 1.0mg/kg) for up to 2 weeks on the basis of the severity of disease and organ system involvement and the dosage is tapered to 7.5mg/day. Patients experiencing a mild or moderate extra-renal flare may temporarily increase their prednisone dose by up to 20mg per day and taper this dose over 4 weeks, if judged clinically appropriate by the investigator. IV corticosteroids in equivalent doses are allowed if gastrointestinal involvement temporarily precludes treatment with oral corticosteroids.

Anti-Malarial Medications

[0245] Patients taking anti-malarial medications at study entry maintain a constant dosage throughout the study. Patients not previously on anti-malarial medications may be enrolled in the study but should not initiate anti-malarial medications unless experiencing a disease flare that is unresponsive to corticosteroids. Table 4 lists anti-malarial medications and dose ranges expected to be used during the course of the study.

Table 4. Anti-malarial medications

Anti-malarial Medication	Dose Range (oral)
Hydroxychloroquine	200-400 mg daily
Chloroquine	500 mg every day or every other day
Quinacrine	100 mg every day

Antihypertensive Therapy

[0246] All patients who are not currently taking either an ACE inhibitor or an angiotensin-receptor blocker should be started on one at screening. Patients are on either an ACE inhibitor or angiotensin-receptor blocker for at least 10 days prior to randomization. Combination therapy with the two agents is not allowed.

[0247] During screening, every effort is made to adequately control patients' blood pressures. The dose of the ACE inhibitor or angiotensin-receptor blocker may be titrated upward to the maximum recommended dose in the current package insert to achieve adequate blood pressure control as recommended by the Eighth Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (James, P.A. *et al.* (2014) *JAMA* 311:507-520). If adequate blood pressure control is not achieved, patients may be started on additional antihypertensive agents but not on agents that affect proteinuria (e.g., nondihydropyridine calcium channel blockers, aldosterone

antagonists, direct renin antagonists). Additional agents that specifically target the renin-angiotensin system are not initiated during the study. Suggested dose ranges for specific ACE inhibitors and angiotensin-receptor blockers are listed in Table 5. If patients are intolerant to ACE inhibitors and angiotensin-receptor blockers, they may use either a direct renin inhibitor or aldosterone antagonists, but not in combination.

Table 5. Suggested Dose Ranges for ACE Inhibitors and Angiotensin-Receptor Blockers

ACE Inhibitor or Angiotensin-Receptor Blocker	**Dose Range (Oral), mg/day**
ACE Inhibitors	
Benazepril	10-80
Ramipril	2.5-10
Lisinopril	10-80
Enalapril	10-40
Quinapril	10-80
Captopril	75-450
Perindopril	4-16
Trandolapril	1-8
Moexipril	7.5-30
Angiotensin-Receptor Blockers	
Eprosartan	400-600
Valsartan	80-320
Olmesartan	20-40
Candesartan	8-32
Telmisartan	20-80
Losartan	25-100
Irbesartan	75-300

Study Objectives and Outcome Measures

[0248] The primary objective of this proof-of-concept study is to measure complete renal response (CRR) at 52 weeks with administration of obinutuzumab. The ability of obinutuzumab plus MMF to achieve a CRR at week 52 is compared to placebo plus MMF

and assessed by improvements in renal function, urinary sediment, and proteinuria. Secondary objectives include evaluations of the safety of obinutuzumab in this patient population, the ability of obinutuzumab to induce an overall response (CRR+PRR) at Week 52, the ability of obinutuzumab to improve time-to-response (CRR+PRR) over the course of 52 weeks, and the ability of obinutuzumab to improve biomarkers of LN disease activity (*e.g.*, reduced anti-dsDNA antibody levels, increased C3 and C4 levels; see Tew, G.W. *et al.* (2010) *Lupus* 19:146-157).

[0249] The primary efficacy outcome measure is the proportion of subjects who achieve a CRR, evaluated at 52 weeks. In this study, CRR is defined by attainment of all of the following:

(a) Normalization of serum creatinine as evidenced by the following:

(i) Serum creatinine less than or equal to the upper limit of normal (ULN) range of central laboratory values if the baseline (Day 1) is not within the normal range of the central laboratory values; and

(ii) Serum creatinine ≤ 15% above baseline and less than or equal to the ULN range of central laboratory values if baseline (Day 1) serum creatinine is within the normal range of the central laboratory values;

(b) Inactive urinary sediment (as evidenced by < 10 RBCs/high-power field (HPF) and the absence of red cell casts); and

(c) Urinary protein to creatinine ratio < 0.5.

[0250] Any patient who switches to rescue medication prior to Week 52 is considered a non-responder. The proportions of patients achieving CRR across treatment groups is compared using a Cochrane-Mantel-Haenzel (CMH) test with race (Afro-Caribbean/African-American versus others) and region (United States versus non-United States) as stratification factors. If the test result is in favor of the obinutuzumab group at $\alpha<0.1$-level (one-sided), a shift toward better renal response associated with the obinutuzumab group is concluded.

[0251] The secondary efficacy outcome measures are the following:

(a) Proportional analysis of patients who achieve an overall response at Week 52 (CRR + PRR);

(b) Time to overall response (CRR + PRR) over the course of 52 weeks;

(c) Percent reduction or increase from baseline and mean and median assessments of biomarkers of LN disease activity (e.g., reduction in anti-dsDNA antibody levels, increased C3 and C4 levels);

(d) Proportion of patients that achieve a PRR at week 52 as defined by attainment of all of the following:

(i) serum creatinine ≤ 15% above baseline levels;

(ii) RBCs/HPF ≤ 50% above baseline and no red cell casts;

(iii) 50% improvement in the urine protein to creatinine ratio, with one of the following conditions met:

(A) if the baseline urine protein to creatinine ratio is ≤ 3.0, then a urine protein to creatine ratio of < 1.0;

(B) if the baseline protein to creatinine ratio is >3.0, then a urine protein to creatinine ratio of < 3.0;

(e) Proportion of patients who achieve a CRR at Week 24;

(f) Time to CRR, over the course of 52 weeks;

(g) Proportion of patients that achieve a modified CRR (mCRR1) at Week 52 employing the primary-efficacy measure definition and removing the urinary sediment analysis criteria

mCRR1 refers to attainment of normalization of serum creatinine as evidenced by the following:

(i) Serum creatinine less than or equal to the ULN range of central laboratory values;

(ii) Serum creatinine ≤ 15% above baseline and less than or equal to the ULN range of central laboratory values if baseline (Day 1) serum creatinine is within the normal range of the central laboratory values;

(iii) Urinary protein to creatinine ratio < 0.5;

(h) Proportion of patients that achieve a second CRR (mCRR2) at Week 52 as defined by attainment of the following:

(i) Normalization of serum creatinine as evidenced by the following:

(A) Serum creatinine ≤ the ULN range of central laboratory values;

(B) Serum creatinine ≤ 15% above baseline if baseline (Day 1) serum creatinine is above the normal range of the central laboratory values OR S ≤ the ULN range of central laboratory values if baseline (Day 1) serum creatinine is within the normal range of the central laboratory values;

(ii) Inactive urinary sediment (as evidenced by < 10 RBCs/HPF and the absence of red cell casts);

(iii) Urinary protein to creatinine ratio < 0.5.

[0252] The pharmacodynamic (PD) objective is to compare changes in CD19+ B cells in the peripheral blood following treatment with obinutuzumab versus placebo. Levels of circulating CD19+ B-cells are measured at screening and Days 15, 28, 84, 168, 364, and 728.

[0253] The pharmacokinetic (PK) objectives are to characterize the pharmacokinetics of obinutuzumab in the LN population and to assess potential PK interactions between obinutuzumab and concomitant medications, including mycophenolate mofetil (MMF). Non-linear mixed-effects modeling (with software NONMEM) is used to analyze the dose-concentration-time data of obinutuzumab. The PK profile data is used to further develop a PK model, including the effect of major covariates (*e.g.*, sex, race/ethnicity, weight, biochemical and hematological parameters at baseline, degree of underlying disease), on the main parameters (*e.g.*, clearance). Derivation of individual measures of exposure, such as area under the concentration-time curve (AUC) and maximum concentration observed (C_{max}) depend on the final PK model used. Serum obinutuzumab is summarized (mean, minimum, maximum, SD, and geometric mean) and reported.

[0254] The exploratory objectives for the study include evaluation of pre-dose levels of exploratory biomarkers (including but not limited to B-cell subsets and levels of protein and/or mRNA in serum, blood, and urine) and potential associations with outcome, evaluation of changes in exploratory biomarkers (including but not limited to B-cell subsets and levels of protein and/or mRNA in serum, blood, and urine) over time in patients dosed with obinutuzumab versus placebo, evaluation of the occurrence of extrarenal flares, evaluation of the impact of therapy on patient and physician-reported outcomes, and assessment of renal biopsy histopathology (*e.g.*, for the presence of CD19+ B cells at the screening and/or subsequent biopsies). The exploratory outcome measures include:

(a) levels of circulating B-cell subsets at Screen and Days 15, 28, 84, 168, 364, and 728;

(b) levels of exploratory biomarkers (including but not limited to B-cell subsets and levels of protein and/or mRNA in serum, blood, and urine) at Screen and Days 1, 15, 28, 84, 168, 252, 364, 532, and 728;

(c) proportion of patients experiencing a Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)-2K flare;

(d) proportion of patients experiencing a renal flare over 52 weeks and 104 weeks;

(e) proportion of patients achieving CRR, mCRR1, mCRR2 at additional timepoints (including Week 12 and 36);

(f) Physician's Global Assessment (visual analog scale captured in screening, at the baseline visit, and at several timepoints during study conduct); and

(g) renal biopsy evaluations.

Laboratory, Biomarker, and Other Biological Samples

[0255] The following laboratory assessments are recorded during the study:

(a) Hematology: hemoglobin, hematocrit, RBC, mean corpuscular volume, mean corpuscular hemoglobin, WBC (absolute and differential), and quantitative platelet count;

(b) Blood chemistry: AST/SGOT, ALT/SGPT, alkaline phosphatase, amylase, lipase, total protein, albumin, cholesterol, total bilirubin, urea, uric acid, creatinine, random glucose, potassium, sodium, chloride, calcium, phosphorus, lactic dehydrogenase, CPK, and triglycerides;

(c) Urinalysis: dipstick for blood, nitrate, protein, and glucose and urine microscopy;

(d) 24-hour urine collection (analyzed for total protein, total creatinine, and creatinine clearance) to be performed at randomization and at Months 3, 6, 9, and 12;

(e) Flow cytometry: B cell (including CD19, CD27, CD38, and IgD), T cell (CD3, CD4, CD8), and NK cells (CD16, CD56);

(f) Autoantibody profile: anti-nuclear antibody (ANA), anti-dsDNA, anti-Sm, anti-RNP, anti-Ro, anti-La, and anti-C1q;

(g) Anti-dsDNA antibody: measured by ELISA at all visits as part of SLEDAI-2K assessment;

(h) Quantitative immunoglobulin: total Ig levels including IgG, IgM, and IgA isotypes;

(i) Complement: C3, C4, and CH50;

(j) Antibody titers: antibody titers to common antigens (rubella, tetanus, influenza, *S. pneumoniae*); and

(k) Pregnancy test: urine pregnancy test performed at screening and prior to each study drug infusion. Infusion is not administered unless test is negative. At all other timepoints, urine pregnancy test is performed on the basis of menstrual history and pregnancy risk.

[0256] The following samples are sent for analysis: cells from blood and urine for B-cell and lupus-related biomarkers (including but not limited to CD19+ B cells and mRNA associated with B-cell activity), serum and urine for B-cell and lupus-related biomarkers (including but not limited to B-cell activating factor or BAFF), and renal biopsy slides for immunohistopathology assessment.

Infusions

[0257] Prior to each infusion of either study drug or placebo, patients receive prophylactic treatment with acetaminophen (650–1000 mg) and diphenhydramine (50 mg; or equivalent dose of a similar agent) by mouth, given 30–60 minutes before the start of the infusion period. The patients who are receiving obinutuzumab receive 80 mg methylprednisolone IV and patients who are receiving placebo receive placebo-methylprednisolone IV given 30–60 minutes before the start of the obinutuzumab/placebo infusion. If a patient experiences a mild infusion-related reaction (IRR) that is deemed by the investigator to be clinically significant, the infusion rate should be reduced to half of the initial infusion rate (in compliance with non-Hodgkin's lymphoma protocol infusion rates and schedules). After the reaction has resolved, the infusion should be kept at the reduced rate for an additional 30 minutes. If the reduced rate is tolerated, then the infusion rate may be increased to the next closest rate on the infusion schedule. Patients who experience a severe IRR should have their infusion interrupted immediately and should receive aggressive symptomatic treatment. The infusion should not be restarted until all of the symptoms have disappeared. Upon restarting the infusion, the rate should be half of that which precipitated the reaction. Instructions for administration of obinutuzumab infusions are provided in Table 6 below.

Table 6. Administration of obinutuzumab infusions.

First Infusion (Day 1)	Subsequent Infusions
Begin infusion at an initial rate of 50 mg/hr. If no infusion reaction occurs, increase the infusion rate in 50-mg/hr increments every 30 minutes to a maximum of 400mg/hr. If an infusion reaction develops, stop or slow the infusion. Administer infusion-reaction medications and supportive care in accordance with institutional protocol. Resume the infusion at a 50% reduction in rate (the rate being used at the time that the hypersensitivity or infusion-related reaction occurred) if the reaction has resolved.	If a patient experienced an infusion reaction during the prior infusion, start at the same rate as the first infusion (50 mg/hr) and follow those directions as noted. If the patient tolerated the prior infusion well, begin infusion at a rate of 100mg/hr. If no infusion reaction occurs, increase the infusion rate in 100-mg/hr increments every 30 minutes to a maximum of 400mg/hr. If an infusion reaction develops, stop or slow the infusion. Administer infusion-reaction medications and supportive care in accordance with institutional protocol. Resume the infusion at a 50% reduction in rate (the rate being used at the time that the hypersensitivity or infusion-related reaction occurred) if the reaction has resolved.

[0258] All renal biopsies and reports that are obtained as part of study entry are photomicrographed and sent to an online central reading portal for oversight of the

histopathologic assessment performed by the local renal histopathologist. An expert panel assesses these biopsies and adjudicates a final interpretation. Every effort is made to complete this process while patients are in screening but is not mandatory for completion of screening activities. All new biopsies obtained during screening or during the study are processed in a manner to enable immunohistochemical staining of the tubulointerstitium for the presence of B cells. The study encourages but does not mandate the performance of repeat renal biopsies for patients that have not achieved a CRR, and seeks to enrich for study centers that perform repeat renal biopsies.

Example 2: Obinutuzumab outperforms Rituximab at Inducing B-Cell Cytotoxicity in Rheumatoid Arthritis and Systemic Lupus Erythematosus patient samples through Fc gamma receptor-dependent and independent effector mechanisms

[0259] A proportion of Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) patients treated with standard doses of rituximab (RTX) display inefficient B cell deletion and poor clinical responses which can be augmented by delivering higher doses, indicating that standard-dose RTX is a sub-optimal therapy in these patients. To investigate whether better responses could be achieved with other anti-CD20 mAbs, a comparison was made between RTX with Obinutuzumab (OBZ), a new-generation, glycoengineered type II anti-CD20 mAb in a series of in vitro assays measuring B cell cytotoxicity in SLE and RA samples. It was found that OBZ was at least 2-fold more efficient than RTX at inducing B-cell cytotoxicity in in-vitro whole blood assays. Dissecting this difference it was found that RTX elicited more potent complement-dependent cellular cytotoxicity (CDC) than OBZ. In contrast, OBZ was more effective at evoking Fc gamma receptor (FcγR)-mediated effector mechanisms including activation of NK cells and neutrophils. OBZ was also more efficient at inducing direct cell death. This was true for all CD19+ B-cells as a whole and in naïve (IgD+CD27-); and switched (IgD-CD27+) memory B-cells specifically, a higher frequency of which is associated with poor clinical response after RTX.

Materials and Methods

Patients

[0260] All participants of this study provided consent according to the declaration of Helsinki approved by the local research ethics committee. All patients with RA satisfied the American College of Rheumatology (ACR)/European League Against Rheumatism classification criteria (Aletaha D. et al. 2010 Ann Rheum Dis. 2010;69(9):1580-8) and all patients with SLE met the ACR classification criteria (Petri M. et al. Arthritis Rheum. 2012;64(8):2677-86).

Antibodies and Reagents

[0261] Anti-CD20 mAbs used in the studies included RTX, OBZ and non-glycoengineered, wild type glycosylated OBZ (OBZ_{Gly}) and in some experiments OBZ with a mutated Fc portion (P329G LALA) that does not engage any Fc related effector functions (Herter S. et al. Cancer Research. 2015;75(15 Supplement):2460), OBZ-PG LALA. Roche Innovation Center Zürich, Switzerland generated all anti-CD20 mAbs except RTX, which was a kind gift from the pharmacy of University College Hospital, U.K. AT10, an FcγRII antagonist (Greenman J. et al. Mol Immunol. 1991;28(11):1243-54), was produced in-house.

Flow Cytometry and B Cell Isolation

[0262] Fluorochrome-conjugated mAb were procured from Becton Dickinson biosciences or Biolegend, U.K.): CD3 (phycoerythrin [PE]-Cy 7), CD15 (fluorescein isothiocyanate, FITC): CD16 (Allophycoyanin, APC), CD19 (Alexa Fluor 700), CD45 (PE), CD56 (PE), CD 107a (Brilliant Violet 421), CD11b (PE), CD62L (APC), propidium iodide and Annexin V (FITC). Flow cytometry was performer using a Becton Dickinson LSR Fortessa cell analyzer. Lymphocytes were identified based on forward- and side-scatter characteristics. B cells were identified as CD19+ or CD20+, T cells as CD3+ and NK cells as CD3-56+. Neutrophils were identified based on forward- and side-scatter characteristics and CD15 positivity. The mean fluorescent intensity (MFI) of CD11b and CD62L in samples incubated with mAbs was compared with that in samples incubated without antibodies.

[0263] In all experiments, peripheral blood mononuclear cells (PBMC) were separated from whole blood samples by Ficoll-Hypaque density gradient and B-cells were isolated from PBMC using EasySep™ Human B Cell Enrichment Kit (Cambridge, U.K.).

Whole Blood B-cell Depletion Assays

[0264] Briefly, 300µl of freshly drawn whole blood anticoagulated with heparin was incubated with or without mAbs at 1µg/mL for 24 hours at 37°C and 5% CO_2. Samples were then stained with anti-CD3, anti-CD19 and anti-CD45 before lysing red cells and analyzing on flow cytometer, as described previously (Reddy V. et al. Arthritis & rheumatology.

2015;67(8):2046-55). The % B-cell depletion was calculated from the proportion of B cells to T cells remaining after treatment and defined as the cytotoxicity index (CTI) as described previously (Mossner E. et al. Blood. 2010;115(22):4393-402, and Reddy V. et al. Arthritis & rheumatology. 2015;67(8):2046-55).

Surface Fluorescence-quenching Assays

[0265] Surface fluorescence-quenching assays were performed as described previously (Beers S.A. et al. Blood. 2008;112(10):4170-7, and Reddy V. et al. Arthritis & rheumatology. 2015;67(8):2046-55) to assess internalization of mAbs by B-cells. Isolated B-cells were incubated for 6 hours with Alexa-488 conjugated mAbs at a concentration of 5 μg/mL before analyzing by flow cytometry.

Complement-dependent Cellular Cytotoxicity Assays

[0266] CDC assays were performed as previously described (Cragg M.S. et al. Blood. 2004;103(7):2738-43). Isolated B cells were incubated with mAbs at a concentration of 10μg/mL for 30 minutes at 37°C and 5% CO_2. Samples were stained with fluorescence conjugated anti-CD19 antibodies, Annexin V (Av) and propidium iodide (PI) and the frequency of CD19+Av+PI+ cells assessed by flow cytometry. Freshly collected normal healthy human serum was used as a source of complement. To define the activity relating to complement, part of the serum was heat inactivated (HIS) at 56°C for 30 minutes. The ability of mAbs to activate complement and lyse target cells was assessed by the relative frequency of CD19+Av+PI+ cells in samples incubated either with normal healthy serum or HIS.

Direct Cell Death

[0267] Isolated B-cells were incubated in RPMI supplemented with 10% heat inactivated foetal calf serum with or without mAbs at a concentration of 10μg/mL for 6 hours at 37°c and 5% CO_2. The frequency of CD19+Av+ cells in samples with mAbs compared with that in samples without mAbs represented the ability of mAbs to induce direct cell death.

NK Cell Degranulation Assays

[0268] NK cell degranulation was assessed using samples from the whole blood B-cell depletion assay by measuring the expression of CD107a or LAMP-1 (a lysosome associated membrane protein 1), which is up regulated upon activation of NK cells and correlates with NK cell mediated ADCC (Alter G. et al. J Immunol Methods. 2004;294(1-2):15-22, and Aktas E. et al. Cell Immunol. 2009;254(2):149-54). Therefore, the frequency of CD3-56+107a+ NK cells in samples with mAbs were compared with that in samples incubated without mAbs. Activation of NK cells is associated with an increased activity of

metalloproteinase, which cleaves CD16 reducing its expression upon NK cell activation (Romee R. et al. Blood. 2013;121(18):3599-608). Therefore the extent of CD16 loss was also used as an indirect measure of NK cell activation (Grzywacz B. et al. Leukemia. 2007;21(2):356-9; author reply 9, and Bowles J.A. et al. Blood. 2006;108(8):2648-54).

Neutrophil Activation Assays

[0269] Neutrophil activation was assessed in the whole blood assay by measuring increases in CD11b or decreases in CD62L on CD15+neutrophils by flow cytometry (Golay J. et al. Blood. 2013;122(20):3482-91, and Wittmann S. et al. Cytometry A. 2004;57(1):53-62). The ability of mAbs to induce neutrophil activation was assessed by comparing the mean fluorescent intensity (MFI) of CD11b and CD62L on CD15+ neutrophils in samples incubated with or without mAbs.

Statistical Analysis

[0270] Data was analyzed using Graph Pad Prism Software version 5.0. Mann Whitney test or Wilcoxon matched-pairs signed rank test were used to compare between groups as appropriate. Spearman correlation coefficient was used to analyze for correlation.

Results

Type II mAbs are more efficient than Type I at inducing B-cell cytotoxicity

[0271] To assess the effect of Type I and II mAbs on B cell cytotoxicity in RA and SLE samples, whole blood B-cell depletion assays were performed as described previously (Reddy V. et al. Arthritis & rheumatology. 2015;67(8):2046-55). OBZ was > 2-fold more efficient than RTX at deleting B-cells from patients with RA (n=31) and SLE (n=34) with both non-glycomodified OBZ_{Gly} and OBZ more efficient than RTX in all samples tested (**FIG. 2A**). In both RA and SLE, the median CTI of OBZ was significantly greater than the CTI of OBZ_{Gly} and RTX and the CTI of OBZ_{Gly} was significantly higher than the CTI of RTX in both RA and SLE. In RA, the median (interquartile range) CTI of RTX, OBZ_{Gly} and OBZ was 29 (13-50), 60 (47-70) and 67 (60-77), respectively and in SLE was 19 (11-39), 40 (31-53) and 59 (52-70), respectively. Thus, in both RA and SLE, there was a hierarchy of mAb-induced B cell depletion: RTX < OBZ_{Gly}< OBZ. The remarkable inter-sample variability in B-cell depletion, particularly with RTX was also noted. The superior efficiency of OBZ_{Gly} (having a non-glycomodified Fc similar to RTX) suggests that its type II nature accounts for the difference between the two types of mAbs in the efficiency of B-cell depletion in the whole

blood assay; whereas the increased efficiency of OBZ compared to OBZ_{Gly} is attributable to afucosylation of the Fc portion.

B-cells internalize RTX more rapidly than OBZ

[0272] Next, it was investigated whether the superior efficiency of type II mAbs in B cell depletion was consistent with their type II nature, and so it assessed whether B-cells from patients with RA and SLE internalized RTX to a greater extent than OBZ. It was found that RTX internalized more extensively than OBZ after 6 hours of incubation with a median (range) percentage of surface accessible RTX vs OBZ of: 55 (51 - 57) versus 83 (81 - 84), respectively in RA (n=5); and 60 (49 – 77) versus 76 (70 – 80), respectively in SLE (n=8) (**FIG. 2B**). To assess the role of FcγRIIb in this internalization, experiments were performed in the presence of the FcγRII-blocking mAb AT10, which partially inhibited internalization of RTX and to a smaller extent, OBZ (**FIG. 2B**), similar to previous observations using a non-glycomodified Type II antibody variant of OBZ (Reddy V. et al. Arthritis & rheumatology. 2015;67(8):2046-55).

RTX is more efficient than OBZ at inducing complement-dependent cellular cytotoxicity

[0273] The ability of these mAbs to elicit CDC was also investigated. It was found that the frequency of lysed B cells (CD19+Av+PI+) was significantly greater in samples incubated with RTX in the presence of normal healthy serum (NHS) compared to heat inactivated serum (HIS) with a median (range) difference of 10.9% (8.1 - 21) whereas the difference for OBZ was 4.8% (0.9 - 6.5) (**FIG. 2C**). The mean±SD fold increase in lysed cells in samples incubated with NHS vs HIS was 1.9±0.5 and 1.2±0.2 for RTX and OBZ, respectively (**FIG. 2D**). Thus, the data suggests that RTX was superior to OBZ at evoking CDC.

OBZ is more efficient than RTX at activating NK cells

[0274] These CDC results were consistent with the type I and II nature of the mAbs but at odds with the superior efficiency of type II mAbs in the whole blood assay. Next, the ability of the mAbs to elicit FcγR-mediated effector mechanisms was investigated; first assessing NK activation in the whole blood B-cell depletion assay. Gating as indicated in **FIG. 3E**, allowed assessment of NK degranulation (CD107a increase) relative to expression of CD16. The highest proportion of CD107a+ NK (CD3-CD56+) cells was seen in the CD56+CD16- fraction (**FIG. 3A-3G**) suggesting that degranulating NK cells had down regulated CD16 as previously reported (Grzywacz B. et al. Leukemia. 2007;21(2):356-9; author reply 9).

[0275] Having established these parameters, equivalent assays were performed comparing RTX and OBZ. After 24 hours of incubation in the absence of mAbs there was no significant difference in the frequency of NK cells, CD107a+ NK cells, CD16+ NK cells or B-cells between patients with RA (n=18) and SLE (n=23) (**Fig. 4A**). However, the median (range) frequency of CD3-CD56+CD107a+ activated NK cells was significantly higher in samples incubated with OBZ compared to RTX 5.1% (1.9 - 22) vs 2.8% (0.3 - 14) and 5.5% (0.6 - 12) vs 4.3% (1.2 - 8.9), respectively) but there was significantly lower median (range) frequency of CD16+ NK cells, in both RA and SLE 69 (36 - 94) vs 89 (83 – 97) and 66 (42 – 91) vs 84 (61 – 95), respectively (**FIG. 4B**). Also, there was a significantly higher fold-increase in the frequency of CD3-CD56+CD107a+ NK cells in samples incubated with OBZ compared to those incubated with RTX in SLE (**FIG. 4B**). Furthermore, it was found that NK cell activation, as assessed by either gain of CD107a or loss of CD16; or the fold increase in the frequency of CD3-CD56+CD107a+ NK cells, was greater in RA compared to SLE (**FIG. 4B**). NK cell activation, as assessed by the frequency of CD3-CD56+CD107a+ NK cells by RTX and OBZ, correlated significantly with that in samples incubated without mAbs with r^2=0.89, $p<0.05$; r^2=0.78, $p<0.05$, respectively, in RA (**FIG. 4C**) and r^2=0.52, $p<0.05$; r^2=0.36, $p<0.05$, respectively, in SLE (**FIG. 4D**). However, correlations were stronger in RA compared to SLE. Taken together, these data suggest disease related defects in the activation of NK cells in SLE may contribute to inefficient B-cell depletion noted in whole blood depletion assays (**FIG. 2A**) and that baseline activation status of NK cells may influence response to activation by mAbs in both RA and SLE (**FIG. 4B and 4C**).

[0276] It was next investigated whether differential activation of NK cells by RTX and OBZ was due to type I and type II characteristics and/or due to the effect of Fc engineering using either OBZ with wild-type glycosylation (OBZ_{Gly}) or completely lacking FcγR engagement (OBZ-PG LALA), consequently, less/in- efficient at inducing ADCC and CDC (Hessell A.J. et al. Nature. 2007;449(7158):101-4). Significant differences were not found in the frequency of CD3-CD56+CD107a+ or CD3-CD56+CD16+ NK cells in samples incubated without mAbs compared to samples incubated with OBZ-PG LALA in both RA (n=6) and SLE (n= 12) showing that FcγR-engagement is essential as expected. In these samples also OBZ was more efficient than OBZ_{Gly} and RTX at depleting B cells in the whole blood assay in both RA (n=18) and SLE (n=23) (**FIG. 5A**), but an increasing hierarchy was noted in the frequency of, and fold-increase in, CD3-CD56+CD107a+ NK cells as follows: no mAbs = OBZ-PG LALA < RTX < OBZ (**FIG. 5B and 5C**). The frequency of CD3-CD56+CD16+NK cells was significantly lower in samples incubated with OBZ when

compared with other samples (**FIG. 5D**). The frequency of CD3-CD56+CD16+NK cells was also lower in samples incubated with OBZ_{Gly} compared to RTX in RA, but not SLE (**FIG. 5D**).

[0277] Thus, the ability of mAbs to up-regulate the expression of CD107a on CD3-CD56+ NK cells was greater in RA compared with SLE, such that the mean fold difference in samples incubated with RTX, OBZ-PG LALA, OBZ_{Gly} and OBZ when compared with samples incubated without mAbs was 1.2, 1.5, 1.9 and 3.1, respectively, in RA and 1.5, 0.8, 1.4 and 1.8, respectively, in SLE (**FIG. 5C**). Although the pattern of B-cell depletion achieved by mAbs in RA and SLE (**FIG. 5A**) was similar to the pattern of NK cell activation by mAbs (**FIG. 5B and 5D**) there was no direct correlation between % B-cell depletion achieved by mAbs and the frequency of CD3-CD56+CD107a+ NK cells in individual samples (data not shown).

OBZ is more efficient than RTX at activating neutrophils

[0278] In addition to NK cells, neutrophils have also been proposed as mAb effector cells (Golay J. et al. Blood. 2013;122(20):3482-91). Therefore, next, the ability of mAbs to induce neutrophil activation was assessed by measuring the expression of CD11b and CD62L, as described previously (Wittmann S. et al. Cytometry A. 2004;57(1):53-62) and shown in **FIG. 9**. CD11b forms part of the β integrin (Mac-1) complex and several genetic variants of this complex have been associated with lupus-related phagocytic defects (Bologna L. et al. J Immunol. 2011;186(6):3762-9). Upon neutrophil activation the surface expression of CD11b is up regulated whereas the expression of the adhesion molecule CD62L is down regulated (Golay J. et al. Blood. 2013;122(20):3482-91, and Wittmann S. et al. Cytometry A. 2004;57(1):53-62). It was found that the MFI of CD11b in samples incubated with mAbs was significantly higher in both RA (n=10) and SLE (n=22) (**FIG. 6A**) when compared with samples incubated without mAbs. In both RA and SLE, significant correlations were noted between the MFI of CD11b in samples incubated in the absence of mAbs and that in samples incubated with RTX (r^2=0.81, 0.82, respectively) whereas significant correlation for OBZ was noted in SLE (r^2=0.81), but not RA (**FIG. 6B**). A hierarchy was also noted in the ability of mAbs to up-regulate CD11b such that the MFI of CD11b was lower in samples incubated with RTX < OBZ_{Gly} < OBZ, as in the case of NK cell activation. Also, the MFI of CD62L was greater in samples incubated with RTX > OBZ_{Gly} > OBZ (**FIG. 6C**). In both RA and SLE, significant correlations were noted between the MFI of CD62L in samples incubated in the absence of mAbs and that in samples incubated with RTX (r^2=0.93, 0.91, respectively) and OBZ (r^2=0.64, 0.71, respectively) (**FIG. 6D**). Thus, the hierarchy of mAbs in their ability

to activate neutrophils was OBZ > OBZ_{Gly} > RTX. Taken together, these data suggested that type II mAbs are superior to RTX in activating neutrophils in the whole blood assay for both RA and SLE samples. OBZ-PG LALA did not elicit significant changes for either marker in both RA (n=7) and SLE (n=12) compared to samples incubated in the absence of mAbs.

OBZ is more efficient than RTX at inducing direct cell death

[0279] Next, direct cell death (DCD) was assessed, using the Annexin V assay as shown in **FIG. 10**. The ability of OBZ to induce direct cell death was greater than that of RTX for both CD19+ cells as a whole and also B-cell subpopulations; IgD+CD27- naïve cells and IgD-CD27+ switched memory cells, **FIG. 7A** (RA, n=5 and SLE, n=4). The proportion of Annexin V+ cells was highest for DN cells > IgD+CD27+ unswitched memory cells > IgD-CD27+ switched memory cells > IgD+CD27- naïve cells, even in samples incubated without mAbs. Nonetheless, OBZ was superior to RTX at inducing DCD.

B-cell subpopulations: expression of CD20, FcγRIIb and internalization of mAbs

[0280] It was next investigated whether differences between B-cell subpopulations in the expression of CD20, FcγRIIb and/or their ability to internalize mAbs provided explanations for the differential sensitivity to mAb-induced DCD. B-cell subpopulations displayed varying ability to internalize mAbs such that IgD-CD27+ switched memory cells internalized mAbs less than other B-cell subpopulations; and IgD+CD27+ unswitched memory cells internalized mAbs to a greater extent than other B-cell subpopulations. Antagonizing the effects of FcγRIIb with AT10 significantly reduced internalization in both cases. When compared to naïve and IgD-CD27+ switched memory cells, IgD+CD27+ unswitched memory cells had significantly greater expression of CD20 and FcγRIIb and displayed significantly greater ability to internalize mAbs whereas naïve and IgD-CD27+ switched memory cells had significantly lower expression of CD20 and FcγRIIb and displayed significantly lower levels of internalization. DN cells had remarkably variable levels of expression of CD20 and FcγRIIb, but internalized RTX to a significantly greater extent than IgD-CD27+ switched memory cells. B cells from both RA and SLE samples consistently displayed low levels of OBZ internalization. Taking these data together, there was no clear relationship between the susceptibility of B-cell subpopulations to mAb-induced DCD and the ability to internalize mAbs or to express CD20 or FcγRIIb.

[0281] Here, it was shown that Obinutuzumab, a type II anti-CD20 mAb with a glycomodified Fc demonstrated at least 2-fold greater potency at deleting B-cells from whole blood samples of patients with both RA and SLE compared to the RTX. This increased activity of OBZ was affected predominantly through Fc gamma receptor (FcγR)-mediated

effector mechanisms and DCD. In contrast, RTX recruited complement more efficiently for CDC, but was rapidly internalized and significantly less efficient at evoking ADCC and DCD. The subsequent analysis revealed that the expression of the CD20 target molecule was less on IgD-CD27+ switched memory and DN cells; perhaps accounting for their relative resistance to removal by RTX.

CLAIMS

What is claimed is:

1. A method for treating or delaying progression of lupus nephritis in an individual that has lupus, comprising administering to the individual at least a first antibody exposure to a type II anti-CD20 antibody and a second antibody exposure to the type II anti-CD20 antibody, the second antibody exposure not being provided until from about 18 weeks to about 26 weeks after the first antibody exposure;

wherein the first antibody exposure comprises one or two doses of the type II anti-CD20 antibody, the first antibody exposure comprising a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody;

wherein the second antibody exposure comprises one or two doses of the type II anti-CD20 antibody, the second antibody exposure comprising a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody; and

wherein the type II anti-CD20 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:1, HVR-H2 sequence of SEQ ID NO:2, and HVR-H3 sequence of SEQ ID NO:3, and a light chain comprising HVR-L1 sequence of SEQ ID NO:4, HVR-L2 sequence of SEQ ID NO:5, and HVR-L3 sequence of SEQ ID NO:6.

2. The method of claim 1, wherein the first antibody exposure comprises a first dose of between about 900mg and about 1100mg of the type II anti-CD20 antibody and a second dose of between about 900mg and about 1100mg of the type II anti-CD20 antibody.

3. The method of claim 1 or claim 2, wherein the first antibody exposure comprises a first dose of the type II anti-CD20 antibody and a second dose of the type II anti-CD20 antibody, and wherein the second dose of the first antibody exposure is not provided until from about 1.5 weeks to about 2.5 weeks after the first dose of the first antibody exposure.

4. The method of claim 3, wherein the first antibody exposure comprises a first dose of the type II anti-CD20 antibody and a second dose of the type II anti-CD20 antibody, and wherein the second dose of the first antibody exposure is not provided until about 2 weeks after the first dose of the first antibody exposure.

5. The method of claim 3 or claim 4, wherein the first dose of the first antibody exposure is about 1000mg of the type II anti-CD20 antibody.

6. The method of any one of claims 3-5, wherein the second dose of the first antibody exposure is about 1000mg of the type II anti-CD20 antibody.

7. The method of any one of claims 1-6, wherein the second antibody exposure comprises a first dose of between about 900mg and about 1100mg of the type II anti-CD20 antibody and a second dose of between about 900mg and about 1100mg of the type II anti-CD20 antibody.

8. The method of any one of claims 1-7, wherein the second antibody exposure comprises a first dose of the type II anti-CD20 antibody and a second dose of the type II anti-CD20 antibody, and wherein the second dose of the second antibody exposure is not provided until from about 1.5 weeks to about 2.5 weeks after the first dose of the second antibody exposure.

9. The method of claim 8, wherein the second antibody exposure comprises a first dose of the type II anti-CD20 antibody and a second dose of the type II anti-CD20 antibody, and wherein the second dose of the second antibody exposure is not provided until about 2 weeks after the first dose of the second antibody exposure.

10. The method of claim 8 or claim 9, wherein the first dose of the second antibody exposure is about 1000mg of the type II anti-CD20 antibody.

11. The method of any one of claims 8-10, wherein the second dose of the second antibody exposure is about 1000mg of the type II anti-CD20 antibody.

12. The method of any one of claims 1-11, wherein the first antibody exposure and the second antibody exposure are administered intravenously.

13. The method of any one of claims 1-12, wherein the individual has class III or class IV lupus nephritis.

14. The method of any one of claims 1-12, wherein the individual is at risk for developing class III or class IV lupus nephritis.

15. A method for treating or delaying progression of lupus nephritis in an individual that has lupus, comprising administering to the individual an effective amount of a type II anti-CD20 antibody; wherein the type II anti-CD20 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:1, HVR-H2 sequence of SEQ ID NO:2, and HVR-H3 sequence of SEQ ID NO:3, and a light chain comprising HVR-L1 sequence of SEQ ID NO:4, HVR-L2 sequence of SEQ ID NO:5, and HVR-L3 sequence of SEQ ID NO:6; and wherein the individual has class III or class IV lupus nephritis.

16. The method of claim 14, wherein the type II anti-CD20 antibody is administered intravenously.

17. The method of any one of claims 1-16, wherein the individual does not have class III (C) or class IV (C) lupus nephritis.

18. The method of any one of claims 1-17, wherein the individual has class V lupus nephritis.

19. The method of any one of claims 1-18, further comprising administering to the individual an effective amount of an immunosuppressive agent.

20. The method of claim 19, wherein the immunosuppressive agent comprises mycophenolic acid, a derivative thereof, or a salt thereof.

21. The method of claim 20, wherein the immunosuppressive agent comprises mycophenolate mofetil.

22. The method of any one of claims 1-21, further comprising administering to the individual an effective amount of a glucocorticoid or corticosteroid.

23. The method of claim 22, wherein the glucocorticoid or corticosteroid comprises methylprednisolone.

24. The method of claim 22, wherein the glucocorticoid or corticosteroid comprises prednisone.

25. The method of any one of claims 1-24, further comprising administering to the individual an effective amount of an antihistamine.

26. The method of claim 25, wherein the antihistamine comprises diphenhydramine.

27. The method of any one of claims 1-26, further comprising administering to the individual an effective amount of a non-steroidal anti-inflammatory drug (NSAID).

28. The method of claim 27, wherein the NSAID comprises acetaminophen.

29. The method of any one of claims 1-28, further comprising administering to the individual an effective amount of an antihypertensive agent.

30. The method of claim 29, wherein the antihypertensive agent is an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin-receptor blocker.

31. The method of any one of claims 1-30, further comprising administering to the individual a standard of care treatment.

32. The method of claim 31, wherein the standard of care treatment comprises treatment with one or more of an angiotensin-converting enzyme (ACE) inhibitor, an angiotensin-receptor blocker, cyclophosphamide, mycophenolate mofetil, azathioprine, and a glucocorticoid or corticosteroid.

33. The method of any one of claims 1-32, wherein the method results in a complete renal response (CRR) in the individual.

34. The method of any one of claims 1-33, wherein the method results in a depletion of circulating peripheral B cells in the individual.

35. The method of claim 34, wherein the circulating peripheral B cells are CD19+ B cells.

36. The method of any one of claims 1-35, wherein the type II anti-CD20 antibody is a humanized or human antibody.

37. The method of any one of claims 1-36, wherein the type II anti-CD20 antibody is afucosylated.

38. The method of any one of claims 1-37, wherein the heavy chain of the type II anti-CD20 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:7.

39. The method of any one of claims 1-38, wherein the light chain of the type II anti-CD20 antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:8.

40. The method of any one of claims 1-39, wherein the type II anti-CD20 antibody is obinutuzumab.

41. The method of any one of claims 1-40, wherein the individual is a human.

42. A kit for treating or delaying progression of lupus nephritis in an individual that has lupus, comprising:

(a) a container comprising a type II anti-CD20 antibody, wherein the type II anti-CD20 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:1, HVR-H2 sequence of SEQ ID NO:2, and HVR-H3 sequence of SEQ ID NO:3, and a light chain comprising HVR-L1 sequence of SEQ ID NO:4, HVR-L2 sequence of SEQ ID NO:5, and HVR-L3 sequence of SEQ ID NO:6; and

(b) a package insert with instructions for treating or delaying progression of lupus nephritis in an individual, wherein the instructions indicate that at least a first antibody exposure to a type II anti-CD20 antibody and a second antibody exposure to the type II anti-CD20 antibody are administered to the individual, the second antibody exposure not being provided until from about 18 weeks to about 26 weeks after the first antibody exposure; wherein the first antibody exposure comprises one or two doses of the type II anti-CD20 antibody, the first antibody exposure comprising a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody; and wherein the second antibody exposure comprises one or two doses of the type II anti-CD20 antibody, the second antibody exposure comprising a total exposure of between about 1800mg and about 2200mg of the type II anti-CD20 antibody.

43. The kit of claim 42, further comprising a container comprising:

(c) a second medicament, wherein the type II anti-CD20 antibody is a first medicament; and

(d) instructions on the package insert for administering the second medicament to the subject.

44. The kit of claim 43, wherein the second medicament is an immunosuppressive agent, a glucocorticoid, a corticosteroid, an anti-malarial agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone.

45. A kit for treating or delaying progression of lupus nephritis in an individual that has lupus, comprising:

(a) a container comprising a type II anti-CD20 antibody, wherein the type II anti-CD20 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:1, HVR-H2 sequence of SEQ ID NO:2, and HVR-H3 sequence of SEQ ID NO:3, and a light chain comprising HVR-L1 sequence of SEQ ID NO:4, HVR-L2 sequence of SEQ ID NO:5, and HVR-L3 sequence of SEQ ID NO:6; and

(b) a package insert with instructions for treating or delaying progression of class III or class IV lupus nephritis in an individual.

46. The kit of claim 45, further comprising a container comprising:

(c) a second medicament, wherein the type II anti-CD20 antibody is a first medicament; and

(d) instructions on the package insert for administering the second medicament to the subject.

47. The kit of claim 46, wherein the second medicament is an immunosuppressive agent, a glucocorticoid, a corticosteroid, an anti-malarial agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone.

48. A method for treating or delaying progression of rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) in an individual, comprising administering to the individual an effective amount of an anti-CD20 antibody, wherein the antibody comprises a heavy chain variable region comprising an HVR-H1 sequence of SEQ ID NO:1, an HVR-H2 sequence of SEQ ID NO:2, and an HVR-H3 sequence of SEQ ID NO:3, and a light chain variable region comprising an HVR-L1 sequence of SEQ ID NO:4, an HVR-L2 sequence of SEQ ID NO:5, and an HVR-L3 sequence of SEQ ID NO:6.

49. The method of claim 47, wherein the antibody is administered intravenously.

50. The method of claim 47 or claim 48, wherein the method results in a depletion of circulating peripheral B cells in the individual.

51. The method of claim 49, wherein the circulating peripheral B cells are CD19+ B cells.

52. The method of any one of claims 47-50, wherein the antibody is a humanized or human antibody.

53. The method of any one of claims 47-51, wherein the antibody is afucosylated.

54. The method of any one of claims 47-52, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:7.

55. The method of any one of claims 47-53, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO:8.

56. The method of any one of claims 47-54, wherein the antibody is obinutuzumab.

57. The method of any one of claims 47-54, wherein the antibody comprises a modified Fc region.

58. The method of claim 56, wherein the Fc region comprises a modification for attenuating effector function.

59. The method of claim 56, wherein the Fc region is a human IgG1 Fc region.

60. The method of claim 58, wherein the Fc region comprises L234A, L235A and P329G amino acid substitutions, numbering according to EU index.

61. The method of any one of claims 48-60, wherein the individual is a human.

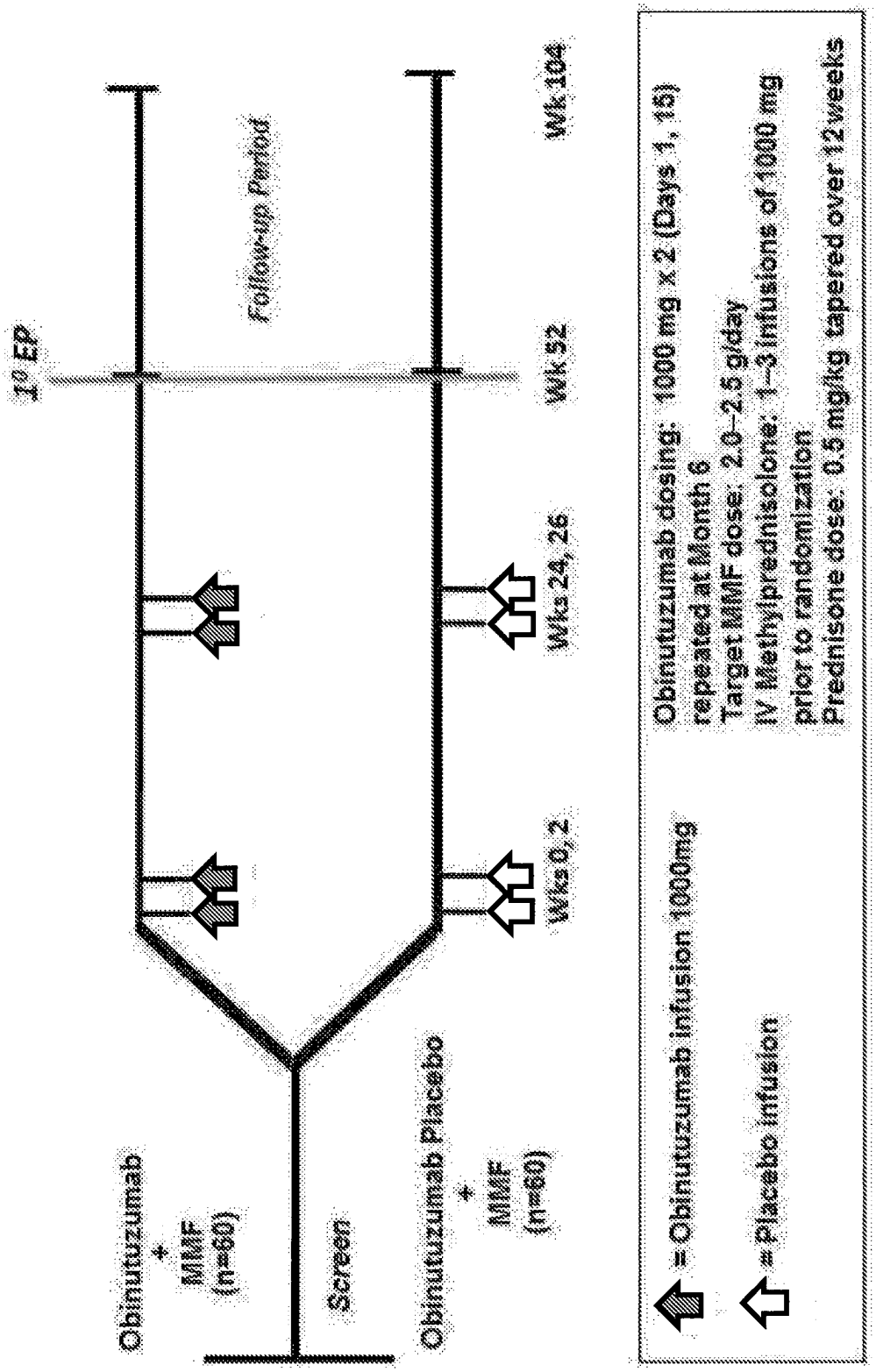

Obinutuzumab + MMF (n=60)
Screen
Obinutuzumab Placebo + MMF (n=60)
Wks 0, 2
Wks 24, 26
1° EP
Follow-up Period
Wk 52
Wk 104
Obinutuzumab dosing: 1000 mg x 2 (Days 1, 15)
repeated at Month 6
Target MMF dose: 2.0–2.5 g/day
IV Methylprednisolone: 1–3 infusions of 1000 mg
prior to randomization
Prednisone dose: 0.5 mg/kg tapered over 12 weeks
= Obinutuzumab infusion 1000mg
= Placebo infusion

FIG. 1

FIG. 2B

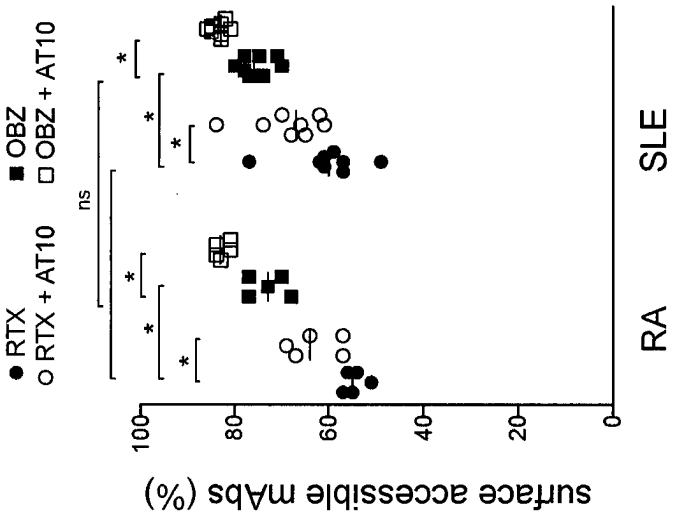

RTX
RTX + AT10
OBZ
OBZ + AT10
ns
*
surface accessible mAbs (%)
100
80
60
40
20
0
RA
SLE

FIG. 2A

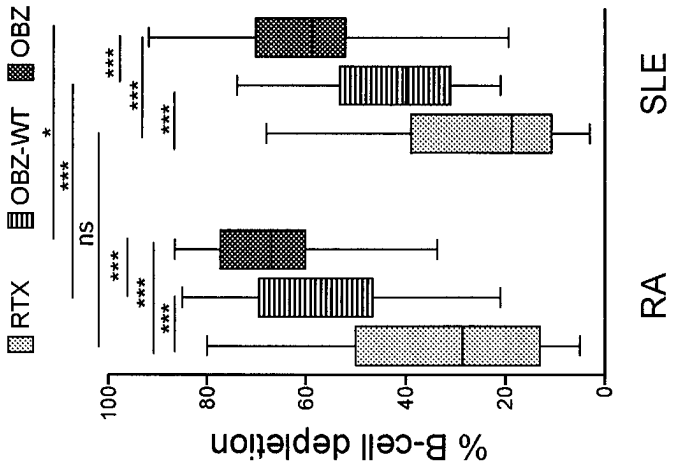

RTX
OBZ-WT
OBZ
ns
*
***
% B-cell depletion
100
80
60
40
20
0
RA
SLE

FIG. 2D

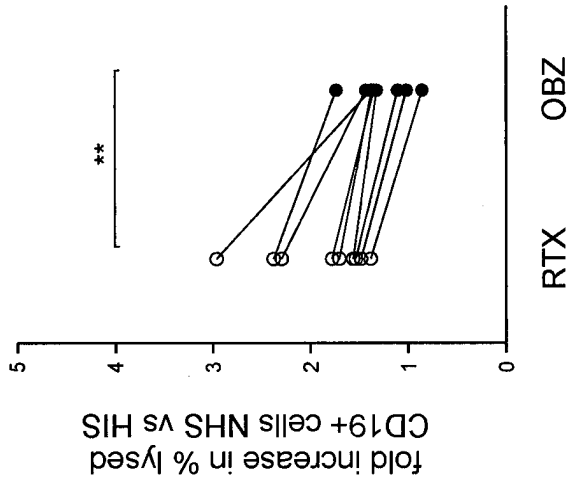

fold increase in % lysed
CD19+ cells NHS vs HIS
5
4
3
2
1
0
**
RTX
OBZ

FIG. 2C

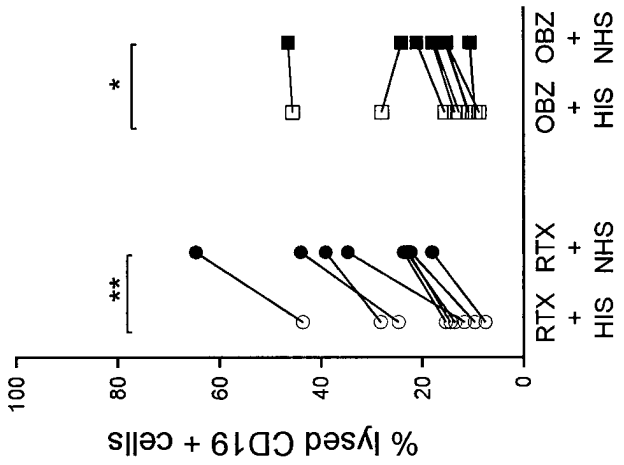

% lysed CD19 + cells
100
80
60
40
20
0
**
*
RTX + HIS
RTX + NHS
OBZ + HIS
OBZ + NHS

FIG. 3A

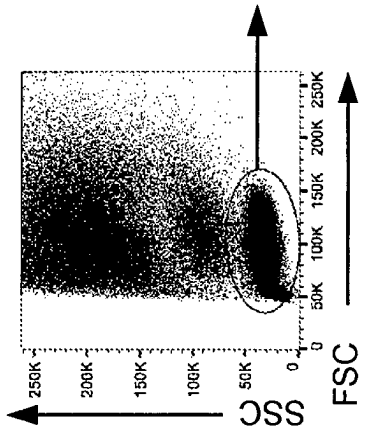


FIG. 3B

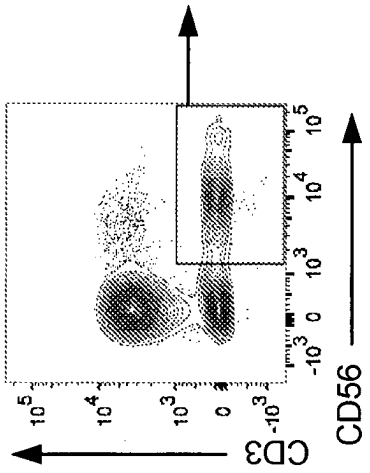


FIG. 3C

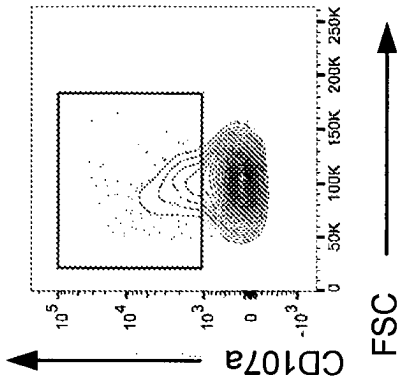
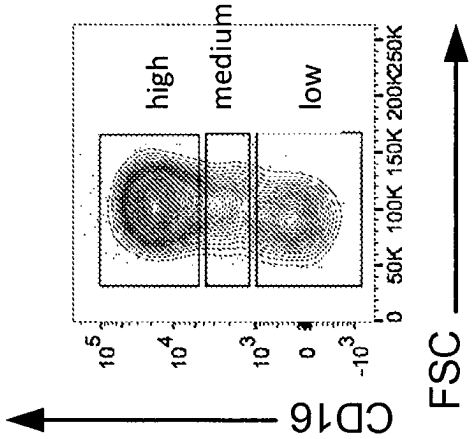


FIG. 3D



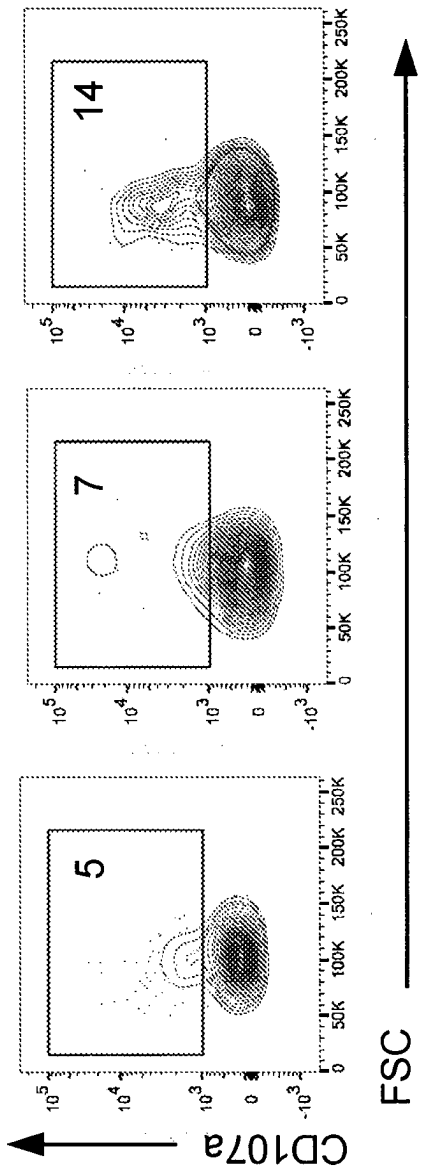


FIG. 3E
FIG. 3F
FIG. 3G
5
7
14
CD107a
FSC
0
50K
100K
150K
200K
250K
10^5
10^4
10^3
0
-10^3

FIG. 4A

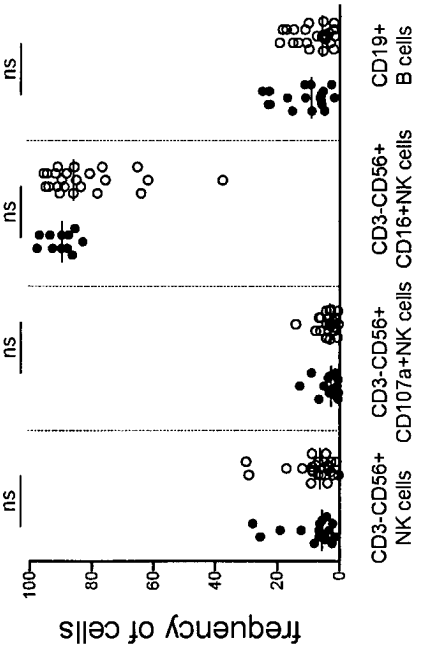


FIG. 4B

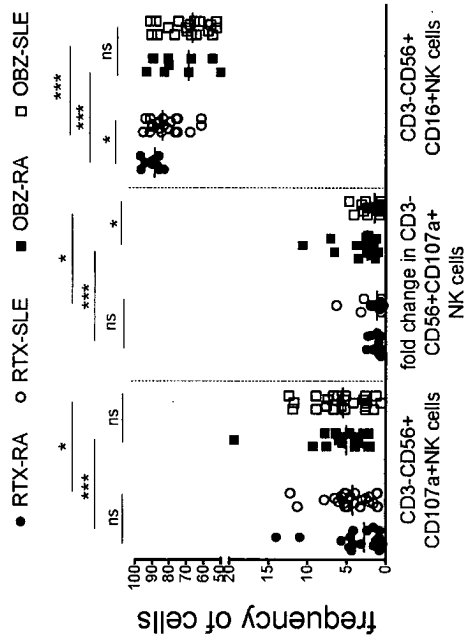


FIG. 4C

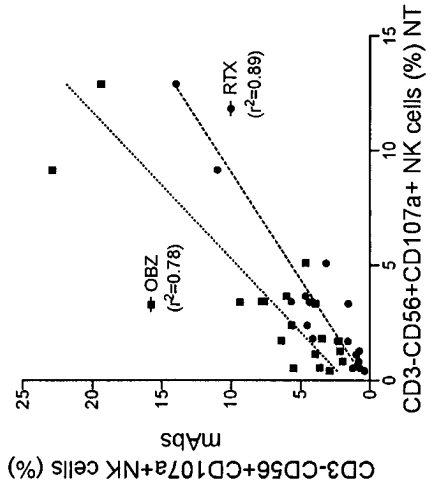


FIG. 4D

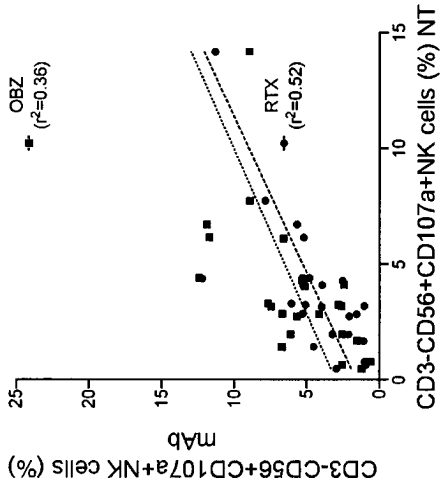


FIG. 5A

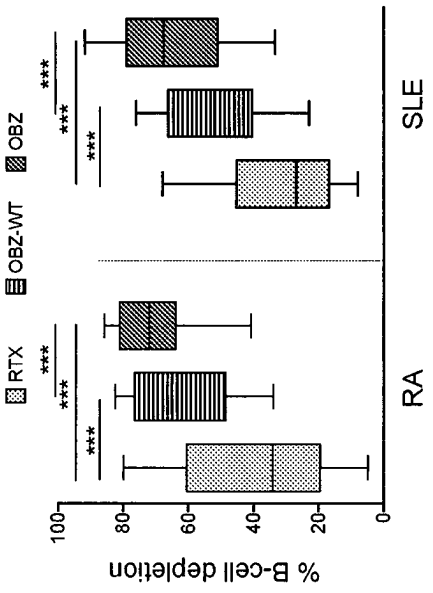


FIG. 5B

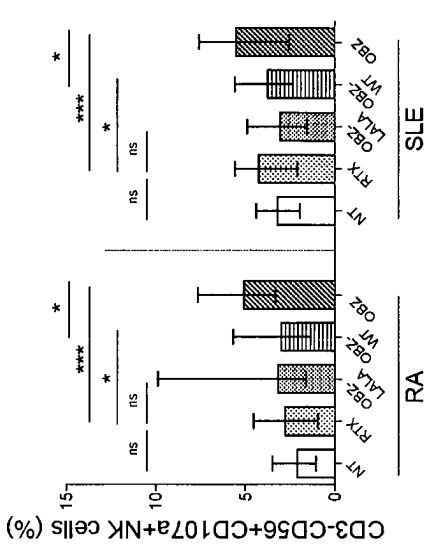


FIG. 5C

FIG. 5D

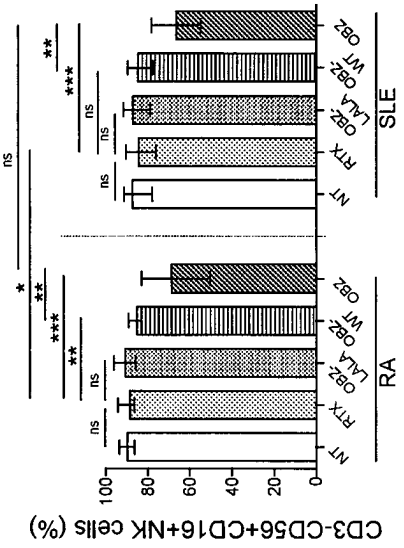


FIG. 6A

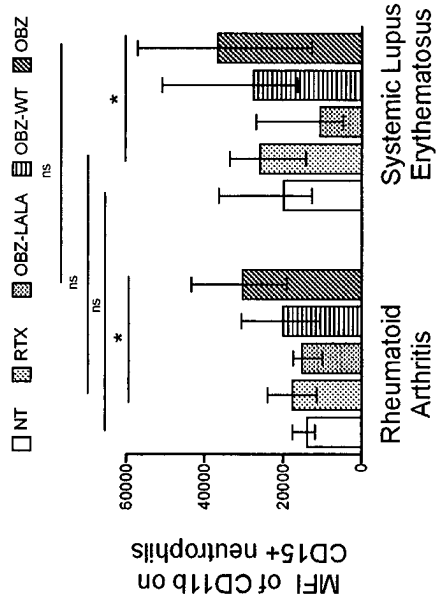


FIG. 6B

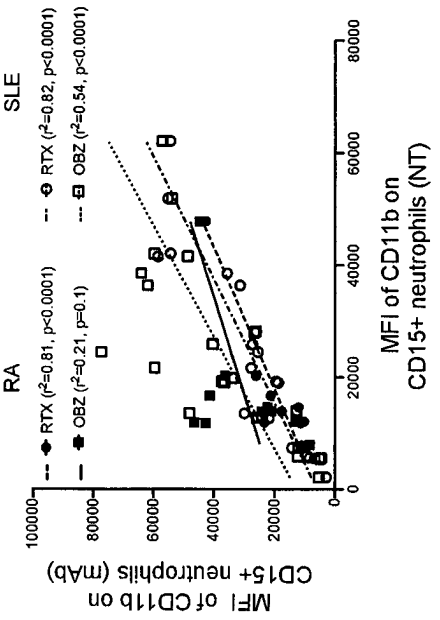


FIG. 6C

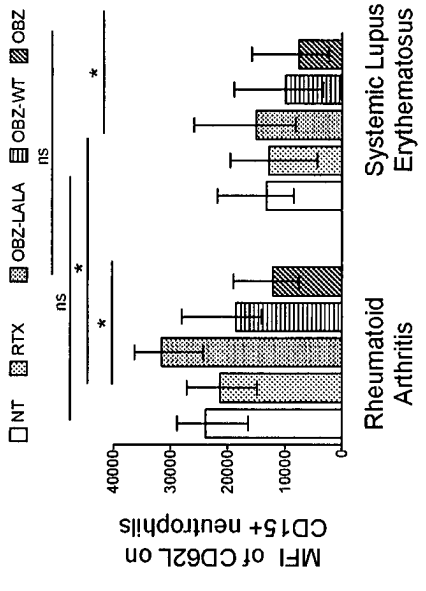


FIG. 6D

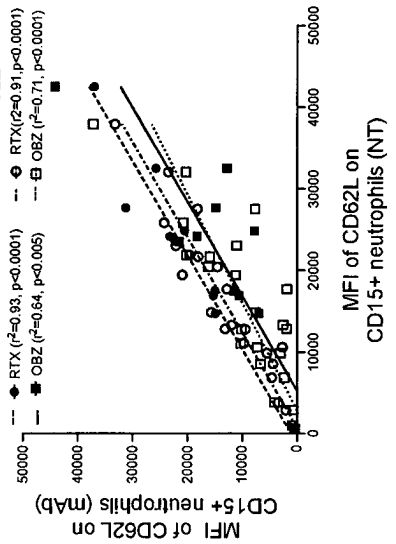


FIG. 7A

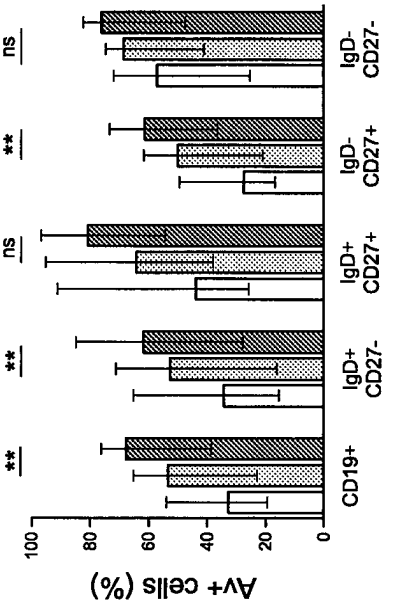


FIG. 7B

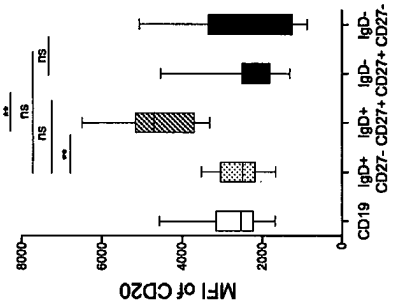


FIG. 7C

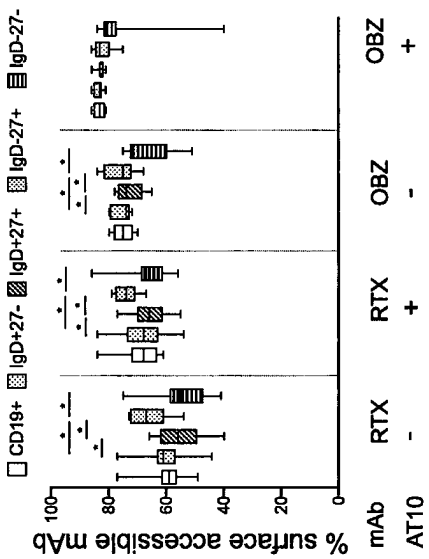
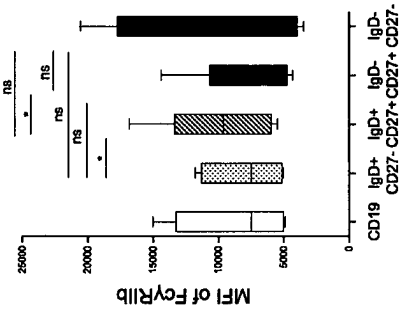


FIG. 7D



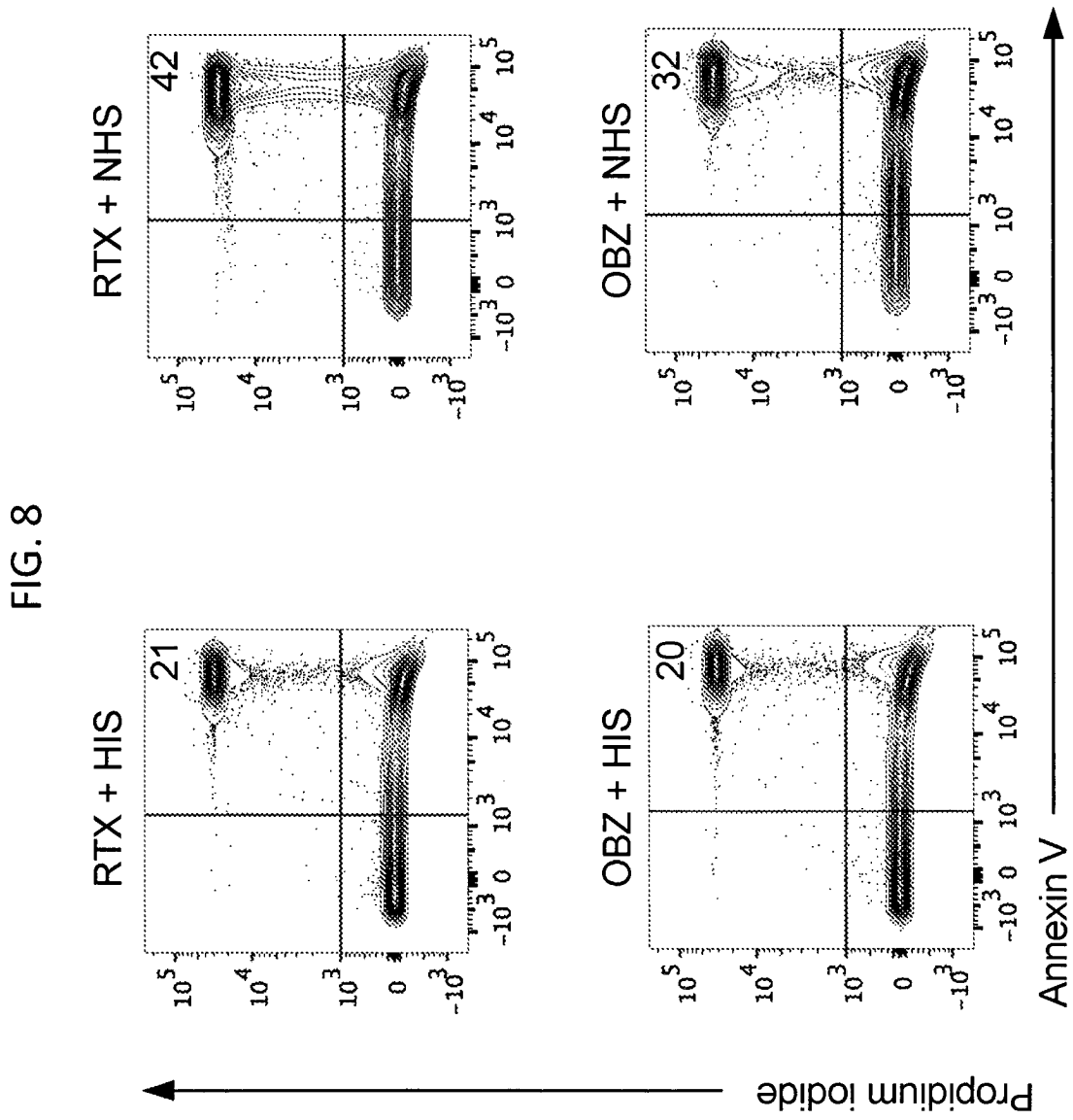

RTX + NHS
42
OBZ + NHS
32
RTX + HIS
21
OBZ + HIS
20
Propidium Iodide
Annexin V

FIG. 8

FIG. 9

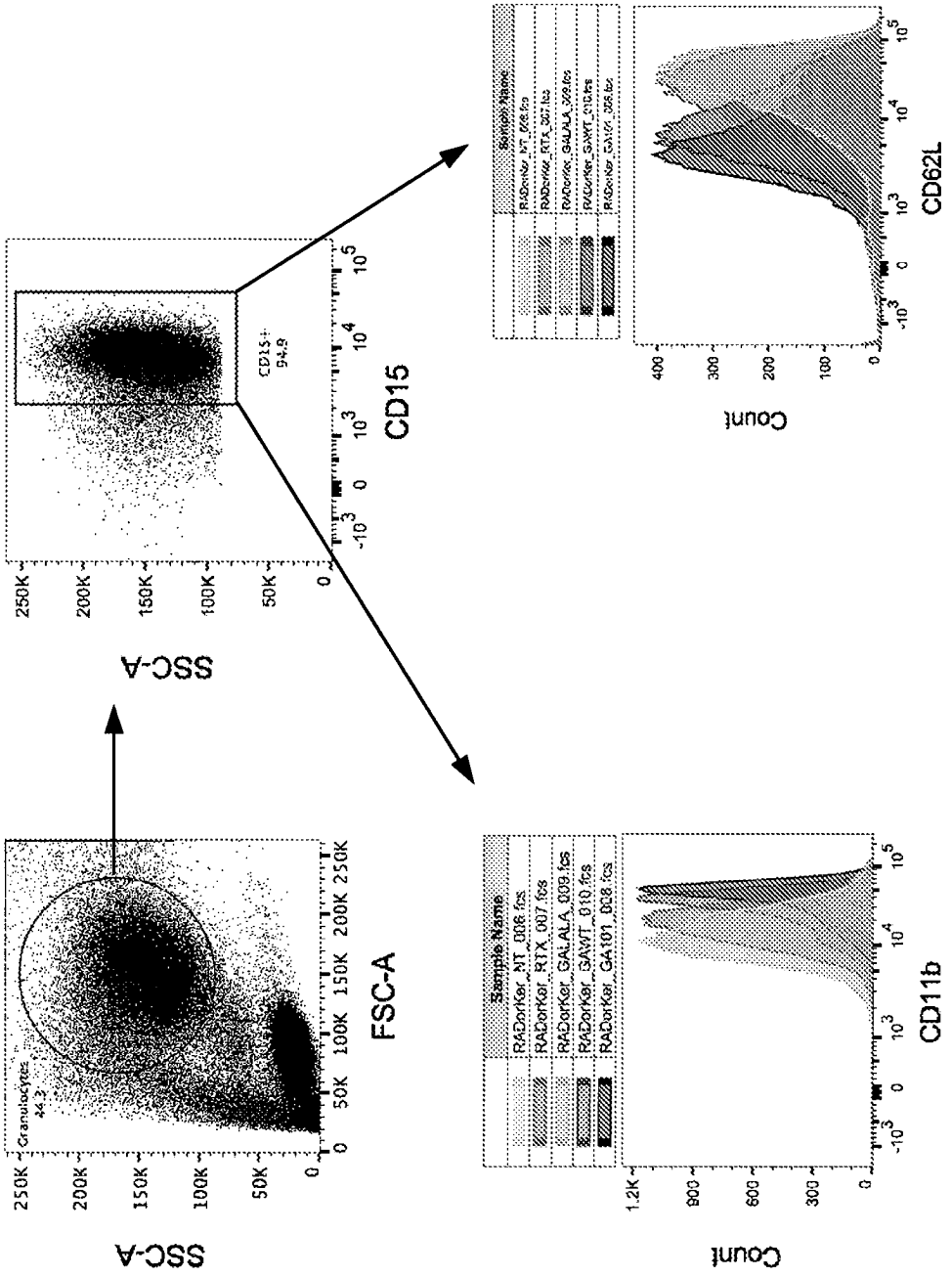

SSC-A
FSC-A
Granulocytes
44.3
CD15
CD15+
94.9
CD62L
CD11b
Count
Sample Name
RADonKer_NT_006.fcs
RADonKer_RTX_007.fcs
RADonKer_GALALA_009.fcs
RADonKer_GAWT_010.fcs
RADonKer_GA101_008.fcs

FIG. 10

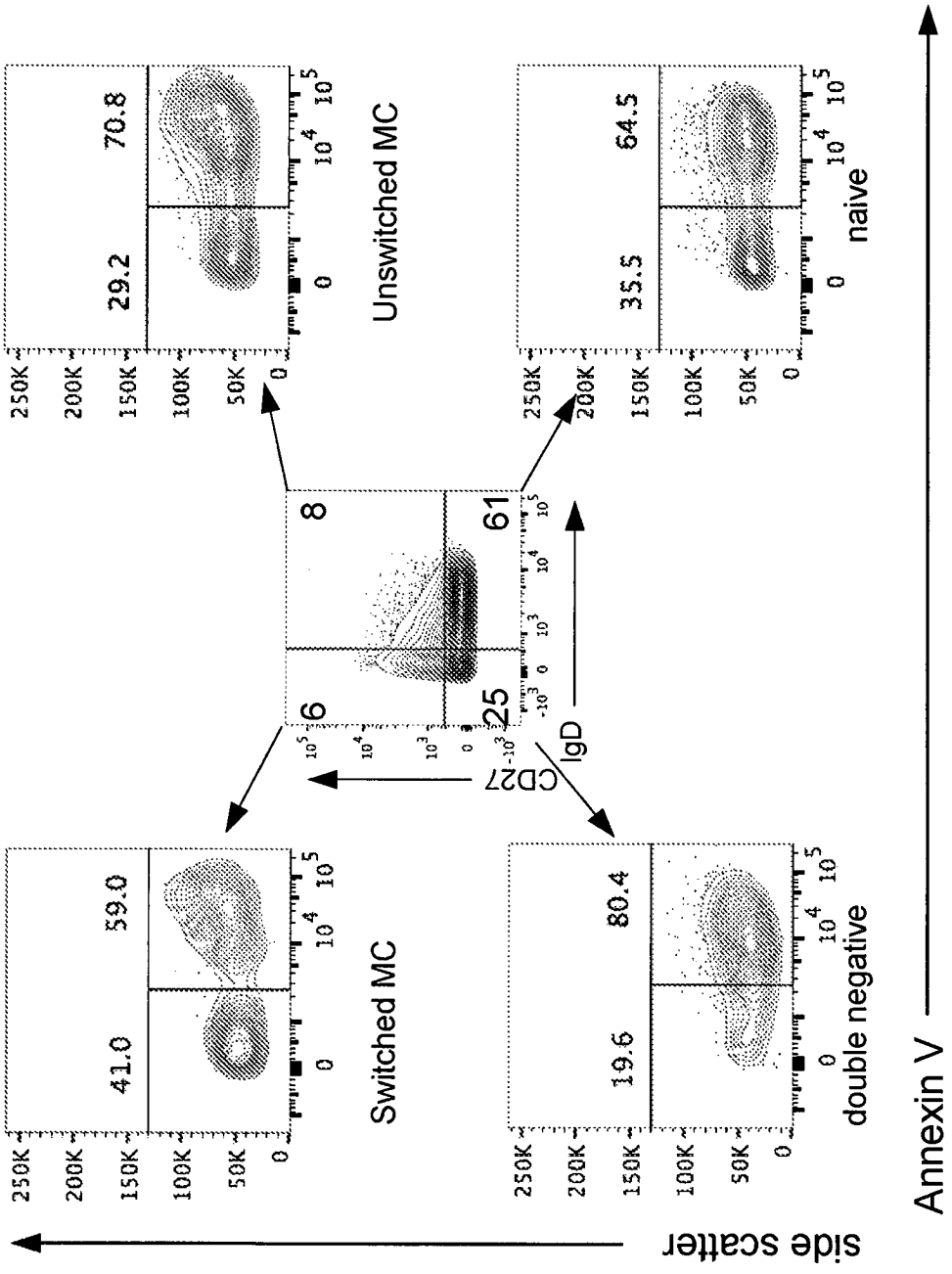
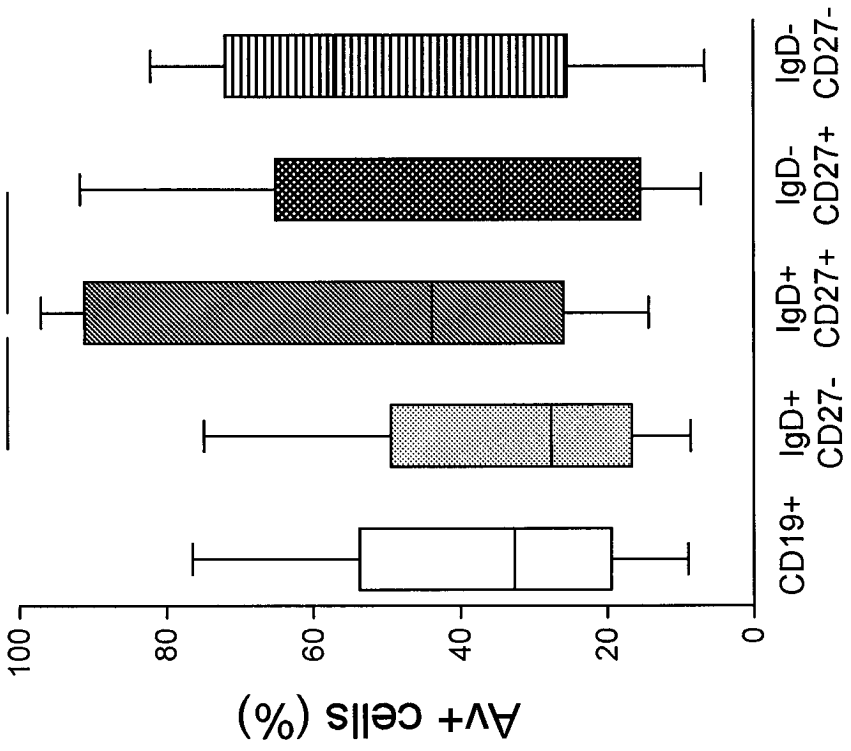

Unswitched MC
naive
Switched MC
double negative
side scatter
Annexin V
CD27
IgD
8
61
6
25
70.8
29.2
64.5
35.5
59.0
41.0
80.4
19.6

FIG. 11


AV+ cells (%)
100
80
60
40
20
0
CD19+
IgD+ CD27-
IgD+ CD27+
IgD- CD27+
IgD- CD27-
*
**
ns

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/031683

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 197 918 A1 (HOFFMANN LA ROCHE [CH]; ROCHE GLYCART AG [CH]) 23 June 2010 (2010-06-23) the whole document in particular, pages 2, 16 and 17; sequences 1,2	42,43
Y	WO 2007/031875 A2 (GLYCART BIOTECHNOLOGY AG [CH]; UMANA PABLO [CH]; MOSSNER EKKEHARD [CH]) 22 March 2007 (2007-03-22) the whole document in particular, pages 114-116	1-14
Y	WO 2013/090478 A1 (PIKAMAB INC [US]) 20 June 2013 (2013-06-20) the whole document in particular, page 41	1-14

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 July 2016	21/09/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Pérez-Mato, Isabel

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2005/120437 A2 (GENENTECH INC [US]; BRUNETTA PAUL G [US]) 22 December 2005 (2005-12-22) the whole document in particular, pages 9, 23 and 24 and example 1	1-14,44
Y	E. MOSSNER ET AL: "Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity", BLOOD, vol. 115, no. 22, 3 June 2010 (2010-06-03) , pages 4393-4402, XP055190313, ISSN: 0006-4971, DOI: 10.1182/blood-2009-06-225979 the whole document in particular, page 4400	1-14
Y	M. WEIDENBUSCH ET AL: "Beyond the LUNAR trial. Efficacy of rituximab in refractory lupus nephritis", NEPHROLOGY DIALYSIS TRANSPLANTATION., vol. 28, no. 1, 3 July 2012 (2012-07-03), pages 106-111, XP055287406, GB ISSN: 0931-0509, DOI: 10.1093/ndt/gfs285 the whole document in particular, abstract and pages 108-109	1-14
Y	BRAD H. ROVIN ET AL: "Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: The lupus nephritis assessment with rituximab study", ARTHRITIS & RHEUMATISM, vol. 64, no. 4, 27 April 2012 (2012-04-27) , pages 1215-1226, XP055287443, ISSN: 0004-3591, DOI: 10.1002/art.34359 the whole document in particular, page 1216	1-14,44

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/031683

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

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

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

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

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

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

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-14, 42-44

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-14, 42-44

   directed to a method for treating lupus nephritis comprising administering at least a first type II anti-CD20 antibody and a second type II anti-CD20 antibody at a certain dosage and schedule, wherein the type II anti-CD20 antibody has CDRs of SEQ ID NOs:1-6, and to kits for said treatment.

   ---

2. claims: 15-41, 45-47

   directed to a method for treating lupus nephritis (class III or class IV) comprising administering a type II anti-CD20 antibody having CDRs of SEQ ID NOs:1-6, and to kits for said treatment.

   ---

3. claims: 48-61(partially)

   directed to a method for treating rheumatoid arthritis comprising administering a type II anti-CD20 antibody having CDRs of SEQ ID NOs:1-6.

   ---

4. claims: 48-61(partially)

   directed to a method for treating systemic lupus erythematosus comprising administering a type II anti-CD20 antibody having CDRs of SEQ ID NOs:1-6.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/031683

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2197918 A1	23-06-2010	AR 068818 A1	09-12-2009
		AU 2008295140 A1	12-03-2009
		BR PI0816458 A2	12-03-2013
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		JP 2008501706 A	24-01-2008
		PA 8635501 A1	02-06-2006

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/031683

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		SV 2006002131 A	26-01-2006
		US 2006024295 A1	02-02-2006
		US 2007025988 A1	01-02-2007
		US 2010303810 A1	02-12-2010
		WO 2005120437 A2	22-12-2005

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 107592812 A
(43)申请公布日 2018.01.16

(21)申请号 201680026687.3
(22)申请日 2016.05.10
(30)优先权数据
62/159,876 2015.05.11 US
62/300,052 2016.02.25 US
(85)PCT国际申请进入国家阶段日
2017.11.08
(86)PCT国际申请的申请数据
PCT/US2016/031683 2016.05.10
(87)PCT国际申请的公布数据
WO2016/183104 EN 2016.11.17
(71)申请人 豪夫迈·罗氏有限公司
地址 瑞士巴塞尔
(72)发明人 P·布鲁内塔
(74)专利代理机构 北京市中咨律师事务所 11247
代理人 柴云峰 黄革生
(51)Int.Cl.
A61K 45/06(2006.01)
A61K 39/395(2006.01)
A61P 13/12(2006.01)
A61P 37/02(2006.01)

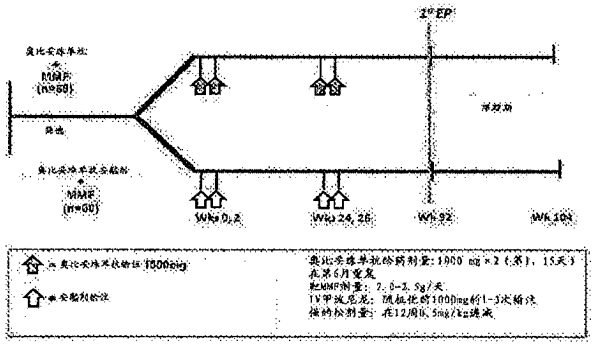
权利要求书4页 说明书62页
序列表14页 附图17页

(54)发明名称

治疗狼疮性肾炎的组合物和方法

(57)摘要

本发明提供了治疗或延缓具有狼疮的个体的狼疮性肾炎进展的方法。在一些实施方案中，所述方法包括向个体施用有效量的II型抗CD20抗体。本发明还提供了治疗或延缓个体中类风湿性关节炎(RA)或系统性红斑狼疮(SLE)进展的方法。在一些实施方案中，所述方法包括施用有效量的抗CD20抗体。



CN 107592812 A

1.一种治疗或延缓具有狼疮的个体的狼疮性肾炎的进展的方法，包括向所述个体施用对II型抗CD20抗体的至少第一次抗体暴露和对II型抗-CD20抗体的第二次抗体暴露，第二次抗体暴露在第一次抗体暴露后约18周至约26周才提供；

其中所述第一次抗体暴露包括一个或两个剂量的II型抗CD20抗体，所述第一次抗体暴露包括约1800mg至约2200mg所述II型抗-CD20抗体的总暴露；

其中所述第二次抗体暴露包括一个或两个剂量的II型抗CD20抗体，所述第二次抗体暴露包括约1800mg至约2200mg所述II型抗CD20抗体的总暴露；和

其中II型抗CD20抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链，以及包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链。

2.权利要求1的方法，其中所述第一次抗体暴露包括约900mg至约1100mg的II型抗CD20抗体的第一剂量和约900mg至约1100mg的II型抗-CD20抗体的第二剂量。

3.权利要求1或权利要求2的方法，其中所述第一次抗体暴露包括第一剂量的II型抗CD20抗体和第二剂量的II型抗-CD20抗体，并且其中第一次抗体暴露的第二剂量在第一次抗体暴露的第一剂量后约1.5周至约2.5周才提供。

4.权利要求3的方法，其中所述第一次抗体暴露包括第一剂量的II型抗-CD20抗体和第二剂量的II型抗CD20抗体，并且其中第一次抗体暴露的第二剂量在第一次抗体暴露的第一剂量后约2周才提供。

5.权利要求3或权利要求4的方法，其中第一次抗体暴露的第一剂量为约1000mg的II型抗-CD20抗体。

6.权利要求3-5中任一项的方法，其中第一次抗体暴露的第二剂量为约1000mg的II型抗-CD20抗体。

7.权利要求1-6中任一项的方法，其中所述第二次抗体暴露包括约900mg至约1100mg的所述II型抗CD20抗体的第一剂量和约900mg至约1100mg的所述II型抗CD20抗体的第二剂量。

8.权利要求1-7中任一项的方法，其中所述第二次抗体暴露包括第一剂量的II型抗CD20抗体和第二剂量的II型抗CD20抗体，并且其中第二次抗体暴露的第二剂量在第二次抗体暴露的第一剂量后约1.5周至约2.5周才提供。

9.权利要求8的方法，其中所述第二次抗体暴露包括第一剂量的II型抗-CD20抗体和第二剂量的II型抗-CD20抗体，并且其中第二次抗体暴露的第二剂量在第二次抗体暴露的第一剂量后约2周才提供。

10.权利要求8或9的方法，其中第二次抗体暴露的第一剂量为约1000mg的II型抗-CD20抗体。

11.权利要求8-10中任一项的方法，其中第二次抗体暴露的第二剂量为约1000mg的II型抗-CD20抗体。

12.权利要求1-11中任一项的方法，其中静脉内施用第一次抗体暴露和第二次抗体暴露。

13.权利要求1-12中任一项的方法，其中所述个体具有III类或IV类狼疮性肾炎。

14.权利要求1-12中任一项的方法，其中所述个体处于发展III类或IV类狼疮性肾炎的

风险中。

15.一种治疗或延缓具有狼疮的个体的狼疮性肾炎进展的方法，包括向所述个体施用有效量的II型抗CD20抗体；其中II型抗CD20抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链，以及包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链；并且其中个体具有III类或IV类狼疮性肾炎。

16.权利要求14的方法，其中静脉内施用所述II型抗CD20抗体。

17.权利要求1-16中任一项的方法，其中所述个体不具有III类(C)或IV类(C)狼疮性肾炎。

18.权利要求1-17中任一项的方法，其中所述个体具有V类狼疮性肾炎。

19.权利要求1-18中任一项的方法，还包括向所述个体施用有效量的免疫抑制剂。

20.权利要求19的方法，其中所述免疫抑制剂包括霉酚酸、其衍生物或其盐。

21.权利要求20的方法，其中所述免疫抑制剂包括霉酚酸酯。

22.权利要求1-21中任一项的方法，还包括向所述个体施用有效量的糖皮质激素或皮质类固醇。

23.权利要求22的方法，其中所述糖皮质激素或皮质类固醇包括甲泼尼龙。

24.权利要求22的方法，其中所述糖皮质激素或皮质类固醇包括强的松。

25.权利要求1-24中任一项的方法，还包括向所述个体施用有效量的抗组胺药。

26.权利要求25的方法，其中所述抗组胺药包括苯海拉明。

27.权利要求1-26中任一项的方法，还包括向所述个体施用有效量的非甾体抗炎药(NSAID)。

28.权利要求27的方法，其中所述NSAID包括对乙酰氨基酚。

29.权利要求1-28中任一项的方法，还包括向所述个体施用有效量的抗高血压剂。

30.权利要求29的方法，其中所述抗高血压剂是血管紧张肽转化酶(ACE)抑制剂或血管紧张肽受体阻断剂。

31.权利要求1-30中任一项的方法，还包括向所述个体施用护理标准治疗。

32.权利要求31的方法，其中所述护理标准治疗包括用血管紧张肽转化酶(ACE)抑制剂、血管紧张肽受体阻断剂、环磷酰胺、霉酚酸酯、硫唑嘌呤和糖皮质激素或皮质类固醇中的一种或多种进行治疗。

33.权利要求1-32中任一项的方法，其中所述方法导致个体中的完全肾反应(CRR)。

34.权利要求1-33中任一项的方法，其中所述方法导致个体中循环的外周B细胞的消耗。

35.权利要求34的方法，其中所述循环的外周B细胞是CD19+B细胞。

36.权利要求1-35中任一项的方法，其中所述II型抗CD20抗体是人源化或人抗体。

37.权利要求1-36中任一项的方法，其中所述II型抗-CD20抗体是无岩藻糖基化的。

38.权利要求1-37中任一项的方法，其中所述II型抗CD20抗体的重链包括：包含SEQ ID NO:7的氨基酸序列的重链可变区。

39.权利要求1-38中任一项的方法，其中所述II型抗CD20抗体的轻链包括：包含SEQ ID NO:8的氨基酸序列的轻链可变区。

40.权利要求1-39中任一项的方法，其中所述II型抗-CD20抗体是奥比妥珠单抗。

41.权利要求1-40中任一项的方法，其中所述个体是人。

42.一种治疗或延缓具有狼疮的个体的狼疮性肾炎进展的药盒，包含：

(a)包含II型抗CD20抗体的容器，其中II型抗CD20抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链，以及包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链；和

(b)包装插页，其具有用于治疗或延缓个体中狼疮性肾炎进展的说明书，其中说明书指示至少向所述个体施用对II型抗CD20抗体的第一次抗体暴露和对II型抗CD20抗体的第二次抗体暴露，第二次抗体暴露在第一次抗体暴露后约18周至约26周才提供；其中所述第一次抗体暴露包括一个或两个剂量的II型抗CD20抗体，所述第一次抗体暴露包括约1800mg至约2200mg所述II型抗-CD20抗体的总暴露；并且其中所述第二次抗体暴露包括一个或两个剂量的II型抗CD20抗体，所述第二次抗体暴露包括约1800mg至约2200mg所述II型抗-CD20抗体的总暴露。

43.权利要求42的药盒，还包括容器，所述容器包括：

(c)第二药物，其中II型抗-CD20抗体是第一药物；和

(d)用于向受试者施用第二药物的包装插页上的说明。

44.权利要求43的药盒，其中所述第二药物是免疫抑制剂、糖皮质激素、皮质类固醇、抗疟剂、细胞毒性剂、整联蛋白拮抗剂、细胞因子拮抗剂或激素。

45.一种治疗或延缓具有狼疮的个体的狼疮性肾炎进展的药盒，包含：

(a)包含II型抗CD20抗体的容器，其中II型抗CD20抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链，以及包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链；和

(b)具有治疗或延缓个体中III类或IV类狼疮性肾炎进展的说明书的包装插页。

46.权利要求45的药盒，还包括容器，所述容器包含：

(c)第二药物，其中II型抗-CD20抗体是第一药物；和

(d)用于向受试者施用第二药物的包装插页上的说明。

47.权利要求46的药盒，其中所述第二药物是免疫抑制剂、糖皮质激素、皮质类固醇、抗疟剂、细胞毒性剂、整联蛋白拮抗剂、细胞因子拮抗剂或激素。

48.一种治疗或延缓个体中类风湿性关节炎(RA)或系统性红斑狼疮(SLE)进展的方法，包括向个体施用有效量的抗CD20抗体，其中所述抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链可变区，以及包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链可变区。

49.权利要求47的方法，其中静脉内施用所述抗体。

50.权利要求47或48的方法，其中所述方法导致个体中循环的外周B细胞的消耗。

51.权利要求49的方法，其中循环的外周B细胞是CD19+B细胞。

52.权利要求47-50中任一项的方法，其中所述抗体是人源化抗体或人抗体。

53.权利要求47-51中任一项的方法，其中所述抗体是无岩藻糖基化的。

54.权利要求47-52中任一项的方法，其中所述重链可变区包含SEQ ID NO:7的氨基酸

序列。

55.权利要求47-53中任一项的方法，其中所述轻链可变区包含SEQ ID NO:8的氨基酸序列。

56.权利要求47-54中任一项的方法，其中所述抗体是奥比妥珠单抗。

57.权利要求47-54中任一项的方法，其中所述抗体包含修饰的Fc区。

58.权利要求56的方法，其中所述Fc区包含用于衰减效应子功能的修饰。

59.权利要求56的方法，其中所述Fc区是人IgG1Fc区。

60.权利要求58的方法，其中所述Fc区包含根据EU指数编号的L234A、L235A和P329G氨基酸取代。

61.权利要求48-60中任一项的方法，其中所述个体是人。

治疗狼疮性肾炎的组合物和方法

[0001] 相关申请的交叉引用

[0002] 本申请要求2015年5月11日提交的美国临时申请序列号62/159,876和2016/2月25日提交的62/300,052的优先权;其各自通过整体引用并入本文。

[0003] 以ASCII文本文件提交序列表

[0004] 以下以ASCII文本文件提交的内容通过整体引用并入本文:序列表的计算机可读形式(CRF)(文件名:146392032240SeqList.txt,记录日期:2016年5月5日,大小:37KB)。

技术领域

[0005] 本文提供了通过施用II型抗CD20抗体来治疗或延缓具有狼疮的个体中狼疮性肾炎进展的方法。本文还提供了通过施用抗CD20抗体治疗或延缓个体中类风湿性关节炎(RA)或系统性红斑狼疮(SLE)进展的方法。

背景技术

[0006] 狼疮是涉及攻击结缔组织的抗体的自身免疫性疾病。估计这种疾病影响近一百万美国人,主要是年龄在20-40岁之间的妇女。狼疮的主要形式是系统性的(系统性红斑狼疮;SLE)。SLE在年龄20-60岁之间的发生率为700名妇女中约1名。SLE能够影响任何器官系统,并且能够导致严重的组织损伤。未经治疗的狼疮可能是致命的,因为它从攻击皮肤和关节发展到包括肺、心脏和肾脏在内的器官,其中肾脏疾病(称为狼疮性肾炎)是主要关注的。狼疮主要表现为一系列爆发,间歇期有很少或没有疾病表现。

[0007] LN是与SLE中致病性相关的最严重的损伤区域之一,并且占该疾病的死亡率和发病率的至少50%。目前,已经诊断为SLE或LN的患者没有真正的治愈性治疗。从实践的角度来看,医生通常采用许多强大的免疫抑制药物,例如大剂量皮质类固醇,例如强的松或硫唑嘌呤或环磷酰胺,它们在爆发期间给予,但也可以持续给予经历经常频繁爆发的人。即使具有减少症状并延长寿命的有效治疗,许多这些药物对被治疗的患者具有潜在的有害副作用。因此,仍然需要对LN具有较少的有害副作用的更有效的治疗。

[0008] 已经在用于治疗狼疮性肾炎的功效的临床研究中测试了两种抗CD20抗体。I型抗CD20抗体-利妥昔单抗未能满足其总反应的主要终点(针对完全肾反应或CRR加权),但导致部分肾反应(PRR)增加了15.3%(Rovin,BH等人,(2012)Arthritis Rheum.64:1215-1226)。另一种I型抗CD20抗体-奥瑞珠单抗因为严重感染事件的不平衡而被部分终止(Mysler,E.F.等人,(2013)Arthritis Rheum.65:2368-2379)。

[0009] 与利妥昔单抗相比,II型抗-CD20抗体-奥比妥珠单抗(obinutuzumab)已显示出产生优良的B细胞消耗。与利妥昔单抗治疗相比,用奥比妥珠单抗处理在食蟹猴中观察到明显更大的B细胞消耗(Mössner,E.等人,(2010)Blood 115:4393-4402)。因此,仍然需要测试II型抗CD20抗体在治疗或预防狼疮患者中的功效。

[0010] 本文引用的所有参考文献,包括专利申请和出版物,通过整体引用并入本文。

[0011] 发明概述

[0012] 在某些方面，本文提供了用于治疗或延缓个体中狼疮性肾炎进展的方法，其包括向所述个体施用对于II型抗CD20抗体的至少第一次抗体暴露和对于II型抗CD20抗体的第二次抗体暴露。在一些实施方案中，个体具有狼疮。在一些实施方案中，直到第一次抗体暴露后约18周至约26周，才提供第二次抗体暴露。在一些实施方案中，直到第一次抗体暴露后约4.5个月至约6.5个月才能提供第二次抗体暴露。在一些实施方案中，第一次抗体暴露包括一个或两个剂量的II型抗CD20抗体，第一次抗体暴露包括约1800mg至约2200mg的II型抗CD20抗体的总暴露。在一些实施方案中，第二次抗体暴露包括一个或两个剂量的II型抗-CD20抗体，第二次抗体暴露包括约1800mg至约2200mg的II型抗-CD20抗体的总暴露。在一些实施方案中，II型抗CD20抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链，以及包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链。在一些实施方案中，个体处于发展III类或IV类狼疮性肾炎的风险中。在一些实施方案中，所述方法用于预防具有狼疮的个体中的狼疮性肾炎。在一些实施方案中，所述方法用于在具有SLE的个体中预防狼疮性肾炎。在一些实施方案中，所述方法用于治疗或延缓具有SLE的个体的狼疮性肾炎的进展。

[0013] 在一些实施方案中，第一次抗体暴露包括第一剂量的II型抗-CD20抗体和第二剂量的II型抗-CD20抗体，并且第一次抗体暴露的第二剂量直到从在第一次抗体暴露的第一剂量后约1.5周至约2.5周才提供。在一些实施方案中，第一次抗体暴露包括第一剂量的II型抗-CD20抗体和第二剂量的II型抗-CD20抗体，并且第一次抗体暴露的第二剂量直到第一次抗体暴露的第一剂量后约2周才提供。在一些实施方案中，第一次抗体暴露包括第一剂量的II型抗-CD20抗体和第二剂量的II型抗CD20抗体，并且第二剂量的第一剂抗体暴露直到第一次抗体暴露的第一剂量后约10天至约17天才提供。在一些实施方案中，第一次抗体暴露包括第一剂量的II型抗-CD20抗体和第二剂量的II型抗-CD20抗体，并且第一次抗体暴露的第二剂量直到第一次抗体暴露的第一剂量后约14天才提供。在一些实施方案中，第一次抗体暴露的第一剂量为约1000mg的II型抗-CD20抗体。在一些实施方案中，第一次抗体暴露的第二剂量为约1000mg的II型抗-CD20抗体。在一些实施方案中，第二次抗体暴露包括II型抗CD20抗体的约900mg至约1100mg之间的第一剂量和II型抗-CD20抗体的约900mg至约1100mg之间的第二剂量。在一些实施方案中，第二次抗体暴露包括第一剂量的II型抗-CD20抗体和第二剂量的II型抗-CD20抗体，并且第二次抗体暴露的第二剂量直到第二次抗体暴露的第一剂量后约1.5周至约2.5周才提供。在一些实施方案中，第二次抗体暴露包括第一剂量的II型抗CD20抗体和第二剂量的II型抗-CD20抗体，并且第二次抗体暴露的第二剂量直到第二次抗体暴露的第一剂量后约2周才提供。在一些实施方案中，第二次抗体暴露包括第一剂量的II型抗-CD20抗体和第二剂量的II型抗-CD20抗体，并且第二次抗体暴露的第二剂量直到第二次抗体暴露的第一剂量后约10天至约17天才提供。在一些实施方案中，第二次抗体暴露包括第一剂量的II型抗-CD20抗体和第二剂量的II型抗-CD20抗体，并且第二次抗体暴露的第二剂量直到第二次抗体暴露的第一剂量后约14天才提供。在一些实施方案中，第二次抗体暴露的第一剂量为约1000mg的II型抗-CD20抗体。在一些实施方案中，第二次抗体暴露的第二剂量为约1000mg的II型抗-CD20抗体。在一些实施方案中，静脉内施用第一次抗体暴露和第二次抗体暴露。在一些实施方案中，个体具有III类或IV类狼疮性肾炎。在一些实施方案中，个体处于发展III类或IV类狼疮性肾炎的风险中。

[0014] 在某些方面，本文提供了用于治疗或延缓具有狼疮的个体的狼疮性肾炎进展的方法，包括向所述个体施用有效量的II型抗-CD20抗体；其中II型抗CD20抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链，以及包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链；并且其中个体具有III类或IV类狼疮性肾炎。在一些实施方案中，个体处于发展III类或IV类狼疮性肾炎的风险中。在一些实施方案中，所述方法用于预防具有狼疮的个体中的狼疮性肾炎。在一些实施方案中，所述方法用于在具有SLE的个体中预防狼疮性肾炎。在一些实施方案中，所述方法用于治疗或延缓具有SLE的个体的狼疮性肾炎的进展。

[0015] 在某些方面，本文提供了用于治疗或延缓具有狼疮的个体的狼疮性肾炎进展的方法，其包括向个体施用约1000mg的剂量的II型抗CD20抗体，其中II型抗CD20抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链和包含SEQ ID NO:4的HVR-L1的序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链，并且其中该剂量在第1、15、168和128天施用于个体。在某些方面，本文提供了用于治疗或延缓具有狼疮的个体的狼疮性肾炎进展的方法，其包括向个体施用约1000mg的剂量的II型抗CD20抗体，其中II型抗CD20抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:1的HVR-H2序列，SEQ ID NO:3的HVR-H3序列的重链，以及包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链，并且其中该剂量在0、2、24和26周施用于个体。在一些实施方案中，第0周对应于第1天。在一些实施方案中，个体具有III类或IV类狼疮性肾炎。在一些实施方案中，II型抗CD20抗体是奥比妥珠单抗。

[0016] 在任何上述实施方案的一些实施方案中，静脉内施用II型抗CD20抗体。在任何上述实施方案的一些实施方案中，个体不具有III类(C)或IV类(C)狼疮性肾炎。在任何上述实施方案的一些实施方案中，个体具有V类狼疮性肾炎。在任何上述实施方案的一些实施方案中，所述方法还包括向个体施用有效量的免疫抑制剂。在一些实施方案中，免疫抑制剂包括霉酚酸、其衍生物或其盐。在一些实施方案中，免疫抑制剂包括霉酚酸酯。在任何上述实施方案的一些实施方案中，所述方法还包括向个体施用有效量的糖皮质激素或皮质类固醇。在一些实施方案中，糖皮质激素或皮质类固醇包括甲泼尼龙。在一些实施方案中，糖皮质激素或皮质类固醇包括泼尼松。在任何上述实施方案的一些实施方案中，所述方法还包括向个体施用有效量的抗组胺药。在一些实施方案中，抗组织胺包括苯海拉明。在任何上述实施方案的一些实施方案中，所述方法还包括向个体施用有效量的非甾体抗炎药(NSAID)。在一些实施方案中，NSAID包括对乙酰氨基酚。在任何上述实施方案的一些实施方案中，所述方法还包括向个体施用护理标准治疗。在一些实施方案中，护理标准治疗包括用血管紧张肽转化酶(ACE)抑制剂，血管紧张肽受体阻断剂，环磷酰胺，霉酚酸酯，硫唑嘌呤和糖皮质激素或皮质类固醇中的一种或多种进行治疗。在一些实施方案中，在对II型抗CD20抗体的第一次抗体暴露于之后和/或在对II型抗-CD20抗体的第二次抗体暴露之后施用护理标准治疗。在任何上述实施方案的一些实施方案中，所述方法还包括向个体施用有效量的抗高血压药。在一些实施方案中，抗高血压药是血管紧张肽转化酶(ACE)抑制剂或血管紧张肽受体阻断剂。在任何上述实施方案的一些实施方案中，该方法导致个体中的完全肾反应(CRR)。在任何上述实施方案的一些实施方案中，该方法导致个体中循环的外周B细胞的消耗。在一些实施方案中，循环的外周B细胞是CD19+ B细胞。在任何上述实施方案的一些实施方案中，II

型抗CD20抗体是人源化或人抗体。在任何上述实施方案的一些实施方案中，II型抗-CD20抗体是非岩藻糖基化的。在任何上述实施方案的一些实施方案中，II型抗CD20抗体是非岩藻糖基化的(例如，如美国专利号8，883，980中所述)。在任何上述实施方案的一些实施方案中，II型抗CD20抗体的重链包括：包含SEQ ID NO:7的氨基酸序列的重链可变区。在任何上述实施方案的一些实施方案中，II型抗CD20抗体的轻链包括：包含SEQ ID NO:8的氨基酸序列的轻链可变区。在任何上述实施方案的一些实施方案中，II型抗CD20抗体是奥比妥珠单抗。在任何上述实施方案的一些实施方案中，个体或患者是人。

[0017] 在某些方面，本文提供了用于治疗或延缓具有狼疮的个体的狼疮性肾炎进展的药盒或制品，包括：(a)包括II型抗-CD20抗体的容器，其中II型抗CD20抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链和包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链；和(b)具有用于治疗或延缓个体中狼疮性肾炎进展的说明书的包装插页，其中说明书指示将对于II型抗CD20抗体的至少第一次抗体暴露和对于II型抗CD20抗体的第二次抗体暴露施用于个体，第二次抗体暴露在第一次抗体暴露后约18周至约26周后才提供；其中所述第一次抗体暴露包括一个或两个剂量的II型抗CD20抗体，所述第一次抗体暴露包括约1800mg至约2200mg的II型抗-CD20抗体的总暴露；并且其中所述第二次抗体暴露包括一个或两个剂量的II型抗CD20抗体，所述第二次抗体暴露包括约1800mg至约2200mg的II型抗-CD20抗体的总暴露。在一些实施方案中，药盒或制品还包括(c)第二药物，其中II型抗CD20抗体是第一药物；和(d)用于将第二药物施用于受试者的包装插页的说明。在一些实施方案中，第二药物是免疫抑制剂、糖皮质激素、皮质类固醇、抗疟剂、细胞毒性剂、整联蛋白拮抗剂、细胞因子拮抗剂或激素。在一些实施方案中，II型抗CD20抗体的重链包括：包含SEQ ID NO:7的氨基酸序列的重链可变区。在一些实施方案中，II型抗CD20抗体的轻链包括：包含SEQ ID NO:8的氨基酸序列的轻链可变区。在一些实施方案中，II型抗CD20抗体是奥比妥珠单抗。在一些实施方案中，药盒或制品用于在具有SLE的个体中预防狼疮性肾炎。在一些实施方案中，药盒或制品用于治疗或延缓具有SLE的个体的狼疮性肾炎的进展。

[0018] 在某些方面，本文提供了用于治疗或延缓个体中类风湿性关节炎(RA)或系统性红斑狼疮(SLE)进展的方法，包括向个体施用有效量的抗CD20抗体，其中抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链可变区，以及包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链可变区。在一些实施方案中，静脉内施用抗体。在一些实施方案中，该方法导致个体中循环的外周B细胞的消耗。在一些实施方案中，循环的外周B细胞是CD19+ B细胞。在一些实施方案中，抗体是人源化或人抗体。在一些实施方案中，抗体是非岩藻糖基化的。在一些实施方案中，重链可变区包含SEQ ID NO:7的氨基酸序列。在一些实施方案中，轻链可变区包含SEQ ID NO:8的氨基酸序列。在一些实施方案中，重链可变区包含SEQ ID NO:7的氨基酸序列，轻链可变区包含SEQ ID NO:8的氨基酸序列。在一些实施方案中，抗体是奥比妥珠单抗。在一些实施方案中，抗体包含修饰的Fc区。在一些实施方案中，Fc区包括用于衰减效应子功能的修饰。在一些实施方案中，Fc区是人IgG1 Fc区。在一些实施方案中，人IgG1 Fc区包括根据EU指数编号的L234A、L235A和P329G氨基酸取代。在任何上述实施方案的一些实施方案中，个体或患者是人。

[0019] 在某些方面，本文提供了用于治疗或延缓个体中类风湿性关节炎(RA)或系统性红斑狼疮(SLE)进展的组合物，所述组合物包括抗CD20抗体，其中所述抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链可变区，以及包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链可变区。在一些实施方案中，静脉内施用组合物。在一些实施方案中，施用组合物导致个体中循环外周B细胞的消耗。在一些实施方案中，循环的外周B细胞是CD19+ B细胞。在一些实施方案中，抗体是人源化或人抗体。在一些实施方案中，抗体是非岩藻糖基化的。在一些实施方案中，重链可变区包含SEQ ID NO:7的氨基酸序列。在一些实施方案中，轻链可变区包含SEQ ID NO:8的氨基酸序列。在一些实施方案中，重链可变区包含SEQ ID NO:7的氨基酸序列，轻链可变区包含SEQ ID NO:8的氨基酸序列。在一些实施方案中，抗体是奥比妥珠单抗。在一些实施方案中，抗体包含修饰的Fc区。在一些实施方案中，Fc区包括用于衰减效应子功能的修饰。在一些实施方案中，Fc区是人IgG1 Fc区。在一些实施方案中，人IgG1 Fc区包括根据EU指数编号的L234A、L235A和P329G氨基酸取代。

[0020] 在某些方面，本文提供了抗CD20抗体在制备用于治疗个体中的类风湿性关节炎(RA)或系统性红斑狼疮(SLE)的药物中的用途，其中所述抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链可变区，以及包含SEQ ID NO:4的HVR -L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链可变区。

[0021] 在某些方面，本文提供了用于治疗或延缓个体中类风湿性关节炎(RA)或系统性红斑狼疮(SLE)进展的药盒或制品，其包含：(a)包括抗CD20抗体的容器，其中所述抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链可变区，以及包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链可变区；和(b)具有用于施用有效量的抗CD20抗体以治疗或延缓个体中类风湿性关节炎(RA)或系统性红斑狼疮(SLE)进展的说明书的包装插页。在一些实施方案中，包装插页包括用于静脉内施用抗体的说明书。在一些实施方案中，抗体是人源化或人抗体。在一些实施方案中，抗体是非岩藻糖基化的。在一些实施方案中，重链可变区包含SEQ ID NO:7的氨基酸序列。在一些实施方案中，轻链可变区包含SEQ ID NO:8的氨基酸序列。在一些实施方案中，重链可变区包含SEQ ID NO:7的氨基酸序列，轻链可变区包含SEQ ID NO:8的氨基酸序列。在一些实施方案中，抗体是奥比妥珠单抗。在一些实施方案中，抗体包含修饰的Fc区。在一些实施方案中，Fc区包括用于衰减效应子功能的修饰。在一些实施方案中，Fc区是人IgG1 Fc区。在一些实施方案中，人IgG1 Fc区包括根据EU指数编号的L234A、L235A和P329G氨基酸取代。

[0022] 应当理解，本文所述的各种实施方案的一个、一些或全部属性可以组合以形成本发明的其它实施方案。本发明的这些和其它方面对于本领域技术人员将变得显而易见。通过下面的详细描述进一步描述本发明的这些和其它实施方案。

附图说明

[0023] 图1示出了对检查奥比妥珠单抗+霉酚酸酯vs.安慰剂+霉酚酸酯的二期研究的研究设计。EP＝终点；MMF＝霉酚酸酯

[0024] 图2A-2D示出了奥比妥珠单抗或利妥昔单抗在RA和SLE患者样本中引起的全血B细胞消耗、内化和补体依赖性细胞毒性。图2A示出了RA(n=31)和SLE(n=34)患者的全血样本。将样本用或不用抗CD20mAb、RTX、OBZ_{Gly}和OBZ温育24小时，然后进行流式细胞术以分析B细胞死亡。值是三个重复孔的平均值。框中的水平线代表中位数，框代表四分位数范围，并且框须代表范围。图2B示出了表面可及的mAb的频率。通过用来自先前用抗FcγRII阻断mAb，AT10温育的RA(n=5)和SLE(n=8)患者的分离的B细胞温育6小时后，以流式细胞术评价频率。水平线代表中位数。图2C示出了由RTX和OBZ诱导的CDC。将分离的B细胞(健康对照(HC)，n=2；RA，n=2和SLE，n=3)与正常健康血清(NHS)或热灭活血清(HIS)用RTX或OBZ温育30分钟后分析裂解的CD19+ Av+ PI+ B细胞的频率。图2D示出了用NHS对HIS温育的样品中CD19+ Av+ PI+细胞的倍数增加，表示mAb对CDC的效率。RTX，利妥昔单抗；OBZ，奥比妥珠单抗；RA，类风湿关节炎；SLE，系统性红斑狼疮。HC，健康对照*$p<0.05$；**，$p<0.005$；***，$p<0.0001$和ns，不显著。

[0025] 图3A-3G示出流式细胞术门控策略来评估NK细胞脱粒，描述CD107a和CD16的NK细胞表达之间的关系。将全血样本用或不用mAb温育24小时，然后通过流式细胞术分析。基于前向和侧向散射特性和CD56的表达而不是CD3鉴定NK细胞。CD3-CD56+ CD107a+细胞的频率表示活化/脱粒的NK细胞。FSC，前向散射；SSC，侧向散射。图3A示出了前向散射与侧向散射的流式细胞术门控。图3B示出了CD56与CD3的流式细胞术门控。图3C示出了前向散射对CD107a的流式细胞术门控。图3D示出了前向散射对CD16的流式细胞术门控。基于CD16的相对表达鉴定了CD3-CD56+ NK细胞的三个亚群(框中为高、中、低)。基于以等级方式CD16++<CD16+<CD16-的CD16表达，激活的CD107a+ NK细胞的相对频率在这3个亚群中不同。图3E示出了对于高框的前向散射对CD107a的流式细胞术门控。图3F示出了对于中框的前向散射对CD107a的流式细胞术门控。图3G示出了对于低框的前向散射对CD107a的流式细胞术门控。

[0026] 图4A-4D示出在RA和SLE患者样品中激活NK细胞方面，OBZ比RTX更有效。评估在存在或不存在mAb的情况下温育24小时的具有RA(n=18)和SLE(n=23)的患者的全血样品中的NK细胞活化。*，$p<0.05$；**，$p<0.005$；***，$p<0.0001$；ns，不显著，当p至少<0.05时，Spearman相关系数r^2被认为是显著的。图4A示出了在淋巴细胞门中的CD3-CD56+ NK细胞、CD3-CD56+ CD107a+ NK细胞和CD3-CD56-CD16+ NK细胞的频率作为总NK细胞或CD19+细胞的百分比。水平线代表中位数。图4B示出了来自RA和SLE的患者，用RTX和OBZ温育的样品中，CD3-CD56+ CD107a+ NK细胞的频率和CD3-CD56+ CD107a+ NK细胞频率的倍数增加以及CD3-CD56+ 16+ NK细胞的频率。水平线代表中位数。图4C示出了在来自RA(n=18)患者的用或不用RTX和OBZ温育的样品中CD3-CD56+ CD107a+ NK细胞的频率之间的关系。图4D示出了在来自SLE(n=23)患者的用或不用RTX和OBZ温育的样品中CD3-CD56+ CD107a+ NK细胞的频率之间的关系。

[0027] 图5A-5D示出在RA和SLE患者样品中引发NK细胞介导的细胞毒性方面，奥比妥珠单抗比利妥昔单抗更有效。图5A示出了全血B细胞消耗测定，其显示了来自RA(n=18)和SLE(n=23)患者的样品中RTX、OBZ_{Gly}和OBZ的B细胞消耗百分比。框和框须代表四分位数范围并且该范围，框中的水平线代表中位数。图5B示出了通过流式细胞术分析，用或不用mAb温育24小时后RA和SLE患者的全血样品中CD3-CD56+ CD107a+ NK细胞的频率。图5C示出了通过流式细胞术分析，用或不用mAb温育的全血样品中CD3-CD56+ CD107a+ NK细胞的频率相对增

加。图5D示出了通过流式细胞术分析，用或不用mAb温育24小时后RA (n＝18) 和SLE (n＝23) 患者的全血样品中CD3-CD56+ CD16+ NK细胞的频率。对于条形图，误差条表示中位数和四分位数范围。*p<0.05；**，p<0.005；***，p<0.0001；和ns，不显著。

[0028] 图6A-6D示出在RA和SLE患者样品中激活嗜中性粒细胞方面奥比妥珠单抗比利妥昔单抗更有效。图6A示出了在用或不用mAb (1μg/ml) 温育的RA (n＝10) 和SLE (n＝22) 患者的全血样品温育24小时后，CD11b在CD15+嗜中性粒细胞上的平均荧光强度 (MFI) 。中位数和四分位数范围由误差条表示。图6B示出了在RA或SLE样品中用或不用mAb温育的样品中CD11b在CD15+嗜中性粒细胞上的MFI之间的关系。图6C示出了用或不用在RA和SLE样品中的mAb温育的样品中CD15+嗜中性粒细胞上CD62L的MFI。图6D示出了在用或不用RA (n＝10) 和SLE (n＝22) 样品的mAb温育的样品中CD15+嗜中性粒细胞上CD62L的MFI之间的关系。*p<0.05；**，p<0.005；***，p<0.0001。当p至少<0.05时，Spearman相关系数r^2被认为是显著的。

[0029] 图7A-7D示出来自RA和SLE样品的B细胞亚群中CD20和Fc γ RIIb的直接细胞死亡、内化和表达的评估。图7A示出了基于IgD和CD27的相对表达 (IgD+ CD27-初始细胞；IgD+ CD27+未转换记忆细胞；IgD-CD27+转换的记忆细胞和IgD-CD27-双阴性细胞) ，作为所有CD19+ B细胞和B细胞亚群的比例的膜联蛋白V+细胞的频率；在用或不用mAb温育的RA (n＝5) 和SLE (n＝4) 患者的样品中。图7B示出了来自SLE患者 (n＝9) 的样品中所有CD19+细胞和B细胞亚群上CD20的平均荧光强度 (MFI) 。图7C示出了表面荧光猝灭测定。在所有CD19+ B细胞和B细胞亚群中，用来自SLE患者 (n＝9) 的具有或者不具有用抗Fc γ RIImAb，AT10的先前温育的分离的B细胞温育6小时后的表面可及的mAb的频率。图7D示出了患有SLE (n＝9) 的患者的样品中所有CD19+ B细胞和B细胞亚群中Fc γ RIIb的MFI。对于条形图，误差条表示中位数和四分位数范围。盒和盒须代表四分位数范围，盒中的水平线代表中位数。*p<0.05；**，p<0.005；***，p<0.0001。

[0030] 图8示出了补体依赖性细胞毒性测定的门控策略，以及通过RTX和OBZ的CDC。将分离的B细胞用含有NHS或HIS的mAb在室温下温育30分钟，然后通过流式细胞术分析。An V+ PI+细胞的频率代表细胞死亡。HIS，热灭活血清；NHS，正常健康血清；RTX，利妥昔单抗；OBZ，奥比妥珠单抗；V，Annexin V和PI，碘化丙锭。

[0031] 图9示出了评估嗜中性粒细胞活化的流式细胞术门控策略。温育24小时后，通过流式细胞术分析全血样品。通过前向和侧向散射和CD15阳性识别嗜中性粒细胞。在对CD15为阳性的门控中性粒细胞上分析CD11b和CD62L的平均荧光强度。

[0032] 图10示出了流式细胞术门控策略来评估直接细胞死亡。在37℃和5％CO2下用或不用mAb温育6小时后，通过流式细胞术分析分离的B细胞。CD19+ B细胞分为初始 (IgD+ CD27-) ，未转换记忆细胞 (IgD+ CD27+) ，转换记忆细胞 (IgD-CD27+) 和双阴性细胞 (IgD-CD27-) 。膜联蛋白V+细胞的频率代表直接细胞死亡。

[0033] 图11示出了B细胞亚群中自发性细胞死亡的固有敏感性。通过流式细胞术分析在37℃和5％CO2下用补充有10％胎牛血清的RPMI温育6小时的分离的B细胞。膜联蛋白V+细胞的频率代表CD19+细胞整体以及也在分为初始 (IgD+ CD27-) ，未转换记忆细胞 (IgD+ CD27+) ，转换记忆细胞 (IgD-CD27+) ，和双阴性细胞 (IgD-CD27-) 的B细胞亚群中的直接细胞死亡。Av，膜联蛋白V；*p<0.05；***，p<0.0001；ns，不显著。

[0034] 发明详述

[0035] 在一个方面，本文提供了用于治疗或延缓个体中狼疮性肾炎进展的方法，包括向个体施用对于II型抗CD20抗体的至少第一次抗体暴露和对于II型抗CD20抗体的第二次抗体暴露。在一些实施方案中，个体具有狼疮。在一些实施方案中，在第一次抗体暴露后约18周至约26周之后才提供第二次抗体暴露。在一些实施方案中，第一次抗体暴露包括一个或两个剂量的II型抗CD20抗体，第一次抗体暴露含有约1800mg至约2200mg的II型抗-CD20抗体的总暴露。在一些实施方案中，第二次抗体暴露包括一个或两个剂量的II型抗CD20抗体，第二次抗体暴露含有约1800mg至约2200mg的II型抗-CD20抗体的总暴露。在一些实施方案中，抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链，以及包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链。

[0036] 在另一方面，本文提供了用于治疗或延缓具有狼疮的个体的狼疮性肾炎进展的方法，包括向个体施用有效量的II型抗CD20抗体。在一些实施方案中，抗体包含含有SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链和含有SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链。在一些实施方案中，个体具有III类或IV类狼疮性肾炎。

[0037] 在另一方面，本文提供了用于治疗或延缓个体中类风湿性关节炎(RA)或系统性红斑狼疮(SLE)进展的方法，其包括向个体施用有效量的抗CD20抗体。在一些实施方案中，抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链可变区，和包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链可变区。

[0038] I.一般技术

[0039] 本文所描述或参考的技术和程序通常被本领域技术人员充分理解并使用常规方法通常采用，例如广泛利用的方法描述于Sambrook等人，Molecular Cloning:A Laboratory Manual第3版(2001)Cold Spring Harbor Laboratory Press,Cold Spring Harbor,N.Y.;Current Protocols in Molecular Biology(F.M.Ausubel,等人编(2003))；系列Methods in Enzymology(Academic Press,Inc.):PCR 2:A Practical ApproacH(M.J.MacPherson,B.D.Hames和G.R.Taylor编(1995))，Harlow和Lane编(1988)Antibodies,A Laboratory Manual,and Animal Cell Culture(R.I.Freshney编(1987))；Oligonucleotide Synthesis(M.J.Gait编,1984);Methods in Molecular Biology,Humana Press;Cell Biology:A Laboratory Notebook(J.E.Cellis,编,1998)Academic Press;Animal Cell Culture(R.I.Freshney)编,1987);Introduction to Cell and Tissue Culture(J.P.Mather and P.E.Roberts,1998)Plenum Press;Cell and Tissue Culture:Laboratory Procedures(A.Doyle,J.B.Griffiths,and D.G.Newell编,1993-8)J.Wiley and Sons;Handbook of Experimental Immunology(D.M.Weir和C.C.Blackwell编);Gene TransFcr Vectors for Mammalian Cells(J.M.Miller and M.P.Calos编,1987);PCR:The Polymerase Chain Reaction,(Mullis等人编,1994);Current Protocols in Immunology(J.E.Coligan等人编,1991);Short Protocols in Molecular Biology(Wiley and Sons,1999);Immunobiology(C.A.Janeway and P.Travers,1997)；Antibodies(P.Finch,1997);Antibodies:A Practical ApproacH(D.Catty编,IRL Press,

1988-1989);Monoclonal Antibodies:A Practical ApproacH(P.Shepherd and C.Dean编,Oxford University Press,2000);Using Antibodies:A Laboratory Manual(E.Harlow and D.Lane(Cold Spring Harbor Laboratory Press,1999);The Antibodies(M.Zanetti和J.D.Capra编,Harwood Academic Publishers,1995);和Cancer:Principles and Practice of Oncology(V.T.DeVita等人编,J.B.Lippincott Company,1993)。

[0040] II.定义

[0041] 术语"狼疮性肾炎(LN)"是指在肾脏中的狼疮(例如,系统性红斑狼疮、药物诱发的狼疮、新生儿狼疮或盘状狼疮)的表现。

[0042] 术语"抗体"包括单克隆抗体(包括具有免疫球蛋白Fc区的全长抗体)、具有多表位特异性的抗体组合物、多特异性抗体(例如,双特异性抗体、双抗体和单链分子,以及抗体片段(例如,Fab、F(ab')2和Fv)。术语"免疫球蛋白"(Ig)与"抗体"在本文中互换使用。

[0043] 基本的4链抗体单元是异四聚体糖蛋白,其由两条相同的轻链(L)和两条相同的重链(H)组成。IgM抗体由5个基本的异四聚体单元连同额外的被称为J链的多肽组成,并包含10个抗原结合位点,而IgA抗体包含2-5个基本的4链单元,其可聚合形成与J链组合的多价装配物。在IgG的情况中,4链单元通常是约150,000道尔顿。每个L链通过一个共价二硫键连接到H链,而两个H链取决于H链的同种型通过一个或多个二硫键彼此相连。每个H和L链还具有规律间隔的链内二硫键。每条H链在N-末端具有可变结构域(V_H),接着是对于每条α和Y链为三个恒定结构域(C_H)和对于μ和ε同种型为4个C_H结构域。每条L链在N-末端具有可变结构域(V_L),随后为在其另一端的恒定结构域。V_L与V_H对准,并且C_L与重链(C_HI)的第一个恒定结构域对准。认为特定氨基酸残基形成轻链和重链可变结构域之间的界面。V_H和V_L的配对一起形成单个抗原结合位点。对于不同类别抗体的结构和特性,参见,例如,Basic and Clinical Immunology,第8版,Daniel P.Sties,Abba I.Terr and Tristram G.Parsolw(编),Appleton&Lange,Norwalk,CT,1994,第71页和Chapter 6。基于其恒定结构域的氨基酸序列,来自任何脊椎动物物种的L链可归入给两个完全不同的类型(称为K和λ)之一。取决于其重链(CH)的恒定结构域的氨基酸序列,免疫球蛋白可归入不同的类或同种型。有五类免疫球蛋白:IgA、IgD、IgE、IgG和IgM,分别具有称为α、δ、ε、γ和μ的重链。基于CH序列和功能中相对小的差异,γ和α类进一步分为亚类,例如,人表达下列亚类:IgG1、IgG2A、IgG2B、IgG3、IgG4、IgA1和IgA2。

[0044] 抗体的"可变区"或"可变结构域"指抗体的重链或轻链的氨基末端结构域。重链和轻链的可变结构域可以分别称为"VH"和"VL"。这些结构域一般是抗体的最易变化的部分(相对于同一类的其它抗体)并包含抗原结合位点。

[0045] 术语"可变"是指这样的事实,该可变结构域的某些区段在抗体间序列中广泛地不同。该V结构域介导抗原结合并限定特定抗体对其特定抗原的特异性。然而,变异性并非均匀分布于可变区的整个跨度。相反,其集中在轻链和重链可变结构域中都有的三个称为高变区(HVR)的区段内。可变结构域中更加高度保守的部分称作构架区(FR)。天然重链和轻链的可变结构域各自包含四个FR区,大多采取β-折叠构型,通过3个HVR连接,其形成环状连接,并且在某些情况下形成β-折叠结构的一部分。每条链中的HVR通过FR区保持紧密接近在一起,并与来自另一条链的HVR一起促进抗体的抗原结合位点的形成(参见Kabat等人,Sequences of Immunological Interest,第5版,National Institute of Health,

Bethesda,MD(1991))。恒定结构域不直接参与抗体与抗原的结合,但展现出多种效应子功能,例如抗体在抗体依赖性的细胞毒性中的参与。

[0046] 如本文所用的术语"单克隆抗体"指获自基本上同质性抗体群的抗体,即包括该群体的各个抗体除了可以少量存在的可能天然发生的突变和/或翻译后修饰(例如异构化、酰胺化)之外是相同的。单克隆抗体针对单个抗原位点是高度特异性的。与多克隆抗体制剂(通常包括针对不同决定簇(表位)的不同抗体)相反,每种单克隆抗体针对抗原上的单个决定簇。除了它们的特异性,单克隆抗体的优点还在于它们通过杂交瘤培养合成,未受到其它免疫球蛋白的污染。修饰语"单克隆"表明该抗体的特征为从抗体的基本上同质的群中获得,并且不应被解释为需要通过任何特定方法生产抗体。例如,按照本发明待使用的单克隆抗体可通过多种技术制成,包括例如,杂交瘤方法(例如,Kohler和Milstein.,Nature,256:495-97(1975);Hongo等人,Hybridoma,14(3):253-260(1995),Harlow等人,Antibodies:A Laboratory Manual,(Cold Spring Harbor Laboratory Press,第二版.1988);Hammerling等人,在:Monoclonal Antibodies and T-Cell Hybridomas 563-681(Elsevier,N.Y.,1981)中),重组DNA方法(参见,例如美国专利号4,816,567),噬菌体展示技术(参见,例如Clackson等人,Nature,352:624-628(1991);Marks等人,J.Mol.Biol.222:581-597(1992);Sidhu等人,J.Mol.Biol.338(2):299-310(2004);Lee等人,J.Mol.Biol.340(5):1073-1093(2004);Fcllouse,Proc.Natl.Acad.Sci.USA 101(34):12467-12472(2004);和Lee等人,J.Immunol.Methods 284(1-2):119-132(2004)),和用于在动物中生产具有编码人免疫球蛋白序列的部分或全部人免疫球蛋白基因座或基因的人或人样抗体的技术(参见,例如WO 1998/24893;WO1996/34096;WO 1996/33735;WO 1991/10741;Jakobovits等人,Proc.Natl.Acad.Sci.USA 90:2551(1993);Jakobovits等人,Nature 362:255-258(1993);Bruggemann等人,Year in Immunol.7:33(1993);美国专利号5,545,807;5,545,806;5,569,825;5,625,126;5,633,425;和5,661,016;Marks等人,Bio/Technology 10:779-783(1992);Lonberg等人,Nature 368:856-859(1994);Morrison,Nature 368:812-813(1994);Fishwild等人,Nature Biotechnol 14:845-851(1996);Neuberger,NatureBiotechnol.14:826(1996);和Lonberg和Huszar,Intern.Rev.Immunol.13:65-93(1995))。

[0047] "术语"裸抗体"是指不与细胞毒性部分或放射性标记缀合的抗体。

[0048] 术语"全长抗体"、"完整抗体"或"全抗体"可互换使用,是指与抗体片段相反,以其基本完整形式的抗体。具体地,全抗体包含具有重链和轻链(包括Fc区)的那些。恒定结构域可以是天然序列恒定结构域(例如人天然序列恒定结构域)或其氨基酸序列变体。在一些情况下,完整抗体可以具有一个或多个效应子功能。

[0049] "抗体片段"包括完整抗体的一部分,优选完整抗体的抗原结合和/或可变区。抗体片段的实例包括Fab、Fab'、F(ab')2和Fv片段;双抗体;线性抗体(参见美国专利5,641,870,实施例2;Zapata等人,Protein Eng.8(10):1057-1062[1995]));单链抗体分子和由抗体片段形成的多特异性抗体。木瓜蛋白酶消化抗体产生两个相同的抗原结合片段,称为"Fab"片段,和残余的"Fc"片段,该名称反映了易于结晶的能力。Fab片段由完整的L链以及H链的可变区结构域(V_H),和一条重链的第一个恒定结构域(C_H1)组成。每个Fab片段相对于抗原结合是单价的,即,它具有单个抗原结合位点。抗体的胃蛋白酶处理产生一个单一的大的F(ab')

2片段，其粗略地相当于两个通过二硫键相连的具有不同的抗原结合活性的Fab片段，且仍能够交联抗原。Fab' 片段与Fab片段不同之处在于在CH1结构域的羧基末端具有几个额外的残基，包括来自抗体铰链区的一个或多个半胱氨酸。Fab' -SH是本文对于Fab' 的指定，其中所述恒定结构域的一个或多个半胱氨酸残基携带游离硫醇基。$F(ab')_2$抗体片段最初作为成对的Fab' 片段产生，在它们之间有铰链半胱氨酸。抗体片段的其它化学偶联也是已知的。

[0050] Fc片段包括通过二硫键保持在一起的两条H链的羧基末端部分。抗体的效应子功能是通过Fc区中的序列决定的，所述区域也被在某些类型的细胞上发现的Fc受体(FcR)识别。

[0051] "Fv"是最小抗体片段，其含有完整的抗原识别和结合位点。此片段由紧密、非共价结合的一个重链和一个轻链可变区结构域的二聚体组成。从这两个结构域的折叠散发出六个高变环(3个环分别来自H和L链)，其贡献氨基酸残基用于抗原结合，并赋予抗体抗原结合特异性。然而，即使是单个可变结构域(或仅包含三个对抗原特异的HVR的Fv的一半)也具有识别和结合抗原的能力，尽管以比完整结合位点更低的亲和力。

[0052] "单链Fv"也简称为"sFv"或"scFv"，是包含连接成单个多肽链的V_H和V_L抗体结构域的抗体片段。优选地，所述sFv多肽进一步包含在V_H和V_L结构域之间的多肽接头，其使得sFv能够形成抗原结合所需的结构。对于sFv的综述，参见Pluckthun于The Pharmacology of Monoclonal Antibodies，vol.113，Rosenburg和Moore编，Springer-Verlag，New York，pp.269-315(1994)中。

[0053] 本发明的抗体的"功能性片段"包含完整抗体的一部分，一般包括完整抗体的抗原结合或可变区或抗体的Fc区，其保留或具有修饰的FcR结合能力。抗体片段的实例包括线性抗体、单链抗体分子和由抗体片段形成的多特异性抗体。

[0054] 术语"双抗体"是指小的抗体片段，其通过如下制备：构建SFv片段(见上一段)，其具有在V_H和V_L结构域之间的短接头(约5-10个残基)，使得实现V结构域的链间而非链内配对，由此产生二价片段，即具有两个抗原结合位点的片段。双特异性双抗体是两个"交叉"的sFv片段的异源二聚体，其中两个抗体的V_H和V_L结构域存在于不同的多肽链上。双抗体更详细地描述于例如EP 404,097；WO 93/11161；Hollinger等人，Proc.Natl.Acad.Sci.USA 90:6444-6448(1993)。

[0055] 本文的单克隆抗体特别包括"嵌合"抗体(免疫球蛋白)，其中一部分重链和/或轻链与衍生自特定物种或属于特定抗体类型或亚类的抗体中相应序列相同或同源，而一条或多条链的剩余部分与衍生自另一物种或属于另一抗体类型或亚类的抗体，以及这些抗体的片段中相应序列相同或同源，只要它们展示所希望的生物活性(美国专利号4,816,567；和Morrison等人，Proc.Natl.Acad.Sci.USA 81:6851-6855)(1984))。本文所关注的嵌合抗体包含**PRIMATIZED®**抗体，其中抗体的抗原结合区衍生自通过例如用目的抗原免疫猕猴所产生的抗体。如本文所用，"人源化抗体"用作"嵌合抗体"的子集。

[0056] 非人(例如鼠)抗体的"人源化"形式是包含衍生自非人免疫球蛋白的最小序列的嵌合抗体。在一个实施方案中，人源化抗体是人免疫球蛋白(受体抗体)，其中来自受体的HVR(定义见下文)的残基被来自非人物种的HVR(供体抗体)的残基取代，所述非人物种例如小鼠、大鼠、兔或具有所期望的特异性、亲和力和/或能力的非人灵长类动物。在一些情况下，人免疫球蛋白的构架("FR")残基被相应的非人残基取代。此外，人源化抗体可包含在受

体抗体或供体抗体中未发现的残基。可以作出这些修饰以进一步改进抗体的性能，如结合亲和力。一般而言，人源化抗体将包含基本上所有的至少一个，并且通常两个可变结构域，其中所有的或基本上所有的高变环对应于非人免疫球蛋白序列的那些，并且所有或基本上所有的FR区是人免疫球蛋白序列的那些，尽管FR区可以包括一个或多个个别的FR残基的取代，所述取代提高抗体的性能，如结合亲和力、异构化、免疫原性等。FR中的这些氨基酸取代的数目在H链中通常不超过6个，并在L链中不超过3个。人源化抗体任选地还将包含至少一部分免疫球蛋白恒定区(Fc)，通常是人免疫球蛋白的恒定区。进一步的细节，参见例如，Jones等人，Nature 321:522-525(1986)；Riechmann等人，Nature 332:323-329(1988)；和Presta，Curr.Op.Struct.Biol.2:593-596(1992)。还参见，例如Vaswani和Hamilton，Ann.Allergy，Asthma&Immunol.1:105-115(1998)；Harris，Biochem.Soc.Transactions 23:1035-1038(1995)；Hurle和Gross，Curr.Op.Biotech.5:428-433(1994)；和美国专利号6,982,321和7,087,409。

[0057] "人抗体"是指具有相应于由人产生的抗体的氨基酸序列的抗体和/或已使用如本文所公开的任何用于制备人抗体的技术制造的抗体。人抗体的这种定义明确排除包含非人抗原结合残基的人源化抗体。人抗体可使用本领域已知的多种技术，包括噬菌体展示文库来制备。Hoogenboom和Winter，J.Mol.Biol，227:381(1991)；Marks等人，J.Mol.Biol，222:581(1991)。还可以用于人单克隆抗体制备的方法描述于Cole等人，Monoclonal Antibodies and Cancer Therapy，Alan R.Liss，p.77(1985)；Boerner等人，J.Immunol，147(I):86-95(1991)。还参见van Dijk和van de Winkel，Curr.Opin.Pharmacol，5:368-74(2001)。人抗体可以通过向转基因动物施用抗原来制备，所述转基因动物已被修饰以产生此种抗体来应答抗原攻击，但其内源基因座已被失效，例如，免疫的异种小鼠(关于XENOMOUSE™技术参见例如美国专利号6,075,181和6,150,584)。关于经人B细胞杂交瘤技术生成的人抗体还参见，例如Li等人，Proc.Natl.Acad.Sci.USA，103:3557-3562(2006)。

[0058] 本文中使用时，术语"高变区"、"HVR"或"HV"指抗体可变结构域的区域，其序列高度可变和/或形成结构确定的环。通常，抗体包含6个HVR:3个在VH(H1、H2、H3)中，并且3个在VL(L1、L2、L3)中。在天然抗体中，H3和L3展示这6个HVR的最大多样性，并且尤其H3被认为在赋予抗体精细的特异性方面发挥独特的作用。参见，例如Xu等人，Immunity 13:37-45(2000)；Johnson和Wu，在Methods in Molecular Biology 248:1-25中(Lo，编，Human Press，Totowa，NJ，2003)。事实上，仅由重链组成的天然存在的骆驼科抗体在缺乏轻链的情况下是有功能的且稳定的。参见，例如Hamers-Casterman等人，Nature 363:446-448(1993)；Sheriff等人，Nature Struct.Biol.3:733-736(1996)。

[0059] 本文使用和涵盖了许多HVR描述。Kabat互补决定区(CDR)是基于序列变异性，并且是最常用的(Kabat等人，Sequences of Proteins of Immunological Interest，第5版。Public Health Service，National Institutes of Health，Bethesda，MD.(1991))。Chothia改为指结构环的位置(Chothia 和Lesk，J.Mol.Biol.196:901-917(1987))。AbM HVR代表Kabat HVR与Chothia结构环之间的折衷，并被Oxford Molecular的AbM抗体建模软件所使用。"接触"HVR是基于可获得的复合物晶体结构的分析。这些HVR中每一个的残基在下面注明。

[0060]

环	Kabat	AbM	Chothia	接触
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	
H30-H35B(Kabat 编号)				
H1	H31-H35	H26-H35	H26-H32	
H30-H35(Chothia 编号)				
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0061] HVR可以包括如下的“延伸的HVR”：VL中的24-36或24-34(L1)、46-56或50-56(L2)和89-97或89-96(L3)和VH中的26-35(H1)、50-65或49-65(H2)和93-102、94-102或95-102(H3)。对于这些定义的每个，可变结构域残基是按照上文Kabat等编号。

[0062] 表述“如Kabat中的可变结构域残基编号”或“如Kabat中的氨基酸位置编号”及其变体是指上文Kabat等中抗体编译的用于重链可变结构域或轻链可变结构域的编号系统。使用此编号系统，实际的线性氨基酸序列可包含对应可变结构域的FR或HVR缩短或插入的更少的或额外的氨基酸。例如，重链可变结构域可以在H2的残基52后包括单个氨基酸插入(根据Kabat，残基52a)和在重链FR残基82之后包括插入的多个残基(例如，根据Kabat，残基82a、82b和82c等)。对于给定的抗体，残基的Kabat编号可以通过在抗体序列的同源性区域与“标准”Kabat编号序列的比对来确定。

[0063] “构架”或“FR”残基是如本文中所定义的HVR残基之外的那些可变结构域残基。

[0064] “人共有构架”或“受体人构架”是代表在人免疫球蛋白VL或VH构架序列的选择中最经常发生的氨基酸残基的构架。通常，人免疫球蛋白VL或VH序列的选择来自可变结构域序列的亚组。通常，序列的亚组是如Kabat等人，Sequences of Proteins of Immunological Interest，第5版，Public Health Service，National Institutes of Health，Bethesda，MD(1991)中的亚组。实例包括，对于VL，亚组可以是如上文Kabat等的亚组KI、KII、KIII或KIV。此外，对于VH，亚组可以是如上文Kabat等的亚组I、亚组II或亚组III。或者，人共有构架可以衍生自上述，其中特定残基，例如当通过将供体构架序列与各种人构架序列的集合进行比对，基于其对供体构架的同源性选择人构架残基时。“衍生自”人免疫球蛋白构架或人共有构架的受体人构架可以包括其相同的氨基酸序列，或其可以包含预先存在的氨基酸序列的变化。在一些实施方案中，预先存在的氨基酸变化的数目是10个或更少，9个或更少，8个或更少，7个或更少，6个或更少，5个或更少，4个或更少，3个或更少，或2个或更小。

[0065] “VH亚组III共有构架”包含从Kabat等人，上文的可变重亚组III中的氨基酸序列获得的共有序列。在一个实施方案中，VH亚组III共有构架氨基酸序列包含以下每个序列的至少一部分或全部：EVQLVESGGGLVQPGGSLRLSCAAS(HC-FR1)(SEQ ID NO:35)、

WVRQAPGKGLEWV(HC-FR2),(SEQ ID NO:36)、RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR(HC-FR3,SEQ ID NO:37)、WGQGTLVTVSA(HC-FR4),(SEQ ID NO:38)。

[0066] "VLκI共有构架"包含从Kabat等人,上文的可变轻κ亚组I中的氨基酸序列获得的共有序列。在一个实施方案中,VH亚组I共有构架氨基酸序列包含以下序列中的每一个的至少一部分或全部:DIQMTQSPSSLSASVGDRVTITC(LC-FR1)(SEQ ID NO:39)、WYQQKPGKAPKLLIY(LC-FR2)(SEQ ID NO:NO:40)、GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC(LC-FR3)(SEQ ID NO:41)、FGQGTKVEIKR(LC-FR4)(SEQ ID NO:42)。

[0067] 在例如Fc区的指定位置的"氨基酸修饰"是指指定残基的取代或缺失,或指定残基相邻的至少一个氨基酸残基的插入。与指定残基"相邻"的插入是指在其一到两个残基内的插入。插入可以在指定残基的N-末端或C-末端。本文优选的氨基酸修饰是取代。

[0068] "亲和力成熟的"抗体是在其一个或多个HVR中具有一个或多个改变的抗体,所述改变造成该抗体与不具有这些一个或多个改变的亲本抗体相比对抗原的亲和力改进。在一个实施方案中,亲和力成熟的抗体对靶抗原具有纳摩尔或甚至皮摩尔的亲和力。亲和力成熟的抗体可通过本领域已知的程序产生。例如,Marks等人,Bio/Technology 10:779-783(1992)描述了通过VH和VL结构域改组的亲和力成熟。HVR和/或构架残基的随机诱变描述于,例如Barbas等人,Proc Nat.Acad.Sci.USA 91:3809-3813(1994);Schier等人,Gene 169:147-155(1995);Yelton等人,J.Immunol.155:1994-2004(1995);Jackson等人,J.Immunol.154(7):3310-9(1995);和Hawkins等人,J.Mol.Biol.226:889-896(1992)。

[0069] 如本文所用,术语"特异性结合"或"对于……是特异性的"是指可测量的和可重现的相互作用,诸如靶和抗体之间的结合,其在分子包括生物分子的异源群体存在的情况下决定靶的存在。例如,特异性结合至靶(其可以是表位)的抗体是比其结合其它靶具有更大的亲和力(affinity),亲合力(avidity),更容易和/或具有更长的持续时间结合此靶的抗体。在一个实施方案中,抗体结合不相关靶的程度小于抗体与靶的结合的约10%,例如,如通过放射免疫测定(RIA)所测量的。在某些实施方案中,特异性结合靶的抗体具有≤1μM,≤100nM,≤10nM,≤1nM或≤0.1nM的解离常数(Kd)。在某些实施方案中,抗体特异性结合蛋白上的表位,所述表位在来自不同物种的蛋白之间是保守的。在另一个实施方案中,特异性结合可以包括,但不需要排他性结合。

[0070] 术语"Fc区"在本文中用于定义免疫球蛋白重链的C-末端区,包括天然序列Fc区和变体Fc区。虽然免疫球蛋白重链的Fc区的边界可能发生变化,但是人IgG重链Fc区通常定义为从在位置Cys226的氨基酸残基,或从Pro230,向其羧基末端的延伸。Fc区的C-末端赖氨酸(根据EU编号系统的残基447)可以被移除,例如,在抗体生产或纯化过程中,或通过重组改造编码所述抗体重链的核酸。因此,完整抗体的组合物可包含去除了所有K447残基的抗体群,没有去除K447残基的抗体群,和具有有和无K447残基的抗体的混合物的抗体群。用于本发明的抗体的合适的天然序列Fc区包括人IgG1、IgG2(IgG2A、IgG2B)、IgG3和IgG4。

[0071] "Fc受体"或"FcR"描述结合抗体Fc区的受体。优选的FcR是天然序列人FcR。此外,优选的FcR是这样的FcR,其结合IgG抗体(γ受体),并包括FcγR1、FcγRII和FcγRIII亚类的受体,包括等位基因变体和这些受体的可选剪接形式,FcγRII受体包括FcγRIIA("活化受体")和FcγRIIB("抑制受体"),它们具有相似的氨基酸序列,其区别主要在于其胞质结构域。活化受体FcγRIIA在其胞质结构域中包含基于免疫受体酪氨酸的活化基序(ITAM)。

抑制受体Fc γ RIIB在其细胞质结构域中包含基于免疫受体酪氨酸的抑制基序(I'HM)。(参见M.Daëron,Annu.Rev.Immunol.15:203-234(1997)。FcR的综述见于Ravetch和Kinet,Annu.Rev.Immunol.9:457-92(1991);Capel等人,Immunomethods 4:25-34(1994);和de Haas等,J.Lab.Clin.Med.126:330-41(1995)。其它FcR,包括那些将来要鉴定的FcR在本文中被术语"FcR"涵盖。

[0072] 术语"Fc受体"或"FcR"还包括新生儿受体,FcRn,其负责将母体IgG转移给胎儿。Guyer等人,J.Immunol.117:587(1976)和Kim等人,J.Immunol.24:249(1994)。测量与FcRn结合的方法是已知的(参见,例如Ghetie和Ward,Immunol.Today 18:(12):592-8(1997);Ghetie等人,Nature Biotechnology 15(7):637-40(1997);Hinton等人,J.Biol.Chem.279(8):6213-6(2004);WO 2004/92219(Hinton等人)。可以测定体内与FcRn的结合和人FcRn高亲和力结合多肽的血清半衰期,例如,在转基因小鼠或表达人FcRn的转染的人细胞系中,或在对其施用具有变体Fc区多肽的灵长类中。WO2004/42072(Presta)描述了提高或降低了对FcR结合的抗体变体。还参见,例如Shields等人,J.Biol.Chem.9(2):6591-6604(2001)。

[0073] 如本文所用短语"基本上减少,"或"基本上不同,",表示两个数值(通常一个与分子相关,并且另一个与参照/比较分子相关)之间的足够高的差异程度,使得本领域技术人员会认为这两个值之间的差异在通过所述值(例如,Kd值)测量的生物学特征的背景下具有统计学显著性。作为该值对于参照/比较分子的函数,所述两个值之间的差异是,例如,大于约10%,大于约20%,大于约30%,大于约40%,和/或大于约50%。

[0074] 如本文所用短语"基本上相似"或"基本相同",表示两个数值(例如一个与本发明的抗体相关,并且另一个与参照/比较抗体相关)之间的足够高的相似程度,使得本领域技术人员会认为这两个值之间的差异在通过所述值(例如,Kd值)测量的生物学特征的背景下具有很小的或不具有生物学和/或统计学显著性。作为参照/比较子值的函数,所述两个值之间的差异,例如,小于约50%,小于约40%,小于约30%,小于约20%,和/或小于约10%。

[0075] 如本文所用"载体"包括药学上可接受的载体、赋形剂或稳定剂,其在所采用的剂量和浓度下对暴露于其的细胞或哺乳动物是无毒的。通常,生理学上可接受的载体是PH缓冲水溶液。生理学上可接受的载体的实例包括缓冲剂如磷酸盐、柠檬酸盐和其它有机酸;抗氧化剂,包括抗坏血酸;低分子量(小于约10个残基)多肽;蛋白,如血清白蛋白、明胶或免疫球蛋白;亲水性聚合物如聚乙烯吡咯烷酮;氨基酸,如甘氨酸、谷氨酰胺、天冬酰胺、精氨酸或赖氨酸;单糖、二糖和其它碳水化合物,包括葡萄糖,甘露糖或糊精;螯合剂如EDTA;糖醇如甘露醇或山梨醇;成盐平衡离子,诸如钠;和/或非离子表面活性剂,诸如TWEEN™、聚乙二醇(PEG)和PLURONICS™。

[0076] "包装插页"指通常包括在药物的商业包装中的说明书,它们包含有关涉及此类药物应用的适应征、用法、剂量、施用、禁忌症、与该包装产品联合的其它药物和/或警告等的信息。

[0077] 如本文中使用的,术语"治疗/处理"指设计用于改变临床病理学过程期间所治疗个体或细胞的自然进程的临床干预。治疗的期望效果包括降低疾病进展速率,改善或减轻疾病状态,和消退或改善的预后。例如,若一种或多种与狼疮性肾炎有关的症状被减轻或消除,则个体得到成功"治疗",所述症状包括但不限于升高的血清肌酐、蛋白尿、红细胞管型、肾功能减退、肾病综合征、颗粒管型、显微血尿、肉眼血尿、高血压、肾小管异常、高钾血症、

快速进展性肾小球肾炎(RPGN)和急性肾功能衰竭(ARF)。

[0078] 如本文中使用的,"延缓疾病的进展"意指推迟、阻碍、减缓、延迟、稳定、和/或延迟疾病的形成。根据疾病史和/或治疗的个体,此延迟可以是不同时间长度的。如对于本领域技术人员明显的是,充分或显著的延缓实质上可以涵盖预防,因为个体(例如处于发展该疾病风险的个体)不发展疾病。例如,在发生LN症状和/或病理学之前,个体中SLE的进展可能会延缓,以致LN的发展被延迟或阻止。

[0079] 如本文所用,"完全肾反应(CRR)"是指对包括血清肌酐、非活性尿沉渣和尿蛋白与肌酸酐比率小于0.5的归一化的治疗的反应。

[0080] 如本文所用,"部分肾反应(PRR)"是指对治疗的反应小于CRR,但仍包括减轻一种或多种症状,包括但不限于血肌酐降低、尿沉渣减少、和蛋白尿减少。

[0081] "有效量"至少是实现特定病症的可测量改善或预防需要的最小浓度。本文中的有效量可以随诸如患者的疾病状态、年龄、性别、和重量,和抗体引发个体中期望的应答的能力等因素而变化。有效量也是治疗有益效果超过治疗的任何毒性或不利效果的量。为了预防性使用,有益或期望的结果包括如下的结果,例如消除或降低风险,减轻严重性,或者延缓疾病的发作,包括疾病的生物化学,组织学和/或行为症状,其并发症和疾病形成期间呈现的中间病理学表型。为了治疗性使用,有益或期望的结果包括临床结果,诸如减少源自疾病的一种或多种症状,提高那些患有疾病的对象的生命质量,降低治疗疾病需要的其它药物的剂量,增强另一种药物的效果(诸如经由靶向),延缓疾病的进展,和/或延长存活。在狼疮性肾炎的情况中,药物的有效量在和/或在一定程度上减轻一种或多种与病症有关的症状中可以具有效果。可以在一次或多次施用中施用有效量。出于本发明的目的,药物、化合物、或药物组合物的有效量是足以直接或间接实现预防或治疗性治疗的量。如在临床背景中理解的,药物、化合物、或药物组合物的有效量可以与或不与另一种药物、化合物、或药物组合物一起实现。如此,可以在施用一种或多种治疗剂的背景中考虑"有效量",并且若与一种或多种其它药剂一起,可以实现期望的结果或者实现了期望的结果,则可以认为单一药剂以有效量给予。

[0082] 本文所用的"CD20"是指人B淋巴细胞抗原CD20(也称为CD20,B淋巴细胞表面抗原B1,Leu-16,Bp35,BM5和LF5;该序列的特征在于SwissProt数据库登录号P11836)是具有约35kD的分子量的疏水性跨膜蛋白,其位于前B和成熟B淋巴细胞上。(Valentine,MA等人,J.Biol.Chem.264(19)(1989 11282-11287;Tedder,TF等人,Proc.Natl.Acad.Sci.USA 85(1988)208-12;Stamenkovic,I.,等人,J.Exp.Med.167(1988)1975-80;EinFcld,DA等人,EMBO J.7(1988)711-7;Tedder,TF,等人,J.Immunol.142(1989)2560-8)。相应的人类基因是跨膜4结构域,亚家族A,成员1,也称为MS4A1。该基因编码跨膜4A基因家族的成员。该新生蛋白家族的特征是具有共同的结构特征和相似的内含子/外显子剪接边界,并在造血细胞和非淋巴组织之间显示独特的表达模式。该基因编码在B细胞发育和分化成浆细胞中发挥作用的B淋巴细胞表面分子。这个家族成员在一群家庭成员中被定位到11q12。该基因的选择性剪接导致两个转录物变体,其编码相同的蛋白质。

[0083] 术语"CD20"和"CD20抗原"在本文中可互换使用,并且包括由细胞天然表达或在用CD20基因转染的细胞上表达的人CD20的任何变体、同种型和物种同源物。将本发明的抗体与CD20抗原结合通过灭活CD20来介导杀死表达CD20的细胞(例如肿瘤细胞)。表达CD20的细

胞的杀死可以通过一种或多种以下机制发生:细胞死亡/凋亡诱导、ADCC和CDC。

[0084] 本领域公认的CD20的同义词包括B淋巴细胞抗原CD20、B淋巴细胞表面抗原B1、Leu-16、Bp35、BM5和LF5。

[0085] 根据本发明的术语"抗CD20抗体"是特异性结合CD20抗原的抗体。取决于抗CD20抗体对CD20抗原的结合性质和生物学活性,可以根据Cragg,MS等人,Blood 103(2004)2738-2743;和Cragg,M.S.等人,Blood101(2003)1045-1052,区分两种类型的抗CD20抗体(I型和II型抗-CD20抗体),参见下表1。

[0086] 表1.I型和II型抗CD20抗体的性质

[0087]

I型抗CD20抗体	II型抗CD20抗体
I型CD20表位	II型CD20表位
将CD20定位在脂膜筏	不将CD20定位在脂膜筏
增加的CDC(如果是IgG1同种型)	减少的CDC(如果是IgG1同种型)
ADCC活性(如果是IgG1同种型)	ADCC活性(如果是IgG1同种型)
完全结合能力	降低的结合能力
同型聚集	更强的同型聚集
在交联时诱导凋亡	不用交联的强细胞死亡诱导

[0088] II型抗CD20抗体的实例包括例如,人源化B-Ly1抗体IgG1(WO 2005/044859中公开的嵌合人源化IgG1抗体)、11B8IgG1(如WO 2004/035607中所公开)和AT80IgG1。通常IgG1同种型的II型抗CD20抗体显示出特征性的CDC性质。与IgG1同种型的I型抗体相比,II型抗CD20抗体具有降低的CDC(如果是IgG1同种型)。

[0089] I型抗-CD20抗体的实例包括例如,利妥昔单抗、HI47IgG3(ECACC,杂交瘤)、2C6IgG1(如WO 2005/103081中所公开)、2F2IgG1(WO 2004/035607和WO 2005/103081中所公开)和2H7IgG1(如WO 2004/056312中所公开)。

[0090] 根据本发明的无岩藻糖化的抗CD20抗体优选为II型抗-CD20抗体,更优选WO 2005/044859和WO 2007/031875中描述的无岩藻糖化的人源化B-Ly1抗体。

[0091] "利妥昔单抗"抗体(参考抗体;I型抗-CD20抗体的实例)是基因改造的针对人CD20抗原的嵌合含人γ1鼠恒定区结构域的单克隆抗体。然而,该抗体不是糖改造的,不是无岩藻糖化的,因此具有至少85%的岩藻糖的量。此嵌合抗体含有γ1人恒定区结构域,并且在于1998年4月17日出版并转让给IDEC制药公司(IDEC Pharmaceuticals Corporation)的US

5,736,137(Andersen,K.C.等人)中以名称"C2B8"鉴定。利妥昔单抗被批准用于治疗患有复发的或难治性低度或滤泡性CD20阳性的B细胞非霍奇金淋巴瘤的患者。体外作用机制研究已经显示利妥昔单抗显示出人补体依赖性细胞毒性(CDC)(Reff,M.E.等人,Blood 83(2)(1994)435-445)。另外,其在测定抗体依赖性细胞毒性(ADCC)的测定中显示出活性。

[0092] 本文所用的术语"GA101抗体"是指结合人CD20的以下抗体中的任一种:(1)抗体,包括:包含SEQ ID NO:1的氨基酸序列的HVR-H1,包含SEQ ID NO:2的氨基酸序列的HVR-H2,包含SEQ ID NO:3的氨基酸序列的HVR-H3,包含SEQ ID NO:4的氨基酸序列的HVR-L1,包含SEQ ID NO:5的氨基酸序列HVR-L2,和包含SEQ ID NO:6的氨基酸序列的HVR-L3;(2)包含SEQ ID NO:7的氨基酸序列的VH结构域和包含SEQ ID NO:8的氨基酸序列的VL结构域的抗体,(3)包含SEQ ID NO:9的氨基酸序列和SEQ ID NO:10的氨基酸序列的抗体;(4)称为奥比妥珠单抗的抗体,或(5)包括与SEQ ID NO:9的氨基酸序列具有至少95%、96%、97%、98%或99%序列同一性的氨基酸序列并且包括与SEQ ID NO:10的氨基酸序列具有至少95%、96%、97%、98%或99%序列同一性的氨基酸序列的抗体。在一个实施方案中,GA101抗体是IgG1同种型抗体。在一些实施方案中,抗CD20抗体是人源化B-Ly1抗体。

[0093] 术语"人源化B-Ly1抗体"是指如WO 2005/044859和WO 2007/031875中公开的人源化B-Ly1抗体,其从鼠单克隆抗CD20抗体B-Ly1(鼠重链的可变区(VH):SEQ ID NO:11;鼠轻链的可变区(VL):SEQ ID NO:12-参见Poppema,S.和Visser,L.,Biotest Bulletin 3(1987)131-139)通过与来自IgG1的人恒定结构域的嵌合和随后的人源化(参见WO 2005/044859和WO 2007/031875)而获得。这些"人源化B-Ly1抗体"详细公开在WO 2005/044859和WO 2007/031875中。

[0094] 鼠单克隆抗CD20抗体B-Ly1重链可变区(VH)(SEQ ID NO:11)

```
Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys
Lys
 1               5                  10                  15
Ala Ser Gly Tyr Ala Phe Ser Tyr Ser Trp Met Asn Trp Val Lys
Leu
            20                  25                  30
Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Arg Ile Phe Pro Gly
Asp
        35                  40                  45
Gly Asp Thr Asp Tyr Asn Gly Lys Phe Lys Gly Lys Ala Thr Leu
Thr
    50                  55                  60
Ala Asp Lys Ser Ser Asn Thr Ala Tyr Met Gln Leu Thr Ser Leu
Thr
65                  70                  75                  80
Ser Val Asp Ser Ala Val Tyr Leu Cys Ala Arg Asn Val Phe Asp
Gly
                85                  90                  95
Tyr Trp Leu Val Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
Ala
            100                 105                 110
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[0095]

[0096] 鼠单克隆抗CD20抗体B-Ly1轻链可变区(VH)(SEQ ID NO:12)

Asn Pro Val Thr Leu Gly Thr Ser Ala Ser Ile Ser Cys Arg Ser Ser
1 5 10 15
Lys Ser Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu
20 25 30
Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn

[0097]

35 40 45
Leu Val Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr
50 55 60
Asp Phe Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val
65 70 75 80
Tyr Tyr Cys Ala Gln Asn Leu Glu Leu Pro Tyr Thr Phe Gly Gly Gly

[0098]

85 90 95
Thr Lys Leu Glu Ile Lys Arg
100

[0099] 在一个实施方案中，“人源化B-Ly1抗体”具有选自SEQ ID NO:7、8和13-33的重链可变区(VH)(特别是对应于WO 2005/044859和WO 2007/031875的B-HH2至B-HH9和B-HL8至B-HL17)。在一个具体实施方案中，这种可变结构域选自SEQ ID NO:14、15、7、19、27、29(对应于WO 2005/044859和WO 2007/031875的B-HH2、BHH-3、B-HH6、B-HH8、B-HL8、B-HL11和B-HL13)。在一个具体实施方案中，“人源化B-Ly1抗体”具有SEQ ID NO:8的轻链可变区(VL)(对应于WO 2005/044859和WO 2007/031875的B-KV1)。在一个具体实施方案中，“人源化B-Ly1抗体”具有SEQ ID NO:7的重链可变区(VH)(对应于WO 2005/044859和WO 2007/031875的B-HH6)和SEQ ID NO:8的轻链可变区(VL)(对应于WO 2005/044859和WO 2007/031875的B-KV1)。此外，在一个实施方案中，人源化B-Ly1抗体是IgG1抗体。根据本发明，此类无岩藻糖化人源化B-Ly1抗体是根据WO 2005/044859、WO 2004/065540、WO 2007/031875、Umana，P.等人，Nature Biotechnol.17(1999)176-180和WO 99/154342中描述的方法在Fc区被糖基化改造(GE)。在一个实施方案中，无岩藻糖化糖改造的人源化B-Ly1是B-HH6-B-KV1GE。在一个实施方案中，抗CD20抗体是奥比妥珠单抗(推荐的INN，WHO Drug Information，Vol.26，No.4，2012，p.453)。如本文所用，奥比妥珠单抗是GA101或RO5072759的同义词。这替代了所有以前的版本(例如Vol.25，No.1，2011，p.75-76)，并且以前称为阿替他布(afutuzumab)(推荐的INN，WHO Drug Information，Vol.23，No.2，2009，p.176；Vol.22，No.2，2008，p.124)。在一些实施方案中，人源化B-Ly1抗体是这样的抗体或其抗原结合片段，其包括：包含SEQ ID NO:9的氨基酸序列的重链和包含SEQ ID NO:10的氨基酸序列的轻链。在一些实施方案中，人源化B-Ly1抗体包含：包含SEQ ID NO:9的三个重链CDR的重链可变区和包含SEQ ID NO:10的三个轻链CDR的轻链可变区。

[0100] 重链(SEQ ID NO:9)

[0101]
```
QVQLVQSGAE VKKPGSSVKV SCKASGYAFS YSWINWVRQA PGQGLEWMGR 50
IFPGDGDTDY NGKFKGRVTI TADKSTSTAY MELSSLRSED TAVYYCARNV 100
FDGYWLVYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD 150
YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY 200
ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP SVFLFPPKPK 250
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS 300
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV 350
YILPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL 400
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPG 449
```

[0102] 轻链(SEQ ID NO:10)

[0103]
```
DIVMTQTPLS LPVTPGEPAS ISCRSSKSLL HSNGITYLYW YLQKPGQSPQ 50
LLIYQMSNLV SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP 100
YTFGGGTKVE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK 150
VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE 200
VTHQGLSSPV TKSFNRGEC 219
```

[0104] 在一些实施方案中，人源化B-Ly1抗体是无岩藻糖化糖改造的人源化B-Ly1。这种糖改造的人源化B-Ly1抗体在Fc区中具有改变的糖基化模式，优选具有降低的岩藻糖残基水平。优选地，岩藻糖的量为Asn297处寡糖总量的60％或更少(在一个实施方案中，岩藻糖的量在40％至60％之间，在另一个实施方案中，岩藻糖的量为50％或更少，在又一个实施方案中，岩藻糖的量在30％或更少)。此外，Fc区的寡糖优选被二分(bisected)。这些糖改造的人源化B-Ly1抗体具有增加的ADCC。

[0105] “抗CD20抗体的与Raji细胞(ATCC编号:CCL_86)上CD20的结合力与利妥昔单抗的比值”通过直接免疫荧光测定法(测定平均荧光强度(MFI))，如实施例2中所述，使用与Cy5缀合的抗CD20抗体和与Cy5缀合的利妥昔单抗与Raji细胞(ATCC编号:CCL-86)在FACSArray(Becton Dickinson)中测定，并且计算如下：

[0106] 与Raji细胞(ATCC编号:CCL-86)上CD20的结合力的比值＝

[0107] $$\frac{MFI(Cy5\text{-}抗CD20抗体)}{MFI(Cy5\text{-}利妥昔单抗)} \times \frac{Cy5\text{-}标记比(Cy5\text{-}利妥昔单抗)}{Cy5\text{-}标记比(Cy5\text{-}抗CD20抗体)}$$

[0108] MFI是平均荧光强度。本文所用的“Cy5-标记比”是指每分子抗体的Cy5-标记分子数。

[0109] 典型地，所述II型抗-CD20抗体具有的所述第二抗CD20抗体与Raji细胞(ATCC编号:CCL-86)上CD20的结合力与利妥昔单抗相比较的比值为0.3至0.6，在一个实施方案中为0.35至0.55，在另一个实施方案中为0.4至0.5。

[0110] 在一个实施方案中，所述II型抗CD20抗体(例如GA101抗体)具有增加的抗体依赖性细胞毒性(ADCC)。

[0111] “具有增加的抗体依赖性细胞毒性(ADCC)的抗体”是指如在此所定义的该术语一样，具有根据本领域普通技术人员已知的任何适当的方法测定的增加的ADCC的抗体。一种公认的体外ADCC测定如下：

[0112] 1)该测定使用已知表达由抗体的抗原结合区域识别的靶抗原的靶细胞；

[0113] 2)该测定使用从随机选择的健康供体的血液中分离的人外周血单核细胞(PBMC)作为效应子细胞；

[0114] 3)该测定根据以下方案进行：

[0115] i)PBMC使用标准密度离心程序分离，并以5×10^6个细胞/ml悬浮在RPMI细胞培养基中；

[0116] ii)靶细胞通过标准的组织培养方法生长，从指数生长期收获，活力大于90%，在RPMI细胞培养基中洗涤，用100微居里的^{51}Cr标记，用细胞培养基洗涤两次，并且以10^5细胞/ml的密度重新悬浮在细胞培养基中；

[0117] iii)将100微升上述最终靶细胞悬浮液转移到96孔微量滴定板的每个孔中；

[0118] iv)将抗体在细胞培养基中从4000ng/ml连续稀释至0.04ng/ml，将50μl所得抗体溶液加入到96孔微量滴定板中的靶细胞中，将覆盖上面整个浓度范围内的各个抗体浓度一式三份测试；

[0119] v)对于最大释放(MR)对照，含有标记的靶细胞的板中的另外3个孔接受50微升的2%(VN)非离子型去污剂(Nonidet,Sigma,St.Louis)的水溶液代替抗体溶液(上述点iv)；

[0120] vi)对于自发释放(SR)对照，含有标记的靶细胞的板中的另外3个孔接受50微升的RPMI细胞培养基代替抗体溶液(上面的点iv)；

[0121] vii)然后将96孔微量滴定板以50×g离心1分钟，并在4℃下温育1小时；

[0122] viii)将50微升的PBMC悬浮液(上述点i)加入到每个孔中以产生25:1的效应细胞:靶细胞比例，并将板放置在37℃，5%CO_2气氛的的培养箱中4小时；

[0123] ix)收获每个孔的无细胞上清液，并使用γ计数器定量实验释放的放射性(ER)；

[0124] x)根据式(ER-MR)/(MR-SR)×100计算每抗体浓度的特异性裂解百分比，其中ER是针对该抗体浓度定量的平均放射性(参见上述点ix)，MR是MR对照(见上文点v)定量的平均放射性(见上述点ix)，SR是SR对照(见上述点iv)定量的平均放射性(见上述点ix)；

[0125] 4)“增加的ADCC”被定义为在上述测试的抗体浓度范围内观察到的特异性裂解的最大百分比的增加和/或在上面测试的抗体浓度范围内观察到实现特异性裂解的最大百分比的一半所需的抗体浓度的降低。在一个实施方案中，ADCC的增加是采用上面测定法测定，相对于使用本领域专业技术人员公知的相同的标准生产方法、纯化方法、配制和保存方法，由相同类型宿主细胞产生的，由相同抗体介导的ADCC，但比较抗体(缺少增加的ADCC)不由被改造以过度表达GnTIII和/或被改造以具有从岩藻糖基转移酶8(FUT8)基因(例如，包括为FUT8敲除的改造)的降低的表达的宿主细胞产生的。

[0126] 所述“增加的ADCC”可以通过例如所述抗体的突变和/或糖改造来获得。在一个实施方案中，将抗体糖改造以具有连接到由GlcNAc平分的抗体的Fc区的双分支寡糖，例如在WO 2003/011878(Jean-Mairet等人)；美国专利号6,602,684(Umana等人)；US 2005/0123546(Umana等人)，Umana,P.等人，Nature Biotechnol.17(1999)176-180)中。在另一个实施方案中，通过在蛋白质岩藻糖基化缺陷的宿主细胞(例如，Lec13 CHO细胞)或具有α-1,6-岩藻糖基转移酶基因(FUT8)缺失或FUT基因表达被敲减的细胞中表达抗体，抗体被糖改造以缺乏在结合到Fc区上的碳水化合物上的岩藻糖(参见例如Yamane-Ohnuki等人Biotech.Bioeng.87:614(2004)；Kanda,Y.等人，Biotechnol.Bioeng.,94(4):680-688(2006)；和WO2003/085107)。在另一个实施方案中，抗体序列已经在其Fc区被改造以增强ADCC(例如，在一个实施方案中，这样的改造抗体变体包括Fc区域，其具有在Fc区的位置298、333和/或334的一个或多个氨基酸取代(残基的EU编号)。

[0127] 术语“补体依赖性细胞毒性(CDC)”是指在补体存在下，根据本发明的抗体对人肿瘤靶细胞的裂解。可以通过在补体存在下用根据本发明的抗CD20抗体处理CD20表达细胞的制剂来测定CDC。如果4小时后抗体以100nM的浓度诱导20%或更多肿瘤细胞的裂解(细胞死亡)，则发现CDC。在一个实施方案中，用^{51}Cr或Eu标记的肿瘤细胞进行测定并测量释放的

^{51}Cr或Eu。对照包括肿瘤靶细胞与补体但不含抗体的温育。

[0128] 术语“表达CD20”抗原旨在表示CD20抗原在细胞，例如T细胞或B细胞中的显著水平的表达。在一个实施方案中，用根据本发明的方法治疗的患者在B细胞上表达显著水平的CD20。可以通过本领域已知的标准测定来测定B细胞上的CD20表达，例如，使用免疫组织化学(IHC)检测、FACS或通过相应mRNA的基于PCR的检测来测量CD20抗原表达。

[0129] 如本说明书和所附权利要求中所使用的，单数形式“一”、“一个”和“该”包括复数指代，除非另有明确规定。因此，例如，提到“一个分子”任选地包括两个或更多个这样的分子的组合等。

[0130] 本文所用的术语“约”是指本领域技术人员熟知的相应值的通常误差范围。这里引用“约”一数值或参数包括(和描述)针对该数值或参数本身的实施方案。

[0131] 应当理解，本文描述的本发明的方面和实施方案包括“包括”，“组成”和“基本上由.....组成”方面和实施方案。

[0132] III.方法

[0133] 在一个方面，本文提供了通过施用有效量的II型抗CD20抗体来治疗或延缓具有狼疮的个体的狼疮性肾炎进展的方法。在一些实施方案中，个体具有或具有发展为狼疮性肾炎的风险。在一些实施方案中，狼疮性肾炎是III类或IV类狼疮性肾炎。在一些实施方案中，所述方法包括向个体施用对于II型抗-CD20抗体的第一次抗体暴露和对于II型抗-CD20抗体的第二次抗体暴露，第二次抗体暴露直到第一次抗体暴露后约18周至约26周才提供；其中所述第一次抗体暴露包括一个或两个剂量的II型抗CD20抗体，所述第一次抗体暴露包括约1800mg至约2200mg的所述II型抗-CD20抗体的总暴露；并且其中所述第二次抗体暴露包括一个或两个剂量的II型抗CD20抗体，所述第二次抗体暴露包括约1800mg至约2200mg的所述II型抗-CD20抗体的总暴露。如下所述，在一些实施方案中，抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链和包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链。在一些实施方案中，抗体包含：包含SEQ ID NO:7的氨基酸序列的VH结构域和包含SEQ ID NO:8的氨基酸序列的VL结构域。在一些实施方案中，抗体包含SEQ ID NO:9的氨基酸序列和SEQ ID NO:10的氨基酸序列。在一些实施方案中，抗体包含这样的抗体，其包括与SEQ ID NO:9的氨基酸序列具有至少95%、96%、97%、98%或99%序列同一性的氨基酸序列，并且其包括与SEQ ID NO:10的氨基酸序列具有至少95%、96%、97%、98%或99%序列同一性的氨基酸序列。

[0134] 抗CD20抗体

[0135] 本公开的某些方面涉及抗CD20抗体，例如用于治疗或预防狼疮性肾炎进展的方法中。在一些实施方案中，抗CD20抗体是II型抗体。在一些实施方案中，抗CD20抗体是人或人源化的。在一些实施方案中，抗CD20抗体是无岩藻糖基化的。在一些实施方案中，抗CD20抗体是GA101抗体。

[0136] II型抗-CD20抗体的实例包括例如，人源化B-Ly1抗体IgG1(如WO 2005/044859中公开的嵌合人源化IgG1抗体)，11B8 IgG1(如WO 2004/035607所公开)和AT80 IgG1。通常IgG1同种型的II型抗CD20抗体显示出特征性的CDC性质。与IgG1同种型的I型抗体相比，II型抗CD20抗体具有降低的CDC(如果IgG1同种型)。

[0137] I型抗-CD20抗体的实例包括例如，利妥昔单抗，HI47 IgG3(ECACC，杂交瘤)，2C6IgG1(如WO 2005/103081所公开)，2F2 IgG1(如WO 2004/035607和WO 2005/103081所公

开)和2H7 IgG1(如WO 2004/056312所公开)。

[0138] 在一些实施方案中,抗CD20抗体是本文所述的GA101抗体。在一些实施方案中,抗CD20是结合人CD20的以下抗体中的任一种:(1)抗体,包括:包括GYAFSY(SEQ ID NO:1)的氨基酸序列的HVR-H1,包括FPGDGDTD(SEQ ID NO:2)的氨基酸序列的HVR-H2,包括NVFDGYWLVY(SEQ ID NO:3)的氨基酸序列的HVR-H3,包括RSSKSLLHSNGITYLY(SEQ ID NO:4)的氨基酸序列的HVR-L1,包括QMSNLVS(SEQ ID NO:5)的氨基酸序列的HVR-L2和包括AQNLELPYT(SEQ ID NO:6)的氨基酸序列的HVR-L3;(2)抗体,包括:包含SEQ ID NO:7的氨基酸序列的VH结构域和包含SEQ ID NO:8的氨基酸序列的VL结构域,(3)包含SEQ ID NO:9的氨基酸序列和SEQ ID NO:10的氨基酸序列的抗体;(4)称为奥比妥珠单抗的抗体,或(5)抗体,包括与SEQ ID NO:9的氨基酸序列具有至少95%、96%、97%、98%或99%序列同一性的氨基酸序列,并且其包括与SEQ ID NO:10的氨基酸序列具有至少95%、96%、97%、98%或99%序列同一性的氨基酸序列。在一个实施方案中,GA101抗体是IgG1同种型抗体。在一些实施方案中,抗CD20抗体包含本文所述任何抗体的HVR-H1、HVR-H2、HVR-H3、HVR-L1、HVR-L2和HVR-L3,例如来自SEQ ID NO:7的3HVR,来自SEQ ID NO:8的3HVR,来自SEQ ID NO:9的3HVR和来自SEQ ID NO:10的3HVR,或者表2中提供的氨基酸序列的任何HVR。

[0139] 在一些实施方案中,抗CD20抗体包含:包含SEQ ID NO:7的氨基酸序列的重链可变区(VH)和包含SEQ ID NO:8的氨基酸序列的轻链可变区(VL)。

[0140]

QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWINWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTS
TAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS(SEQ ID NO:7)

[0141]

DIVMTQTPLSLPVTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLVSGVPDRFSGSGSGTDFT
LKISRVEAEDVGVYYCAQNLELPYTFGGGTKVEIKRTV(SEQ ID NO:8).

[0142] 在一些实施方案中,抗CD20抗体包含:包含SEQ ID NO:9的氨基酸序列的重链和包含SEQ ID NO:10的氨基酸序列的轻链。

[0143]

QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWINWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTS
TAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG(SEQ ID NO:9)

[0144]

DIVMTQTPLSLPVTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLVSGVPDRFSGSGSGTDFT
LKISRVEAEDVGVYYCAQNLELPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(SEQ ID NO:10)

[0145]

[0146] 在一些实施方案中,抗CD20抗体是人源化B-Ly1抗体。在一些实施方案中,人源化B-Ly1抗体包含:包含SEQ ID NO:9的三个重链CDR的重链可变区和包含SEQ ID NO:10的三

个轻链CDR的轻链可变区。在一些实施方案中，人源化B-Ly1抗体包含：包含SEQ ID NO:9的序列的重链和包含SEQ ID NO:10的序列的轻链。

[0147] 在一些实施方案中，抗CD20抗体包含与下表2中列出的多肽序列至少80%、85%、90%、95%、96%、97%、98%或99%同一性的氨基酸序列。

[0148] 表2.多肽序列。

[0149]

构建体	多肽序列	SEQ ID NO
B-HH1	QVQLVQSGAEVKKPGSSVKVSCKASG YTFSYSWMSWVRQAPGQGLEWMGRI FPGDGDTDYAQKFQGRVTITADKSTST AYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	13
B-HH2	QVQLVQSGAEVKKPGSSVKVSCKASG YAFSYSWMNWVRQAPGQGLEWMGRI FPGDGDTDYNGKFKGRVTITADKSTST AYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	14
B-HH3	QVQLVQSGAEVKKPGSSVKVSCKASG YAFSYSWMNWVRQAPGQGLEWMGRI FPGDGDTDYNGKFKGRVTITADKSTST AYMELSSLRSEDTAVYLCARNVFDGY WLVYWGQGTLVTVSS	15
B-HH4	QVQLVQSGAEVKKPGASVKVSCKVSG YAFSYSWMNWVRQAPGQGLEWMGRI FPGDGDTDYNGKFKGRVTITADKSTST AYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	16
B-HH5	QVQLVQSGAEVKKPGSSVKVSCKASG YAFSYSWMSWVRQAPGQGLEWMGRI FPGDGDTDYNGKFKGRVTITADKSTST AYMELSSLRSEDTAVYYCARNVFDGY WLVYWGQGTLVTVSS	17
B-HH6	QVQLVQSGAEVKKPGSSVKVSCKASG YAFSYSWINWVRQAPGQGLEWMGRIF PGDGDTDYNGKFKGRVTITADKSTSTA YMELSSLRSEDTAVYYCARNVFDGYW LVYWGQGTLVTVSS	7
B-HH7	QVQLVQSGAEVKKPGSSVKVSCKASG YAFSYSWISWVRQAPGQGLEWMGRIF PGDGDTDYNGKFKGRVTITADKSTSTA YMELSSLRSEDTAVYYCARNVFDGYW LVYWGQGTLVTVSS	18

[0150]

[0151]

构建体	多肽序列	SEQ ID NO
B-HH8	QVQLVQSGAEVKKPGASVKVSCKASGYTFTYSWMNWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS	19
B-HH9	QVQLVQSGAEVKKPGASVKVSCKASGYTFSYSWMNWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS	20
B-HL1	QVQLVQSGAEVKKPGASVKVSCKASGYTFTYSWMHWVRQAPGQGLEWMGRIFPGDGDTDYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS	21
B-HL2	EVQLVQSGAEVKKPGATVKISCKVSGYTFTYSWMHWVQQAPGKGLEWMGRIFPGDGDTDYAEKFQGRVTITADTSTDTAYMELSSLRSEDTAVYYCATNVFDGYWLVYWGQGTLVTVSS	22
B-HL3	EVQLVQSGAEVKKPGATVKISCKVSGYTFTYSWMNWVQQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADTSTDTAYMELSSLRSEDTAVYYCATNVFDGYWLVYWGQGTLVTVSS	23
B-HL4	QMQLVQSGAEVKKTGSSVKVSCKASGYTFTYSWMSWVRQAPGQGLEWMGRIFPGDGDTDYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS	24
B-HL8	EVQLVESGGGLVKPGGSLRLSCAASGFTFSYSWMNWVRQAPGKGLEWVGRIFPGDGDTDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS	25

[0152]

构建体	多肽序列	SEQ ID NO
B-HL10	EVQLVESGGGLVKPGGSLRLSCAASGFAFSYSWMNWVRQAPGKGLEWVGRIFPGDGDTDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS	26
B-HL11	QVQLVESGGGLVKPGGSLRLSCAASGFTFSYSWMNWVRQAPGKGLEWVGRIFPGDGDTDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS	27
B-HL12	EVQLVESGAGLVKPGGSLRLSCAASGFTFSYSWMNWVRQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS	28
B-HL13	EVQLVESGGGVVKPGGSLRLSCAASGFTFSYSWMNWVRQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS	29
B-HL14	EVQLVESGGGLKKPGGSLRLSCAASGFTFSYSWMNWVRQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS	30
B-HL15	EVQLVESGGGLVKPGSSLRLSCAASGFTFSYSWMNWVRQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS	31
B-HL16	EVQLVESGGGLVKPGGSLRVSCAASGFTFSYSWMNWVRQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS	32

[0153]

构建体	多肽序列	SEQ ID NO
B-HL17	EVQLVESGGGLVKPGGSLRLSCAASGFTFSYSWMNWVRQAPGKGLEWMGRIFPGDGDTDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS	33
VH 信号序列	MDWTWRILFLVAAATGAHS	34
B-KV1	DIVMTQTPLSLPVTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLVSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPYTFGGGTKVEIKRTV	8
VL 信号序列	MDMRVPAQLLGLLLLWFPGARC	43

[0154] 在一些实施方案中，抗-CD20抗体(例如II型抗-CD20抗体)是无岩藻糖化糖改造抗体。这样的糖改造抗体在Fc区具有改变的糖基化模式，优选具有降低的岩藻糖残基水平。优选地，岩藻糖的量为Asn297处寡糖总量的60％或更少(在一个实施方案中，岩藻糖的量在40％至60％之间，在另一个实施方案中，岩藻糖的量为50％或更少，在又一个实施方案中，岩藻糖的量为30％或更少)。此外，Fc区的寡糖优选被平分。这些糖改造的人源化抗CD20(例如，B-Ly1)抗体具有增加的ADCC。

[0155] 寡糖组分可以显著影响与治疗性糖蛋白功效相关的性质，包括物理稳定性、对蛋白酶攻击的抗性、与免疫系统的相互作用、药代动力学和特异性生物学活性。这些性质不仅取决于寡糖的存在与否，而且还取决于特定结构。可以对寡糖结构与糖蛋白功能之间进行一些概括。例如，某些寡糖结构通过与特定碳水化合物结合蛋白的相互作用而介导糖蛋白从血液流快速清除，而其他寡糖结构可以被抗体结合并触发不期望的免疫反应。(Jenkins，N.等人，Nature Biotechnol.14(1996)975-81)。

[0156] 哺乳动物细胞是生产治疗性糖蛋白的优选宿主，因为它们具有以对人类应用最相容形式来糖基化蛋白的能力。(Cumming，D.A.，等人，Glycobiology 1(1991)115-30；Jenkins，N.等人，Nature Biotechnol.14(1996)975-81)。细菌对糖基化蛋白质很罕见，并且类似其他类型的常见宿主如酵母、丝状真菌、昆虫和植物细胞，产生与从血流中快速清除、不期望的免疫相互作用相关，在一些特定情况下，降低生物活性的糖基化模式。在哺乳动物细胞中，在过去二十年中是最常用的是中国仓鼠卵巢(CHO)细胞。除了给出合适的糖基化模式之外，这些细胞允许不断地产生遗传稳定的、高产的克隆细胞系。它们可以使用无血清培养基在简单生物反应器中培养至高密度，并允许开发安全可重复的生物过程。其他常用的动物细胞包括幼仓鼠肾(BHK)细胞、NSO-和SP2/0-小鼠骨髓瘤细胞。近来，也测试了由转基因动物的生产。(Jenkins，N.等人，Nature Biotechnol.14(1996)975-981)。

[0157] 所有抗体在重链恒定区中在保守位置含有碳水化合物结构，每个同种型具有一组不同的N-连接碳水化合物结构，其可变地影响蛋白质组装、分泌或功能活性。(Wright，A.和

Morrison,S.L.,Trends Biotech.15(1997)26-32)。所连接的N-连接的碳水化合物的结构取决于加工程度而显著变化,并且可以包括高甘露糖、多分支以及双分支的复合寡糖。(Wright,A.和Morrison,S.L.,Trends Biotech.15(1997)26-32)。通常,在特定糖基化位点处连接的核心寡糖结构存在不均一的加工,使得甚至单克隆抗体以多种糖形存在。同样,已经表明抗体糖基化的主要差异发生在细胞系之间,并且甚至在不同培养条件下生长的给定细胞系中也观察到较小的差异。(LiFcly,M.R.,等人,Glycobiology 5(8)(1995)813-22)。

[0158] 获得大幅度增加的效力,同时保持简单的生产工艺并潜在地避免明显的不良副作用的一种方式是如Umana P.,等人,Nature Biotechnol.17(1999)176-180和US 6,602,684中所述,通过改造其寡糖成分来增强单体抗体的天然的、细胞介导的效应子功能。IgG1型抗体是癌症免疫治疗中最常用的抗体,其是在每个CH2结构域中在Asn297处具有保守的N-连接糖基化位点的糖蛋白。连接到Asn297上的两个复合的双分支寡糖被掩埋在CH2结构域之间,与多肽骨架形成广泛的接触,并且它们的存在对于抗体介导效应子功能如抗体依赖性细胞毒性(ADCC)是必需的(LiFcly,MR等人,Glycobiology 5(1995)813-822;JefFcris,R.等人,Immunol.Rev.163(1998)59-76;Wright,A.和Morrison,SL,Trends Biotechnol.15(1997)26-32)。

[0159] 先前已经表明,β(1,4)-N-乙酰葡糖胺基转移酶I11("GnTII17y")(是催化平分寡糖形成的糖基转移酶)在中国仓鼠卵巢(CHO)细胞中的过度表达,显著增加由改造的CHO细胞产生的抗成神经细胞瘤嵌合单克隆抗体(chCE7)的体外ADCC活性(参见Umana,P.等人,Nature Biotechnol.17(1999)176-180;和WO 99/154342,其全部内容通过整体引用引入本文)。抗体chCE7属于具有高肿瘤亲和性和特异性的一大类非缀合的单克隆抗体,但在缺乏GnTIII酶的标准工业细胞系中生产时效力太低而不能用于临床(Umana,P等人,Nature Biotechnol.17(1999)176-180)。该研究首先显示通过改造生产抗体的细胞来表达GnTIII可以获得ADCC活性的大幅增加,其也可以导致恒定区(Fc)相关的平分的寡糖,包括平分的无岩藻糖化寡糖的比例增加,高于天然存在的抗体中发现的水平。

[0160] 在一些实施方案中,抗CD20抗体(例如II型抗-CD20抗体)包括人Fc区(例如人IgG1 Fc区)。在一些实施方案中,Fc区包括已经修饰的N-连接的寡糖。在一些实施方案中,与具有未修饰的N-连接寡糖的抗体相比,Fc区的N-连接寡糖具有降低的岩藻糖残基。在一些实施方案中,平分的寡糖是平分的复合寡糖。在一些实施方案中,N-连接的寡糖已经被修饰为具有增加的平分无岩藻糖化寡糖。在一些实施方案中,平分的无岩藻糖化寡糖是杂交型。在一些实施方案中,平分的无岩藻糖化寡糖是复合型。关于更详细的描述,参见例如WO 2003/011878(Jean-Mairet等人);美国专利号6,602,684(Umana等人);US 2005/0123546(Umana等人);和美国专利8,883,980(Umana等人)。

[0161] 在一些实施方案中,抗CD20抗体(例如II型抗-CD20抗体)是多特异性抗体或双特异性抗体。

[0162] 抗体制备

[0163] 根据任何上述实施方案的抗体(例如,本公开的II型抗CD20抗体)可以单独或组合地并入如以下1-7节所述的任何特征:

[0164] 1.抗体亲和力

[0165] 在某些实施方案中,本文提供的抗体具有≤1μM,≤100nM,≤10nM,≤1nM,≤

0.1nM，≤0.01nM或≤0.001nM的解离常数(Kd)(例如，10^{-8}M或更小，例如10^{-8}M至10^{-13}M，例如10^{-9}M至10^{-13}M)。

[0166] 在一个实施方案中，通过放射性标记的抗原结合测定(RIA)测量Kd。在一个实施方案中，使用Fab形式的目标抗体及其抗原进行RIA。例如，Fabs对抗原的溶液结合亲和力通过在滴定一系列未标记抗原存在下用最小浓度的(^{125}I)标记的抗原平衡Fab，然后用抗Fab抗体包被的平板捕获结合的抗原而测量(参见例如Chen等人，J.Mol.Biol.293:865-881(1999))。为了建立测定条件，将MICROTITER®多孔板(Thermo Scientific)用在50mM碳酸钠(pH9.6)中的5μg/ml的捕获抗Fab抗体(Cappel Labs)包被过夜，随后用在PBS中的2%(w/v)牛血清白蛋白在室温(约23℃)下封闭2至5小时。在非吸附板(Nunc#269620)中，将100pM或26pM[^{125}I]-抗原与目标Fab的系列稀释液混合(例如，与抗-VEGF抗体，Fab-12的评估一致，Presta等人，Cancer Res.57:4593-4599(1997))。然后将目标Fab温育过夜；然而，温育可以持续较长时间(例如约65小时)以确保达到平衡。此后，将混合物转移到捕获板中，以在室温下温育(例如1小时)。然后除去溶液，并用PBS中的0.1%聚山梨醇酯20(TWEEN-20)将板洗涤8次。当板干燥时，加入150μl/孔的闪烁剂(MICROSCINT-20™；Packard)，并将板在TOPCOUNT™ γ计数器(Packard)上计数10分钟。选择给出小于或等于最大结合的20%的每种Fab的浓度用于竞争性结合测定。

[0167] 根据另一个实施方案，使用BIACORE ®表面等离子体共振测定法测量Kd。例如，使用BIACORE®-2000或BIACORE-3000(BIAcore，Inc.，Piscataway，NJ)的测定，在25℃使用固定的抗原CM5芯片以约10个应答单位(RU)进行。在一个实施方案中，根据供应商的说明书，用N-乙基-N'-(3-二甲基氨基丙基)-碳二亚胺盐酸盐(EDC)和N-羟基琥珀酰亚胺(NHS)活化羧甲基化葡聚糖生物传感器芯片(CM5，BIACORE，Inc.)。在注射前以5μl/分钟的流速用10mM乙酸钠(pH4.8)将抗原稀释至5μg/ml(～0.2μM)，以获得约10个应答单位(RU)的偶联蛋白。注射抗原后，注射1M乙醇胺以封闭未反应的基团。为了动力学测量，在25℃以约25μl/分钟的流速注入在含0.05%聚山梨醇酯20(TWEEN-20™)表面活性剂的PBS(PBST)中的两倍连续稀释的Fab(0.78nM至500nM)。使用简单一对一朗格缪尔(Langmuir)结合模型(BIACORE ®Evaluation Software version 3.2)通过同时拟合结合和解离传感图计算结合速率(kon)和解离速率(Koff)。平衡解离常数(Kd)以koff/kon比计算。参见例如Chen等人，J.Mol.Biol.293:865-881(1999)。如果通过上述表面等离振子共振测定的结合速率超过10^{6}M-1s-1，则可以通过使用荧光猝灭技术测量结合速率，其正如在分光计例如配备了断流装置的分光光度计(stop-flow equipped spectrophometer)(Aviv Instruments)或8000系列SLM-AMINCO™分光光度计(ThermoSpectronic)中用搅拌比色杯(stirred cuvette)测量的，在存在浓度渐增的抗原的条件下，测量PBS，pH 7.2中的20nM抗-抗原抗体(Fab形式)在25℃的荧光发射强度(激发＝295nm；发射＝340nm，16nm带通)的升高或降低。

[0168] 2.抗体片段

[0169] 在某些实施方案中，本文提供的抗体是抗体片段。抗体片段包括但不限于Fab、Fab'、Fab'-SH、F(ab')2、Fv和scFv片段，以及下述其它片段。有关某些抗体片段的综述，参见Hudson等人，Nat.Med.9:129-134(2003)。关于scFv片段的综述，参见例如Pluckthün，The Pharmacology of Monoclonal Antibodies，113，Rosenburg and Moore编，(Springer-Verlag，New York)，pp.269-315(1994)；另参见WO 93/16185；和美国专利号5,571,894和5,

587,458。关于包括补救受体结合表位残基并具有增加的体内半衰期的Fab和F(ab')2片段的讨论,参见美国专利号5,869,046。

[0170] 双抗体是具有两个抗原结合位点的抗体片段,其可以是二价或双特异性的。参见例如EP 404,097;WO 1993/01161;Hudson等人,,Nat.Med.9:129-134(2003);和Hollinger等人,Proc.Natl.Acad.Sci.USA 90:6444-6448(1993)。Habson等人,Nat.Med.9:129-134(2003)中也描述了三抗体和四抗体。

[0171] 单结构域抗体是包括抗体的全部或部分重链可变结构域或全部或部分轻链可变结构域的抗体片段。在某些实施方案中,单结构域抗体是人单结构域抗体(Domantis,Inc.,Waltham,MA;参见例如美国专利号6,248,516B1)。

[0172] 抗体片段可以通过各种技术制备,包括但不限于完整抗体的蛋白水解消化以及如本文所述的通过重组宿主细胞(例如大肠杆菌或噬菌体)生产。

[0173] 3.嵌合和人源化抗体

[0174] 在某些实施方案中,本文提供的抗体是嵌合抗体。例如,在美国专利号4,816,567;和Morrison等人,Proc.Natl.Acad.Sci.USA,81:6851-6855(1984))中描述了某些嵌合抗体。在一个实例中,嵌合抗体包含非人可变区(例如,源自小鼠、大鼠、仓鼠、兔、或非人灵长动物,诸如猴的可变区)和人恒定区。在另外的实例中,嵌合抗体是"类别交换的"抗体,其中类别或亚类已经从亲本抗体的类别或亚类改变。嵌合抗体包含其抗原结合片段。

[0175] 在某些实施方案中,嵌合抗体是人源化的抗体。通常,非人抗体经人源化以降低对人的免疫原性,同时保持亲本非人抗体的特异性和亲和力。一般地,人源化的抗体包含一个或多个可变结构域,其中HVR,例如,CDR(或其部分)源自非人抗体,并且FR(或其部分)源自人抗体序列。人源化的抗体任选地也包含至少一部分人恒定区。在一些实施方案中,人源化的抗体中的一些FR残基被来自非人抗体(例如,HVR残基源自的抗体)的对应的残基取代,例如,以恢复或提高抗体特异性或亲和力。

[0176] 人源化的抗体和制备其的方法在例如,Almagro和Fransson,Front.Biosci.13:1619-1633(2008)中综述,并在例如,Riechmann等人,Nature 332:323-329(1988);Queen等人,Proc.Nat'l Acad.Sci.USA 86:10029-10033(1989);美国专利号5,821,337、7,527,791、6,982,321和7,087,409;Kashmiri等人,Methods 36:25-34(2005)(描述了特异性决定区域(SDR)的移植);Padlan,Mol.Immunol.28:489-498(1991)(描述了"表面重修");Dall'Acqua等人,Methods 36:43-60(2005)(描述了"FR改组");和Osbourn等人,Methods 36:61-68(2005)和Klimka等人,Br.J.Cancer,83:252-260(2000)(描述了FR改组的"导向选择"方法)中进一步描述。

[0177] 可用于人源化的人构架区包括但不限于:使用"最适"方法选择的构架区(参见,例如,Sims等人J.Immunol.151:2296(1993));源自特别亚组的轻链或重链可变区的人抗体的共有序列的构架区(参见,例如,Carter等人Proc.Natl.Acad.Sci.USA,89:4285(1992);和Presta等人J.Immunol.,151:2623(1993));人成熟(体细胞突变的)构架区或人种系构架区(参见,例如,Almagro和Fransson,Front.Biosci.13:1619-1633(2008));和源自筛选FR文库的构架区(参见,例如,Baca等人,J.Biol.Chem.272:10678-10684(1997)和Rosok等人,J.Biol.Chem.271:22611-22618(1996))。

[0178] 4.人抗体

[0179] 在某些实施方案中，本文提供的抗体是人抗体。可以使用本领域已知的多种技术生产人抗体。在van Dijk和van de Winkel,Curr.Opin.Pharmacol.5:368-74(2001)和Lonberg,Curr.Opin.Immunol.20:450-459(2008)中大体地描述了人抗体。

[0180] 可以通过对转基因动物施用免疫原制备人抗体，所述转基因动物已被修饰以应答抗原攻击而生产完整人抗体或具有人可变区的完整抗体。此类动物通常含有人免疫球蛋白基因座的全部或一部分，其取代了内源性疫球蛋白基因座，或存在于染色体外或随机整合到动物的染色体中。在此类转基因小鼠中，内源性免疫球蛋白基因座通常已被失活。获取来自转基因动物的人抗体的方法的综述参见Lonberg,Nat.Biotech.23:1117-1125(2005)。也参见，例如，描述了XENOMOUSE™技术的美国专利号6,075,181和6,150,584；描述了HUMAB®技术的美国专利号5,770,429；描述了K-M MOUSE®技术的美国专利号7,041,870，和描述了VELOCIMOUSE®技术的美国专利申请公开号US 2007/0061900)。来自由此类动物生成的完整抗体的人可变区可例如，通过与不同的人恒定区组合进一步修饰。

[0181] 也可以通过基于杂交瘤的技术生产人抗体。已经描述了用于生产人单克隆抗体的人骨髓瘤和小鼠-人杂骨髓瘤细胞系(参见，例如，Kozbor J.Immunol.,133:3001(1984)；Brodeur等人,Monoclonal Antibody Production Techniques and Applications,pp.51-63(Marcel Dekker,Inc.,New York,1987)；和Boerner等人,J.Immunol.,147:86(1991))。经由人B细胞杂交瘤技术生成的人抗体也在Li等人,Proc.Natl.Acad.Sci.USA,103:3557-3562(2006)中描述。额外的方法包括，例如，在美国专利号7,189,826(描述了从杂交瘤细胞系生产单克隆人IgM抗体)和Ni,Xiandai Mianyixue,26(4):265-268(2006)(描述了人-人杂交瘤)中描述的那些。人杂交瘤技术(Trioma技术)也在Vollmers和Brandlein,Histology and Histopathology,20(3):927-937(2005)以及Vollmers和Brandlein,Methods and Findings in Experimental and Clinical Pharmacol ogy,27(3):185-91(2005)中描述。

[0182] 也可以通过分离选自源自人的噬菌体展示文库的Fv克隆可变结构域序列生成人抗体。此类可变结构域序列然后可以与期望的人恒定结构域组合。下文描述了从抗体文库选择人抗体的技术。

[0183] 5.源自文库的抗体

[0184] 可通过筛选组合文库的具有期望的一种或多种活性的抗体分离本发明的抗体。例如，本领域已知用于生成噬菌体展示文库和筛选此类文库的具有期望的结合特征的抗体的多种方法。此类方法在例如Hoogenboom等人,Methods in Molecular Biology 178:1-37(O'Brien等人，编辑,Human Press,Totowa,NJ,2001)中综述并在，例如，McCafferty等人,Nature 348:552-554;Clackson等人,Nature 352:624-628(1991)；Marks等人,J.Mol.Biol.222:581-597(1992)；Marks和Bradbury,Methods in Molecular Biology 248:161-175(Lo,编辑,Human Press,Totowa,NJ,2003)；Sidhu等人,J.Mol.Biol.338(2):299-310(2004)；Lee等人,J.Mol.Biol.340(5):1073-1093(2004)；Fellouse,Proc.Natl.Acad.Sci.USA 101(34):12467-12472(2004)；和Lee等人,J.Immunol.Methods 284(1-2):119-132(2004)中进一步描述。

[0185] 在某些噬菌体展示方法中，VH和VL基因的库通过聚合酶链式反应(PCR)分别克隆并在噬菌体文库中随机重组，其然后可以筛选抗原结合噬菌体，如Winter等人，

Ann.Rev.Immunol.,12:433-455(1994)中所述。噬菌体通常展示抗体片段,作为单链抗体Fv(scFv)片段或作为Fab片段。来自经免疫的来源的文库提供了对免疫原高亲和力的抗体而不需要构建杂交瘤。备选地,可以克隆初始库(例如,从人)以对广泛范围的非自身以及自身抗原提供单个来源的抗体而没有任何免疫,如Griffiths等人,EMBO J,12:725-734(1993)所述。最后,也可以通过克隆来自干细胞的未重排的V基因区段,并使用含有随机序列的PCR引物以编码高度可变的CDR3区并且实现体外重排,合成地制备初始文库,如Hoogenboom和Winter,J.Mol.Biol.,227:381-388(1992)所述。描述人抗体噬菌体文库的专利公开包括,例如:美国专利号5,750,373,和美国专利公开号2005/0079574、2005/0119455、2005/0266000、2007/0117126、2007/0160598、2007/0237764、2007/0292936和2009/0002360。

[0186] 从人抗体文库分离的抗体或抗体片段在本文被认为是人抗体或人抗体片段。

[0187] 6.多特异性抗体

[0188] 在某些实施方案中,本文提供的抗体是多特异性抗体,例如双特异性抗体。多特异性抗体是具有对于至少两个不同位点的结合特异性的单克隆抗体。在某些实施方案中,结合特异性之一是对于CD20而另一种是对于任何其他抗原。在某些实施方案中,双特异性抗体可结合CD20的两种不同表位。双特异性抗体也可用于将细胞毒性剂定位至表达CD20的细胞。双特异性抗体可以作为全长抗体或抗体片段制备。

[0189] 用于制备多特异性抗体的技术包括,但不限于,重组共表达具有不同特异性的两条免疫球蛋白重链-轻链对(参见,Milstein和Cuello,Nature 305:537(1983))、WO 93/08829,和Traunecker等人,EMBO J.10:3655(1991)),和"旋钮入孔(knob-in-hole)"改造(参见,例如,美国专利号5,731,168)。也可以通过改造静电转向效应以制备抗体Fc异二聚体分子(WO2009/089004A1);交联两个或多个抗体或片段(参见,例如,美国专利号4,676,980,和Brennan等人,Science,229:81(1985));使用亮氨酸拉链生产双特异性抗体(参见,例如,Kostelny等人,J.Immunol.,148(5):1547-1553(1992));使用用于制备双特异性抗体片段的"双抗体"技术(参见,例如,Hollinger等人,Proc.Natl.Acad.Sci.USA,90:6444-6448(1993));和使用单链Fv(sFv)二聚体(参见,例如Gruber等人,J.Immunol.,152:5368(1994));和例如,如Tutt等人J.Immunol.147:60(1991)中所述制备三特异性抗体,来制备多特异性抗体。

[0190] 本文也包括具有三种或更多种功能性抗原结合位点的经改造的抗体,包括"章鱼抗体"(参见,例如US 2006/0025576A1)。

[0191] 本文的抗体或片段也包括"双作用Fab"或"DAF",其包含结合CD20以及另一种不同抗原的抗原结合位点(参见例如,US 2008/0069820)。

[0192] 7.抗体变体

[0193] 在某些实施方案中,设想本文提供的抗体的氨基酸序列变体。例如,提高抗体的结合亲和力和/或其他生物学性质可以是理想的。可以通过将适当的修饰引入编码抗体的核苷酸序列,或者通过肽合成制备抗体的氨基酸序列变体。此类修饰包括,例如,在抗体的氨基酸序列内缺失,和/或插入和/或取代残基。可以使得缺失、插入,和取代的任意组合达到最终的构建体中,前提是最终构建体拥有期望的特征,例如,抗原结合。

[0194] a)取代、插入和缺失变体

[0195] 在某些实施方案中,提供了具有一个或多个氨基酸取代的抗体变体。取代诱变的

目标位点包括HVR和FR。在表A中标题“保守取代”下显示保守取代。在表A中标题“示例性取代”下提供更实质的改变，并如参考氨基酸侧链类别在下文进一步描述的。可以将氨基酸取代引入到目标抗体中并筛选产物的期望的活性，例如，保留的/提高的抗原结合，降低的免疫原性，或提高的ADCC或CDC。

[0196] 表A

[0197]

原始残基	示例性取代	优选的取代
Ala(A)	Val; Leu; Ile	Val
Arg(R)	Lys; Gln; Asn	Lys
Asn(N)	Gln; His; Asp, Lys; Arg	Gln
Asp(D)	Glu; Asn	Glu
Cys(C)	Ser; Ala	Ser
Gln(Q)	Asn; Glu	Asn
Glu(E)	Asp; Gln	Asp
Gly(G)	Ala	Ala
His(H)	Asn; Gln; Lys; Arg	Arg
Ile(I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu(L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys(K)	Arg; Gln; Asn	Arg
Met(M)	Leu; Phe; Ile	Leu
Phe(F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro(P)	Ala	Ala
Ser(S)	Thr	Thr
Thr(T)	Val; Ser	Ser
Trp(W)	Tyr; Phe	Tyr
Tyr(Y)	Trp; Phe; Thr; Ser	Phe
Val(V)	Ile; Leu; Met; Phe; Ala; 正亮氨酸	Leu

[0198]

[0199] 可以根据共同的侧链性质将氨基酸分组：

[0200] (1)疏水的:正亮氨酸、Met、Ala、Val、Leu、Ile;

[0201] (2)中性亲水的:Cys、Ser、Thr、Asn、Gln;

[0202] (3)酸性的:Asp、Glu;

[0203] (4)碱性的:His、Lys、Arg;

[0204] (5)影响链取向的残基:Gly、Pro;

[0205] (6)芳香族:Trp、Tyr、Phe。

[0206] 非保守取代将使得这些类别中之一的成员与另一类别交换。

[0207] 一类取代变体涉及取代亲本抗体(例如人源化的或人抗体)的一个或多个超变区残基。一般而言,选择用于进一步研究的(一种或多种)获得的变体将在某些生物学性质(例如增加的亲和力、降低的免疫原性)中相对于亲本抗体具有修饰(例如提高)和/或具有亲本抗体的基本保留的某些生物学性质。示例性的取代变体是亲和力成熟的抗体,其可以方便地生成,例如,使用诸如那些本文描述的基于噬菌体展示的亲和力成熟技术。简而言之,突变一个或多个HVR残基并且在噬菌体上展示变体抗体并就特别的生物学活性(例如结合亲和力)进行筛选。

[0208] 可以在HVR中制造变异(例如,取代),例如,以提高抗体亲和力。此类变异可以在HVR"热点",即由在体细胞成熟过程中以高频率经历突变的密码子所编码的残基(参见,例如,Chowdhury,Methods Mol.Biol.207:179-196(2008)),和/或接触抗原的残基出进行,测试获得的变体VH或VL的结合亲和力。通过构建并从第二文库重新选择的亲和力成熟已经在,例如Hoogenboom等人Methods in Molecular Biology 178:1-37(O'Brien等人,编,Human Press,Totowa,NJ,(2001).)中描述。在亲和力成熟的一些实施方案中,通过多种方法中的任何一种(例如,易错PCR、链改组、或寡核苷酸定向诱变)将多样性引入到选择用于成熟的可变基因中。然后产生第二文库。然后筛选文库以鉴定具有期望的亲和力的任何抗体变体。引入多样性的另一种方法涉及HVR定向方法,其中随机化若干HVR残基(例如,一次4-6个残基)。可以特异性地鉴定抗原结合中涉及的HVR残基,例如使用丙氨酸扫描诱变或建模。特别地通常靶向CDR-H3和CDR-L3。

[0209] 在某些实施方案中,取代、插入、或缺失可以在一个或多个HVR内发生,只要此类变异不实质性地降低抗体结合抗原的能力。例如,可以在HVR中制备不实质性地降低结合亲和力的保守改变(例如,如本文提供的保守取代)。此类改变可以,例如在HVR中接触残基的抗原之外。在以上提供的变体VH和VL序列的某些实施方案中,每个HVR或未改变,或含有不多于一个、两个或三个氨基酸取代。

[0210] 鉴定可被靶向用于诱变的抗体的残基或区域的可用的方法称为"丙氨酸扫描诱变",如Cunningham和Wells(1989)Science,244:1081-1085所述。在此方法中,鉴定残基或靶残基的组(例如,带电的残基诸如arg、asp、his、lys和glu)并使用中性或带负电的氨基酸(例如丙氨酸或多聚丙氨酸)将其取代以测定是否影响抗体与抗原的相互作用。可以在表现出对于初始取代功能性敏感的氨基酸位置处引入其他取代。备选地,或额外地,抗原抗体复合物的晶体结构鉴定抗体和抗原之间的接触点。可以作为取代候选对象靶向或消除此类接触残基和相邻残基。可以筛选变体以测定其是否含有期望的性质。

[0211] 氨基酸序列插入片段包括长度范围从一个残基至含有100个或更多残基的多肽的氨基或羧基末端融合物,以及单个或多个氨基酸残基的序列内插入片段。末端插入片段的

实例包括具有N末端甲硫氨酰基残基的抗体。抗体分子的其他插入变体包括抗体的N或C末端与酶(例如ADEPT)或增加抗体的血清半寿期的多肽的融合。

[0212] b)糖基化变体

[0213] 在某些实施方案中,本文提供的抗体被改变以增加或降低抗体被糖基化的程度。对抗体添加或缺失糖基化位点可以通过改变氨基酸序列从而使得产生或移除一个或多个糖基化位点而方便地实现。

[0214] 当抗体包含Fc区时,可以改变附着于其的糖类。由哺乳动物细胞生产的天然抗体通常包含分支的、二分支的寡糖,所述寡糖一般通过N连接附着于Fc区的CH2结构域的Asn297。参见,例如,Wright等人TIBTECH15:26-32(1997)。寡糖可包括多种糖类,例如甘露糖、N-乙酰基葡糖胺(GlcNAc)、半乳糖、和唾液酸,以及附着于二分支寡糖结构的"茎干"中的GlcNAc的岩藻糖。在一些实施方案中,可以制造本发明的抗体中寡糖的修饰以创造具有某些提高的性质的抗体变体。

[0215] 在一个实施方案中,提供的抗体变体具有缺少(直接或间接)附着于Fc区的岩藻糖的糖类结构。例如,此类抗体中岩藻糖的量可以为从1%至80%,从1%至65%,从5%至65%或从20%至40%。通过计算糖链内Asn297处的岩藻糖的平均量相对于附着于Asn 297(例如,复合、杂合和高甘露糖结构)的全部糖基结构的总和测定岩藻糖的量,如通过MALDI-TOF质谱法测量,如,例如WO 2008/077546中所述。Asn297指位于Fc区中约297位(Fc区残基的Eu编号)的天冬酰胺残基;然而,Asn297也可位于297位的约±3个氨基酸上游或下游,即位置294和300之间,由于抗体中微小的序列变异。此类岩藻糖化变体可具有提高的ADCC功能。参见,例如,美国专利公开号US 2003/0157108(Presta,L.);US 2004/0093621(Kyowa Hakko Kogyo Co.,Ltd)。涉及"去岩藻糖化的"或"岩藻糖缺陷的"抗体变体的公开的实例包括:US 2003/0157108;WO 2000/61739;WO 2001/29246;US 2003/0115614;US 2002/0164328;US 2004/0093621;US 2004/0132140;US 2004/0110704;US 2004/0110282;US 2004/0109865;WO 2003/085119;WO 2003/084570;WO 2005/035586;WO 2005/035778;WO2005/053742;WO2002/031140;Okazaki等人,J.Mol.Biol.336:1239-1249(2004);Yamane-Ohnuki等人,Biotech.Bioeng.87:614(2004)。能够生产去岩藻糖化的抗体的细胞系的实例包括蛋白质岩藻糖化缺陷的Lec13CHO细胞(Ripka等人Arch.Biochem.Biophys.249:533-545(1986);US专利申请号US 2003/0157108A1,Presta,L;和WO 2004/056312A1,Adams等人,特别在实施例11中),和敲除细胞系,诸如α-1,6-岩藻糖转移酶基因,FUT8,敲除CHO细胞(参见,例如,Yamane-Ohnuki等人,Biotech.Bioeng.87:614(2004);Kanda,Y.等人,Biotechnol.Bioeng.,94(4):680-688(2006);和WO2003/085107)。

[0216] 提供的抗体变体还具有二分的寡糖,例如,其中附着于抗体Fc区的二分支寡糖被GlcNAc二分。此类抗体变体可具有降低的岩藻糖化和/或提高的ADCC功能。此类抗体变体的实例描述于例如WO 2003/011878(Jean-Mairet等人);美国专利号6,602,684(Umana等人);和US 2005/0123546(Umana等人)中。也提供了在附着于Fc区的寡糖中具有至少一个半乳糖残基的抗体变体。此类抗体变体可具有提高的CDC功能。此类抗体变体描述于例如WO 1997/30087(Patel等人);WO 1998/58964(Raju,S.);和WO 1999/22764(Raju,S.)中。

[0217] c)Fc区变体

[0218] 在某些实施方案中,可以将一个或多个氨基酸修饰引入到本文提供的抗体的Fc区

中，从而生成Fc区变体。Fc区变体可以包含人Fc区序列(例如，人IgG1、IgG2、IgG3或IgG4Fc区)，所述序列在一个或多个氨基酸位置处包含氨基酸修饰(例如取代)。

[0219] 在某些实施方案中，本发明设想具有一些但并非全部效应子功能的抗体变体，这使得其是这样的应用的理想的候选者，所述应用中抗体的体内半寿期是重要的然而某些效应子功能(诸如补体和ADCC)是不必要的或有害的。可以进行体外和/或体内细胞毒性测定法以确认CDC和/或ADCC活性的降低/耗尽。例如，可以进行Fc受体(FcR)结合测定法以确保抗体缺少Fc γ R结合(因此可能缺少ADCC活性)，但保留FcRn结合能力。介导ADCC的主要细胞，NK细胞，仅表达Fc γ RIII，然而单核细胞表达Fc γ RI、Fc γ RII和Fc γ RIII。造血细胞上的FcR表达在Ravetch和Kinet，Annu.Rev.Immunol.9:457-492(1991)的464页上的表3中总结。用于评估目的分子的ADCC活性的体外测定法的非限制性实例描述于美国专利号5,500,362(参见，例如Hellstrom,I.等人，Proc.Nat'l Acad.Sci.USA 83:7059-7063(1986))和Hellstrom,I等人，Proc.Nat'l Acad.Sci.USA 82:1499-1502(1985)；5,821,337(参见Bruggemann,M.等人，J.Exp.Med.166:1351-1361(1987))中。备选地，可以采用非放射性测定法(参见，例如，流式细胞术的ACTI ™非放射性细胞毒性测定法(CellTechnology,Inc.Mountain View,CA;和CytoTox96®非放射性细胞毒性测定法(Promega,Madison,WI)。用于此类测定法的有用的效应子细胞包括外周血单核细胞(PBMC)和天然杀伤(NK)细胞。备选地，或额外地，目的分子的ADCC活性可以在体内评估，例如在动物模型中，例如Clynes等人Proc.Nat'l Acad.Sci.USA 95:652-656(1998)中公开的。也可以进行C1q结合测定法以确认抗体不能结合C1q并因此缺少CDC活性。参见，例如，WO 2006/029879和WO 2005/100402中的C1q和C3c结合ELISA。为了评估补体激活，可以进行CDC测定法(参见，例如，Gazzano-Santoro等人，J.Immunol.Methods 202:163(1996)；Cragg,M.S.等人，Blood 101:1045-1052(2003)；和Cragg,M.S.和M.J.Glennie,Blood 103:2738-2743(2004))。FcRn结合和体内清除/半寿期测定也可以使用本领域已知的方法进行(参见，例如，Petkova,S.B.等人，Int'l.Immunol.18(12):1759-1769(2006))。

[0220] 具有降低的效应子功能的抗体包含在Fc区残基238、265、269、270、297、327和329中的一个或多个具有取代的那些抗体(美国专利号6,737,056)。此类Fc突变体包括在氨基酸位置265、269、270、297和327中的两个或多个处具有取代的Fc突变体，包括具有残基265和297取代为丙氨酸的所谓的"DANA"Fc突变体(美国专利号7,332,581)。

[0221] 在某些实施方案中，本文所述的Fc变体还包含用于减弱效应子功能(例如CDC和/或ADCC)的一种或多种氨基酸修饰。在示例性实施方案中，减弱效应子功能的修饰是不改变Fc区糖基化模式的修饰。在某些实施方案中，减弱效应子功能的修饰减少或消除与人效应子细胞的结合，与一种或多种Fc受体的结合和/或与表达Fc受体的细胞的结合。在一个示例性实施方案中，本文所述的Fc变体包含以下修饰：人IgG1的Fc区中的L234A、L235A和P329G，其导致减弱的效应子功能。先前已经显示了取代L234A、L235A和P329G(L234A/L235A/P329G三重变体被称为LALAPG)减少与Fc受体和补体的结合(参见例如美国公开号2012/0251531)。

[0222] 在各种实施方案中，具有降低的效应子功能的Fc变体是指与野生型Fc区实现的效应子功能(例如Fc区不具有降低效应子功能的突变，尽管它可能具有其它突变)相比，将效应功能(例如，CDC、ADCC和/或与FcR等结合的活性)降低至少10%、20%、30%、40%、50%、60%、70%、80%、90%、95%、97%、98%、99%或更多的Fc变体。在某些实施方案中，具有降

低的效应子功能的Fc变体是指与野生型Fc区相比消除所有可检测的效应子功能的Fc变体。用于测量效应子功能的测定法是本领域已知的并在下面描述。

[0223] 可以进行体外和/或体内细胞毒性测定以确认CDC和/或ADCC活性的减少/消除。例如，可以进行Fc受体(FcR)结合测定以确保抗体缺乏FcγR结合(因此可能缺乏ADCC活性)。用于介导ADCC的原代细胞，NK细胞仅表达FcγRIII，而单核细胞表达FcγRI，FcγRII和FcγRIII。在Ravetch和Kinet，Annu.Rev.Immunol.9:457-492(1991)中总结了造血细胞上的FcR表达。美国专利号5,500,362(参见例如Hellstrom，I.等人，Proc.Nat'l Acad.Sci.USA 83:7059-7063(1986))和Hellstrom，I等人，Nat'l Acad.Sci.USA 82:1499-1502(1985)；5,821,337(参见Bruggemann，M.等人，J.Exp.Med.166:1351-1361(1987)))中描述了评估目的分子的ADCC活性的体外测定的非限制性实例。或者，可以使用非放射性测定方法(参见例如ACTI ™非放射性细胞毒性测定用于流式细胞术(CellTechnology，Inc.Gampton View，CA；和CytoTox 96®非放射性细胞毒性测定(Promega，Madison，WI)。用于这种测定的有用的效应子细胞包括外周血单核细胞(PBMC)和自然杀伤(NK)细胞。或者或另外，可以在体内评估目的分子的ADCC活性，例如在动物模型如Clynes等人，Proc.Nat'l Acad.Sci.USA 95:652-656(1998)中所公开的。也可以进行C1q结合测定以确认抗体不能结合C1q，因此缺乏CDC活性。参见WO 2006/029879和WO 2005/100402中的C1q和C3c结合ELISA。为了评估补体活化，可以进行CDC测定(参见，例如，Gazzano-Santoro等人，J.Immunol.Methods 202:163(1996)；Cragg，MS等人，Blood 101:1045-1052(2003)；和Cragg，MS和M.J.Glennie，Blood 103:2738-2743(2004))。

[0224] 描述了具有提高的或减弱的与FcR结合的某些抗体变体。(参见，例如，美国专利号6,737,056；WO 2004/056312和Shields等人，J.Biol.Chem.9(2):6591-6604(2001)。)

[0225] 在某些实施方案中，抗体变体包含具有提高ADCC的一个或多个氨基酸取代的Fc区，例如Fc区的位置298、333和/或334处(残基的EU编号)的取代。

[0226] 在一些实施方案中，在Fc区进行改变，其导致改变的(即提高的或减弱的)C1q结合和/或补体依赖性细胞毒性(CDC)，例如，如美国专利号6,194,551、WO 99/51642和Idusogie等人J.Immunol.164:4178-4184(2000)中所述。

[0227] 具有增加的半寿期和提高的与新生儿Fc受体(FcRn)的结合的抗体在US2005/0014934A1(Hinton等人)中描述，所述新生儿Fc受体负责母体IgG转移至胎儿(Guyer等人，J.Immunol.117:587(1976)和Kim等人，J.Immunol.24:249(1994))。那些抗体包含其中具有提高Fc区与FcRn的结合的一个或多个取代的Fc区。此类Fc变体包括在Fc区残基：238、256、265、272、286、303、305、307、311、312、317、340、356、360、362、376、378、380、382、413、424或434中的一个或多个处具有取代的那些，例如Fc区残基434的取代(美国专利号7,371,826)。

[0228] 关于Fc区变体的其他实例也参见Duncan&Winter，Nature322:738-40(1988)；美国专利号5,648,260；美国专利号5,624,821；和WO 94/29351。

[0229] d)半胱氨酸改造的抗体变体

[0230] 在某些实施方案中，产生半胱氨酸改造的抗体是理想的，例如，“thioMAb”，其中抗体的一个或多个残基被半胱氨酸残基取代。在特别的实施方案中，被取代的残基存在于抗体的易接近的位点处。通过用半胱氨酸取代那些残基，从而将反应性硫醇基团放置于抗体的可接近位点处并可用于缀合抗体与其他部分，诸如药物部分或接头药物部分，以产生免

疫缀合物，如本文另外所述。在某些实施方案中，以下残基中的任何一个或多个可被半胱氨酸取代：轻链的V205(Kabat编号)；重链的A118(EU编号)；和重链Fc区的S400(EU编号)。可以如，例如美国专利号7,521,541中所述地生成半胱氨酸改造的抗体。

[0231] e)抗体衍生物

[0232] 在某些实施方案中，还可进一步修饰本文提供的抗体以含有本领域已知的并且容易获得的额外的非蛋白质部分。适合于抗体的衍生化的部分包括但不限于水溶性聚合物。水溶性聚合物的非限制性实例包括，但不限于，聚乙二醇(PEG)、乙二醇/丙二醇的共聚物、羧甲基纤维素、葡聚糖、聚乙烯醇、聚乙烯吡咯烷酮、聚-1,3-二氧戊环、聚-1,3,6-三噁烷、乙烯/顺丁烯二酸酐共聚物、聚氨基酸(均聚物或随机共聚物)，和葡聚糖或聚(n-乙烯基吡咯烷酮)聚乙二醇、聚丙二醇均聚物、聚环氧丙烷/环氧乙烷共聚物、聚氧乙烷化多元醇(例如丙三醇)、聚乙烯醇，及其混合物。聚乙二醇丙醛由于其在水中的稳定性可在生产中具有优势。聚合物可具有任意分子量，并且可为分支的或无分支的。附着于抗体的聚合物的数目可不同，并且如果附着多于一个聚合物，它们可以是相同或不同的分子。一般而言，用于衍生化的聚合物的数量和/或类型可以基于如下考虑确定，包括，但不限于，待改善的抗体的特别的性质或功能，抗体衍生物是否将在定义的条件下用于疗法中，等。

[0233] 在另一个实施方案中，提供了抗体和可以通过暴露于辐射选择性地加热的非蛋白质部分的缀合物。在一个实施方案中，非蛋白质部分是碳纳米管(Kam等人，Proc.Natl.Acad.Sci.USA 102:11600-11605(2005))。辐射可以具有任何波长，并且包括，但不限于，这样的波长，所述波长不伤害普通细胞，但将非蛋白质部分加热至杀死抗体-非蛋白质部分临近的细胞的温度。

[0234] A.重组方法和组合物

[0235] 可以使用重组方法和组合物制备抗体，例如美国专利号4,816,567中所述。在一个实施方案中，提供了编码本文所述抗CD20抗体的分离的核酸。这样的核酸可以编码抗体的包括VL的氨基酸序列和/或包括VH的氨基酸序列(例如抗体的轻链和/或重链)。在另一个实施方案中，提供了一种或多种包含该核酸的载体(例如，表达载体)。在另一个实施方案中，提供了包括这种核酸的宿主细胞。在一个这样的实施方案中，宿主细胞包括(例如，已经被转化)：(1)载体，其包括编码包括抗体的VL的氨基酸序列和包括抗体的VH的氨基酸序列的核酸，或(2)包括编码包括抗体VL的氨基酸序列的核酸的第一载体和包括编码包括抗体VH的氨基酸序列的核酸的第二载体。在一个实施方案中，宿主细胞是真核生物，例如中国仓鼠卵巢(CHO)细胞或淋巴细胞(例如Y0，NS0，Sp20细胞)。在一个实施方案中，提供了制备抗CD20抗体的方法，其中所述方法包括在适于表达所述抗体的条件下，培养如上所提供的包括编码抗体的核酸的宿主细胞，并任选地从宿主细胞(或宿主细胞培养基)回收抗体。

[0236] 为重组产生抗CD20抗体，将编码抗体(例如，如上所述)的核酸分离并插入一种或多种载体以便进一步克隆和/或在宿主细胞中表达。可以使用常规方法(例如，通过使用能够与编码抗体重链和轻链的基因特异性结合的寡核苷酸探针)，轻易地分离这种核酸并测序。

[0237] 克隆或表达编码抗体的载体的合适宿主细胞包括本文所述的原核或真核细胞。例如，可以在细菌中生产抗体，尤其当不需要糖基化和Fc效应子功能时。对于细菌中表达抗体片段和多肽，参见例如美国专利号5,648,237、5,789,199和5,840,523。(还参见Charlton，Methods in Molecular Biology，Vol.248(B.K.C.Lo编，Humana Press，Totowa，NJ，2003)，

pp.245-254,其描述在大肠杆菌中表达抗体片段)。在表达后,可以将抗体从细菌细胞糊状物分离于可溶性级分中并且可以进一步纯化抗体。

[0238] 除原核生物之外,真核微生物如丝状真菌或酵母是编码抗体的载体的合适克隆宿主或表达宿主,包括其糖基化途径已经"人源化"的真菌和酵母菌株,导致产生具有部分或完全人类糖基化模式的抗体。参见Gerngross,Nat.Biotech.22:1409-1414(2004),和Li等人,Nat.Biotech.24:210-215(2006)。

[0239] 表达糖基化抗体的合适宿主细胞还源自多细胞生物(无脊椎动物和脊椎动物)。无脊椎动物细胞的例子包括植物细胞和昆虫细胞。已经鉴定了可以与昆虫细胞一起使用,尤其用于转染草地贪夜蛾(Spodoptera frugiperda)细胞的众多杆状病毒毒株。

[0240] 也可以利用植物细胞培养物作为宿主。参见例如美国专利号5,959,177、6,040,498、6,420,548、7,125,978和6,417,429(描述了在转基因植物中产生抗体的PLANTIBODIES™技术)。

[0241] 也可以使用脊椎动物细胞作为宿主。例如,适应于悬浮培育的哺乳动物细胞系可以是有用的。有用的哺乳动物宿主细胞系的其他例子是由SV40转化的猴肾CV1系(COS-7);人胚肾系(如Graham等人,J.Gen Virol.36:59(1977)中所述的293或293细胞);幼仓鼠肾细胞(BHK);小鼠支持细胞(如在例如Mather,Biol.Reprod.23:243-251(1980)中所述的TM4细胞);猴肾细胞(CV1);非洲绿猴肾细胞(VERO-76);人宫颈癌细胞(HELA);犬肾细胞(MDCK;水牛鼠肝脏细胞(BRL 3A);人肺细胞(W138);人肝脏细胞(Hep G2);小鼠乳腺瘤细胞(MMT060562);TRI细胞,如在例如Mather等人,Annals N.Y.Acad.Sci.383:44-68(1982)中所述;MRC5细胞和FS4细胞。其他有用的哺乳动物宿主细胞系包括中国仓鼠卵巢(CHO)细胞,包括DHFR-CHO细胞(Urlaub等人,Proc.Natl.Acad.Sci.USA77:4216(1980));和骨髓瘤细胞系如YO、NS0和Sp2/0。关于适于产生抗体的某些哺乳动物宿主细胞系的综述,见例如Yazaki和Wu,Methods in Molecular Biology,Vol.248(B.K.C.Lo编,Humana Press,Totowa,NJ),pp.255-268(2003)。

[0242] B.测定法

[0243] 本文提供的抗CD20抗体可以通过本领域已知的各种测定法来鉴定、筛选或表征其物理/化学性质和/或生物活性。

[0244] I.结合测定和其他测定

[0245] 在一个方面,测试本发明的抗体的抗原结合活性,例如通过已知方法例如ELISA、Western印迹等。可以使用本领域已知的方法测定CD20结合,并公开示例性方法于此。在一个实施方案中,使用放射免疫测定来测量结合。下面提供示例性放射免疫测定。CD20抗体被碘化,并制备含有固定浓度的碘化抗体和降低浓度的连续稀释的未标记CD20抗体的竞争反应混合物。将表达CD20的细胞(例如,用人CD20稳定转染的BT474细胞)加入到反应混合物中。温育后,洗涤细胞以从与细胞结合的CD20抗体中分离游离的碘化CD20抗体。测定结合的碘化CD20抗体的水平,例如通过计数与细胞相关的放射性和使用标准方法测定的结合亲和力。在另一个实施方案中,使用流式细胞术评估CD20抗体与表面表达的CD20结合的能力(例如,在B细胞亚群上)。获得外周白细胞(例如,来自人、食蟹猴、大鼠或小鼠),并用血清封闭细胞。标记的CD20抗体以连续稀释加入,并且还染色T细胞以鉴定T细胞亚群(使用本领域已知的方法)。在样品温育和洗涤后,使用流式细胞术分选细胞,并使用本领域熟知的方法分

析数据。在另一个实施方案中，可以使用表面等离振子共振分析CD20结合。示例性的表面等离子体共振方法在实施例中举例说明。

[0246] 在另一方面，竞争测定可用于鉴定与本文公开的任何抗CD20抗体竞争结合CD20的抗体。在某些实施方案中，这种竞争性抗体结合与本文公开的任何抗CD20抗体结合的相同表位(例如，线性或构象表位)。Morris(1996)"Epitope Mapping Protocols,"在Methods in Molecular Biology vol.66(Humana Press,Totowa,NJ)中提供了用于作图抗体结合的表位的详细的示例性方法。

[0247] 在示例性竞争测定中，将固定的CD20在包括结合CD20的第一标记抗体(例如利妥昔单抗，GA101抗体等)的溶液中温育，并且测试其第二未标记的抗体与第一抗体竞争结合CD20的能力。第二抗体可存在于杂交瘤上清液中。作为对照，将固定的CD20在包括第一标记抗体而不是第二未标记抗体的溶液中温育。在允许第一抗体与CD20结合的条件下温育后，除去过量未结合的抗体，并测量与固定的CD20相关的标记物的量。如果在测试样品中相对于对照样品与固定化CD20结合的标记物的量显著降低，则表明第二抗体与第一抗体竞争结合CD20。参见Harlow和Lane(1988)Antibodies:A Laboratory Manual ch.14(Cold Spring Harbor Laboratory,Cold Spring Harbor,NY)。

[0248] 2.活性测定

[0249] 本公开的抗CD20抗体(例如II型抗体)可以通过本领域已知的一种或多种活性测定来鉴定和/或表征。例如，如本文所述，可以使用补体依赖性细胞毒性(CDC)和/或抗体依赖性细胞毒性(ADCC)。

[0250] 应当理解，任何上述测定可以使用本发明的免疫缀合物代替抗CD20抗体或补加抗CD20抗体来进行。

[0251] 应当理解，可以使用抗CD20抗体和另外的治疗剂来进行上述测定中的任一种。

[0252] C.免疫缀合物

[0253] 本发明还提供包含与一种或多种细胞毒性剂，例如化学治疗剂或药物，生长抑制剂，毒素(例如蛋白质毒素，细菌、真菌、植物或动物来源的酶促活性毒素，或其片段)或放射性同位素缀合的本文的抗CD20抗体的免疫缀合物。

[0254] 在一个实施方案中，免疫缀合物是抗体-药物缀合物(ADC)，其中抗体与一种或多种药物缀合，包括但不限于美坦生类化合物(参见美国专利号5,208,020、5,416,064和欧洲专利EP 0 425 235 B1)；澳瑞他汀，如单甲基澳瑞他汀药物部分DE和DF(MMAE和MMAF)(参见美国专利号5,635,483和5,780,588和7,498,298)；多拉司他汀、加利车霉素或其衍生物(参见美国专利号5,712,374、5,714,586、5,739,116、5,767,285、5,770,701、5,770,710、5,773,001和5,877,296；Hinman等人，Cancer Res.53:3336-3342(1993)；和Lode等人，Cancer Res.58:2925-2928(1998))；蒽环类抗生素如柔红霉素或多柔比星(参见Kratz等人，Current Med.Chem.13:477-523(2006)；Jeffrey等人，Bioorganic&Med.Chem.Letters 16:358-362(2006)；Torgov等人，Bioconj.Chem.16:717-721(2005)；Nagy等人，Proc.Natl.Acad.Sci.USA 97:829-834(2000)；Dubowchik等人，Bioorg.&Med.Chem.Letters 12:1529-1532(2002)；King等人，J.Med.Chem.45:4336-4343(2002)；和美国专利号6,630,579)；甲氨蝶呤；长春地辛；紫杉烷类，如多西他赛、紫杉醇、拉罗他赛、替西他赛和奥他赛；单端孢霉烯和CC1065。

[0255] 在另一个实施方案中，免疫缀合物包括与酶活性毒素或其片段缀合的本文所述的抗体，其包括但不限于白喉A链，白喉毒素的非结合活性片段，外毒素A链(来自绿脓假单胞菌(Pseudomonas aeruginosa))，蓖麻毒素A链，相思豆毒素A链，蒴莲根毒素A链，α-帚曲霉素(sarcin)，油桐(Aleurites fordii)蛋白，香石竹毒蛋白(dianthin protein)、美洲商陆(Phytolaca americana)蛋白(PAP1、PAPII和PAP-S)、苦瓜(momordica charantia)抑制剂、麻疯树毒蛋白(curcin)、巴豆毒蛋白(crotin)、肥皂草(sapaonaria officinalis)抑制剂、多花白树毒蛋白(gelonin)、丝林霉素(mitogellin)、局限曲菌素(restrictocin)、酚霉素(phenomycin)、伊诺霉素(enomycin)和单端孢菌素(tricothecenes)。

[0256] 在另一个实施方案中，免疫缀合物包括与放射性原子缀合以形成放射性缀合物的本文所述的抗体。各种放射性同位素可用于生产放射性缀合物。实例包括At^{211}、I^{131}、I^{125}、Y^{90}、Re^{186}、Re^{188}、Sm^{153}、Bi^{212}、P^{32}、Pb^{212}和Lu的放射性同位素。当使用放射性缀合物进行检测时，可以包括用于闪烁照相研究的放射性原子，例如tc99m或I123，或用于核磁共振(NMR)成像(也称为磁共振成像，MRI)的自旋标记，同样例如碘-123、碘-131、铟-111、氟-19、碳-13、氮-15、氧-17、钆、锰或铁。

[0257] 抗体和细胞毒性剂的缀合物可以使用各种双功能蛋白偶联剂制备，该双功能蛋白偶联剂例如N-琥珀酰亚胺基-3-(2-吡啶基二硫代)丙酸酯(SPDP)、琥珀酰亚胺基-4-(N-马来酰亚氨基甲基)环己烷-1-羧酸酯(SMCC)、亚氨基硫烷(IT)、亚氨酸酯的双官能衍生物(诸如盐酸己二亚胺酸二甲酯)、活性酯类(诸如辛二酸二琥珀酰亚胺基酯)、醛类(诸如戊二醛)、双叠氮化合物(诸如双(对-叠氮苯甲酰基)己二胺)、双重氮衍生物(诸如双(对-重氮苯甲酰基)乙二胺)、二异氰酸酯(诸如甲苯2,6-二异氰酸酯)、和双活性氟化合物成分(诸如1,5-二氟-2,4-二硝基苯)。例如，可如Vitetta,E.S.,等人,Science 238(1987)1098-1104中所述制备蓖麻毒蛋白免疫毒素。碳-14标记的1-异硫氰酸苯甲基-3-甲基二亚乙基三胺五乙酸(MX-DTPA)是用于将放射性核苷酸与抗体缀合的示例性螯合剂。参见WO 94/11026。接头可以是便于在细胞中释放细胞毒性药物的“可切割接头”。例如，可使用酸不稳定接头、肽酶敏感接头、光不稳定接头、二甲基接头或含二硫化物接头(Chari,R.V.,等人,Cancer Research 52(1992)127-131,US 5,208,020)。

[0258] 本文中的免疫缀合物或ADC明确地考虑但不限于用交联剂试剂制备的这种缀合物，该交联剂试剂包括但不限于BMPS、EMCS、GMBS、HBVS、LC-SMCC、MBS、MPBH、SBAP、SIA、SIAB、SMCC、SMPB、SMPH、磺基-MEMS、磺基-GMBS、磺基-MUMS、磺基-MBS、磺基-SIAB、磺基-SMCC和磺基-SMPB和SVSB(琥珀酰亚胺基-(4-乙烯基砜)苯甲酸酯)，其是可商购的(例如，来自Pierce Biotechnology,Inc.,Rockford,IL,USA)。

[0259] 治疗或延缓狼疮性肾炎进展的方法

[0260] 本公开的某些方面涉及用于治疗或延缓具有狼疮的个体的狼疮性肾炎(LN)进展的方法。在一些实施方案中，个体或患者是人。

[0261] LN在本领域中已知为肾脏中的狼疮(例如，系统性红斑狼疮、药物诱发的狼疮、新生儿狼疮或盘状狼疮)的表现。肾脏中最常见的类型的狼疮是系统性红斑狼疮(SLE)。据认为，25-50％的SLE患者在其疾病早期发生尿和/或肾功能异常，多达60％的成年人和80％的儿童最终发展为LN(更多细节参见Cameron,JS(1999)J.Am.Soc.Nephrol.10:413-424)。LN被认为占SLE相关发病率和死亡率的至少50％。

[0262] 此外，在其他类型的狼疮，例如盘状体(Roujeau，JC等人(1984)Acta Derm.Venereol.66:160-163)和药物诱发的狼疮(Smith，PR等人，(1999)Rheumatology(Oxford)38:1017-1018)中也注意到肾脏表现。在一些实施方案中，个体具有SLE、盘状狼疮或药物诱发的狼疮。

[0263] 可以按照当前的美国风湿病学会(American College of Rheumatology，ACR)标准来诊断SLE。可以通过一个British Isles Lupus Activity Group(BILAG)“A”标准或两个BILAG“B”标准；SLE疾病活动指数(SLEDAI)；或系统性红斑狼疮(SLE)应答者指数(SRI)来定义活动疾病，如在下面实施例中所述，并在Furie等人，Arthritis Rheum.61(9):1143-51(2009)中描述。根据Tan等人，“The Revised Criteria for the Classification of SLE”Arth Rheum 25(1982)改编的用来诊断SLE的一些病征、症状或其他指征可以是面颊红疫(例如脸颊上的疫子)、盘状红疫或红色凸起的板块(red raised patches)；光敏性如对阳光起反应，导致皮疹的发生或增加；口腔溃疡如鼻子或口的溃疡(通常无痛)；关节炎如涉及两个或更多个周边关节的非侵蚀性关节炎(其中关节周围的骨头没有损坏的关节炎)；浆膜炎、胸膜炎或心包炎；肾病症如尿中蛋白过量(大于0.5gm/天或测试棒上为3+)和/或细胞管型(源于尿和/或白细胞和/或肾管细胞的异常部分)；神经学病征、症状或其他指征、突然发作(痉挛)和/或在没有药物情况下的精神病或已知能引起这类效应的代谢紊乱；以及血液学病征、症状或其他指征诸如溶血性贫血或白细胞减少症(每立方毫米白细胞计数低于4,000细胞)或淋巴球减少症(每立方毫米少于1,500个淋巴细胞)或血小板减少症(每立方毫米少于100,000个血小板)。必须有两次或更多次机会检测到白细胞减少症和淋巴球减少症。必须在没有已知能诱导血小板减少症的药物的情况下检测到血小板减少症。本公开并不局限于狼疮的这些病征、症状或其他指征。

[0264] 可以测试自身抗体的存在作为狼疮的指征。自身抗体可以包括但不限于抗dsDNA抗体、抗补体抗体和抗核抗体(例如ENA组)。ENA是指可提取的核抗原，即一组核抗原，包括，例如，RNP，Ro/SS-A，La/SS-B，Sm，SCL-70，Jo-1，如描述于McNeilage等人，J.，Clin.Lab.Immunol.15:1-17(1984)；Whittingham，Ann.Acad.Med.17(2):195-200(1988)；Wallace and Hahn，dubois' lupus erythematosus，第7版，Lippincott(2007)；Tang等人，Medicine 89(1):62-67(2010)。ENA抗体与狼疮相关。McNeilage等人，1984；Whittingham 1988；Asherson等人，Medicine 68(6):366-374(1989)；和Tang等人，2010。减少的补体活性也可能与狼疮有关，例如，通过C3水平、C4水平和/或CH50测定测量。

[0265] 如上文参考SLE所述，本领域已知LN经常在患有狼疮的患者(例如系统性红斑狼疮、药物诱发的狼疮、新生儿狼疮或盘状狼疮)中逐渐显现。也就是说，患者没有一种或多种LN症状的临床或病理学表现也可被诊断患有狼疮。然而，由于最终发展为LN的狼疮患者的频率高，患者仍然可能被认为有发展为LN的风险。因此，在一些实施方案中，本公开的方法可用于延缓患有狼疮的患者中LN的进展或预防LN。在一些实施方案中，本公开的方法可用于延迟或预防患有狼疮的患者(例如，在肾脏中缺乏表现的一种形式的狼疮)的LN的发作。

[0266] LN病理可以根据国际肾脏病学/肾脏病理学会(ISN/RPS)2003分类系统进行分类，如下表所示(参见Markowitz GS，D'Agati VD(2007)Kidney Int 71:491-495和Weening，JJ(2004)Kidney Int 65:521-530进一步描述和定义术语)。

[0267] 表3.ISN/RPS 2003狼疮性肾炎分类。

[0268]

I 类	最小肾小球系膜 LN (通过光学显微镜检查正常肾小球，但通过免疫荧光检测系膜免疫沉积)
II 类	系膜增殖性 LN (通过光学显微镜任何程度的纯系膜细胞过多和系膜基质扩张，具有系膜免疫沉积物。通过免疫荧光或电子显微镜可以看到少数分离的上皮下或内皮下沉积物，但不能通过光学显微镜看到)
III 类	局灶性 LN (活性或非活性的局灶性，节段性或全局毛细血管内-或毛细血管外肾小球肾炎，涉及 50%的所有肾小球，通常伴有局灶性内皮下免疫沉积，具有或不具有系膜改变) III(A)：活跃病灶(局灶性增生性 LN) III(A/C)：活性和慢性病灶(局灶性增生性和硬化性 LN) III(C)：具有肾小球瘢痕的慢性非活跃病灶(局灶性硬化性 LN)

[0269]

IV 类	弥漫性 LN (活性或非活性弥漫性，节段性或全局性毛细血管内或毛细血管外肾小球性肾炎，涉及≥50%所有肾小球的，通常具有弥漫性内皮下免疫沉积物，具有或不具有球系膜改变，当 ≥50%涉及的肾小球具有节段性病灶，该类被分为节段性弥漫 (IV-S)，并且当≥ 50%的所涉及的肾小球具有全局病灶时，该类被分为全局弥漫(IV-G)LN。节段性被定义为涉及不到一半的肾小球簇的肾小球病灶。该类包括具有弥漫性丝环沉积物但具有很少或没有肾小球增殖的病例) IV-S(A): 活跃病灶(弥漫性节段性增殖性 LN) IV-G(A): 活跃病灶(弥漫性全局增生性 LN) IV-S(A/C): 活跃和慢性病灶(弥漫性节段性增殖性和硬化性 LN) IV-G(A/C): 活跃和慢性病灶(弥漫性全局增生性和硬化性 LN) IV-S(C): 具有瘢痕的慢性不活跃病灶(弥漫性节段性硬化性 LN) IV-G(C): 具有瘢痕的慢性不活跃病灶(弥漫性全身硬化性 LN)
V 类	膜性 LN (通过光学显微镜和免疫荧光或电子显微镜，全局或节段性上皮免疫沉积物或其形态学后遗症，有或没有系膜改变)
VI 类	晚期硬化性 LN (≥ 90%的无残留活性的肾小球全局硬化)

[0270] LN＝狼疮性肾炎;A＝活跃;C＝慢性;G＝全局;S＝节段性。

[0271] 注意:V类可能与III或IV类联合发生，在这种情况下，两者都将被诊断。V类LN可能显示晚期硬化症。

[0272] 在一些实施方案中，患者具有III类或IV类LN。在一些实施方案中，患者具有III类LN。例如，在一些实施方案中，患者具有III类(A)或III类(A/C)LN。在一些实施例中，患者具有IV类LN。例如，在一些实施方案中，患者具有IV-S(A)、IV-G(A)、IV-S(A/C)或IV-G(A/C)LN)。如上表3所示，V类LN也可能与III类或IV类LN同时发生。在一些实施方案中，本公开的方法用于治疗III类或IV类LN和伴随的V类LN的患者。

[0273] 如上所述，高频率的狼疮患者(例如，SLE)最终发展为LN。在一些实施方案中，患者处于发展LN的风险中。在一些实施方案中，患者处于发展III类或IV类LN的风险中。在一些实施方案中，患者处于发展具有伴随的V类LN的III类或IV类LN的风险中。

[0274] 在一些实施方案中，患者不具有III类(C)LN(例如，如上表3所述)。在一些实施方案中，患者不具有IV类(C)LN，如IV-S(C)或IV-G(C)LN(例如，如上文表3所述)。

[0275] 本领域已知的几种实验室测试可用于诊断和/或监测狼疮性肾炎的存在、进展和/或对治疗的应答。在一些实施方案中，可以测量血清肌酸酐。在一些实施方案中，血清肌酸酐的正常范围可以为约0.6至约1.3mg/dL，在年龄、男性和女性之间、以及实验室和实验室之间都有一些变化。在一些实施方案中，可以例如通过尿液的显微镜检查来测量尿沉渣和/或管型的存在。例如，可以通过显微镜检查来测定尿样中的红细胞数。在一些实施方案中，尿沉渣的正常值可以是每个高功率场(HPF)约4个红细胞(RBC)或更低。尿管型可以包括但不限于红细胞管型、白细胞管型、肾小管上皮细胞管型、蜡状管型、透明管型，颗粒状管型和脂肪型管型。在一些实施方案中，可以测量尿蛋白与肌酸酐比(UPCR)。尿液中蛋白质的存在(蛋白尿)也可以通过包括但不限于尿白蛋白与肌酸酐比率(UACR)和浸液片尿分析的测试来测定。可用于检查肾功能的其他测试和/或措施包括但不限于renal panel，肌酐清除率，钠、钾、氯化物、碳酸氢盐、磷、钙、白蛋白、血尿素氮(BUN)、肌酐、葡萄糖、肾小球滤过率(eGFR)、BUN/肌酐比和阴离子间隙，并且可以包括在适当时测量血液和/或尿液中的上述参数。有关更详细的描述，参见例如the American College of Rheumatology Guidelines for Screening,Case Definition,Treatment and Management of Lupus Nephritis (Hahn,B.等人，(2012)Arthritis Care Res.64:797-808)。

[0276] 在一些实施方案中，本公开的方法包括向个体施用至少暴露于本公开的II型抗-CD20抗体的第一抗体和暴露于II型抗-CD20抗体的第二抗体。可以使用本文所述的任何II型抗CD20抗体，例如GA101抗体，例如奥比妥珠单抗。在一些实施方案中，在第一次抗体暴露后约18周至约26周后才提供第二次抗体暴露。在一些实施方案中，在第一次抗体暴露后约18周，第一次抗体暴露后约19周，第一次抗体暴露后约20周，第一次抗体暴露后约21周，第一次抗体暴露后约22周，第一次抗体暴露后约23周，第一次抗体暴露后约24周，第一次抗体暴露后约25周，或第一次抗体暴露后约26周才提供第二次抗体暴露。在一些实施方案中，在第一次抗体暴露后小于约下述周中任何一种：26、25、24、23、22、21、20或19后才提供第二个抗体暴露。在一些实施方案中，在第一次抗体暴露后大于约下述周中任何一种：18、19、20、21、22、23、24或25后才提供第二次抗体暴露。也就是说，在具有上限为26、25、24、23、22、21、20或19以及独立选择的下限为18、19、20、21、22、23、24、25周的范围中任一之后才提供第二次抗体暴露，其中下限小于上限。

[0277] 本文所述的给药方案使用一致的系统来跟踪剂量之间的时间，从而在第1天向患者施用第一剂量。如本文所述，本公开的抗体暴露可以包括一个或两个剂量。在抗体暴露含有一个剂量的情况下，提及在第一次抗体暴露后(如本文所述)已经过去一段时间后才提供的第二次抗体暴露是指在第一次抗体暴露的剂量(例如，第1天)和第二次抗体暴露的剂量之间经过的时间量。如果第一次抗体暴露包括两个剂量，则在第1天提供第一次抗体暴露的第一剂量。在抗体暴露含有两个剂量的情况下，提及在第一次抗体暴露(如本文所述)一段时间后才提供的第二次抗体暴露是指在第一次抗体暴露的两个剂量中的第一个(例如，第1天)和第二次抗体暴露的两个剂量的第一剂量之间经过的时间量。例如，如果本公开的方法包括具有两个剂量的第一次抗体暴露和具有两个剂量的第二次抗体暴露，并且在第一次抗体暴露后约22周后才提供第二次抗体暴露，则在第一次抗体暴露的第一剂量和第二次抗体暴露的第一剂量之间的时间间隔约为22周。

[0278] 在一些实施方案中，本公开的第一次抗体暴露包括一个或两个剂量的本公开的II

型抗CD20抗体。在一些实施方案中，第一次抗体暴露含有约1800mg至约2200mg的II型抗-CD20抗体的总暴露。在一些实施方案中，第一次抗体暴露含有约1800mg、约1900mg、约2000mg、约2100mg或约2200mg的II型抗-CD20抗体的总暴露。

[0279] 在一些实施方案中，第一次抗体暴露包括两个剂量。在一些实施方案中，第一次抗体暴露包括约900mg至约1100mg的II型抗CD20抗体的第一剂量和约900mg至约1100mg的II型抗-CD20抗体的第二剂量。在一些实施方案中，第一次抗体暴露的第一剂量含有约1000mg的II型抗-CD20抗体。在一些实施方案中，第一次抗体暴露的第二剂量含有约1000mg的II型抗-CD20抗体。在一些实施方案中，在第一次抗体暴露的第一剂量之后约1.5周至约2.5周才提供第一次抗体暴露的第二剂量。在一些实施方案中，在第一次抗体暴露的第一剂量后约2周才提供第一次抗体暴露的第二剂量。

[0280] 在一些实施方案中，本公开的第二次抗体暴露包括一个或两个剂量的本公开的II型抗-CD20抗体。在一些实施方案中，第二次抗体暴露含有约1800mg至约2200mg的II型抗CD20抗体的总暴露。在一些实施方案中，第二次抗体暴露含有约1800mg、约1900mg、约2000mg、约2100mg或约2200mg II型抗CD20抗体的总暴露。

[0281] 在一些实施方案中，第二次抗体暴露包括两个剂量。在一些实施方案中，第二次抗体暴露包括约900mg至约1100mg的II型抗CD20抗体的第一剂量和约900mg至约1100mg的II型抗-CD20抗体的第二剂量。在一些实施方案中，第二次抗体暴露的第一剂量含有约1000mg的II型抗-CD20抗体。在一些实施方案中，第二次抗体暴露的第二剂量含有约1000mg的II型抗-CD20抗体。在一些实施方案中，在第二次抗体暴露的第一剂量后约1.5周至约2.5周后才提供第二次抗体暴露的第二剂量。在一些实施方案中，在第二次抗体暴露的第一剂量后约2周才提供第二次抗体暴露的第二剂量。

[0282] 在一些实施方案中，本公开的II型抗CD20抗体是静脉内施用(例如通过IV输注)。

[0283] 在一些实施方案中，本公开的方法进一步包括施用有效量的免疫抑制剂(例如，结合本文所述的II型抗-CD20抗体)。几种类别的免疫抑制剂是本领域已知的，包括但不限于细胞抑制剂(例如，细胞毒性剂如抗生素，烷化剂(例如，环磷酰胺，也称为cytophosphane)，肌苷单磷酸脱氢酶抑制剂，抗代谢物如蛋白质合成抑制剂，叶酸类似物，嘌呤类似物，嘧啶类似物等)，免疫抑制抗体，糖皮质激素，靶向亲免蛋白的药物(例如他克莫司、西罗莫司、雷帕霉素及其类似物、环孢菌素等)，mTOR活性位点抑制剂，霉酚酸及其衍生物或盐，TNF结合蛋白，干扰素，阿片类，以及其他小分子(如芬戈莫德)。在一些实施方案中，免疫抑制剂包括霉酚酸、霉酚酸的衍生物或霉酚酸的盐。在一些实施方案中，免疫抑制剂包括霉酚酸酯。在一些实施方案中，免疫抑制剂包括**CellCept®** (Roche)。在一些实施方案中，免疫抑制剂包括**Myfortic®** (Novartis)。本公开的免疫抑制剂的有效量是本领域已知的，并且可以通过标准测定容易地确定。例如，霉酚酸酯可以以2.0-2.5g/天施用，如图1所示。在一些实施方案中，霉酚酸酯可以以1000mg/天以分剂量(2次/天)开始施用，并在第4周以分剂量(2次/天)逐步增高剂量至2.0-2.5g/天。

[0284] 在一些实施方案中，免疫抑制剂可以在施用本公开的II型抗CD20抗体之前、期间或之后施用，例如作为对狼疮的治疗。在一些实施方案中，可以在用本公开的II型抗CD20抗体的整个治疗期间施用免疫抑制剂。在一些实施方案中，霉酚酸酯可以如上所述在用II型抗-CD20抗体的整个治疗期间施用。

[0285] 在一些实施方案中，本公开的方法还包括施用有效量的糖皮质激素或皮质类固醇(例如，与本文所述的II型抗CD20抗体结合)。各种天然存在和合成的糖皮质激素/皮质类固醇在本领域中是已知的，包括但不限于倍氯米松、曲安奈德、地塞米松、倍他米松、强的松、甲泼尼龙、泼尼松龙、可的松和皮质醇。在一些实施方案中，糖皮质激素/皮质类固醇包括甲泼尼龙。在一些实施方案中，糖皮质激素/皮质类固醇包括强的松。本公开的糖皮质激素/皮质类固醇的有效量是本领域已知的，并且可以通过标准测定容易地确定。例如，甲泼尼龙可以以750-1000mg的剂量每天一次施用。作为另一个实例，强的松可以以0.5mg/kg口服施用，任选地逐渐增加至7.5mg/天。

[0286] 在一些实施方案中，糖皮质激素可以在施用本公开内容的II型抗CD20抗体之前、期间或之后施用，例如用于治疗LN临床活性。在一些实施方案中，糖皮质激素可以在施用本公开的II型抗CD20抗体之前施用，例如在II型抗CD20抗体之前30-60分钟施用。在一些实施方案中，80mg甲泼尼龙可以在施用本公开的II型抗CD20抗体之前30-60分钟IV施用。在一些实施方案中，泼尼松(例如，口服施用)和/或甲泼尼龙(例如，IV施用)可以随治疗施用随后进行维持治疗(例如霉酚酸酯或环磷酰胺)。

[0287] 在一些实施方案中，本公开的方法还包括施用有效量的抗组胺药(例如，与本文所述的II型抗CD20抗体结合)。本领域已知的和目前临床应用的抗组胺药包括组胺H_1受体和组胺H_2受体拮抗剂或反向激动剂。在一些实施方案中，抗组胺药包括苯海拉明。本公开的抗组胺药的有效量是本领域已知的，并且可以通过标准测定法容易地确定。例如，苯海拉明可以以50mg口服剂量施用。

[0288] 在一些实施方案中，可以在施用本公开的II型抗CD20抗体之前、期间或之后施用抗组胺药，例如作为预防性治疗。在一些实施方案中，可以在施用本公开的II型抗CD20抗体之前，例如在II型抗CD20抗体前30-60分钟施用抗组胺药。在一些实施方案中，50mg苯海拉明可以在施用本公开的II型抗CD20抗体之前30-60分钟口服施用。

[0289] 在一些实施方案中，本公开的方法还包括施用有效量的非甾体抗炎药或NSAID(例如与本文所述的II型抗CD20抗体结合)。本领域已知的NSAID包括乙酸衍生物、丙酸衍生物、水杨酸酯、烯醇酸衍生物、邻氨基苯甲酸衍生物、选择性COX-2抑制剂、磺酰苯胺(sulfonanilides)等。在一些实施方案中，NSAID包括对乙酰氨基酚。本公开的NSAID的有效量是本领域已知的，并且可以通过标准测定容易地确定。例如，对乙酰氨基酚可以以650-1000mg口服剂量施用。

[0290] 在一些实施方案中，NSAID可以在施用本公开的II型抗CD20抗体之前、期间或之后施用，例如作为预防性治疗。在一些实施方案中，NSAID可以在施用本公开的II型抗CD20抗体之前施用，例如在II型抗CD20抗体之前30-60分钟。在一些实施方案中，可以在施用本公开的II型抗-CD20抗体之前30-60分钟口服施用650-1000mg对乙酰氨基酚。

[0291] 在一些实施方案中，本公开的方法进一步包括施用有效量的抗疟剂(例如与本文所述的II型抗CD20抗体结合)。可以使用的抗疟剂的实例包括但不限于羟氯喹、氯喹和奎纳克林。在一些实施方案中，可以在施用本公开的II型抗-CD20抗体之前、期间或之后施用抗疟剂，例如作为对狼疮的一种或多种症状的治疗。

[0292] 在一些实施方案中，本公开的方法进一步包括施用有效量的整联蛋白拮抗剂(例如，结合本文所述的II型抗-CD20抗体)。可以使用的整联蛋白拮抗剂的实例包括但不限于

LFA-1抗体，例如可从Genentech购得的依法珠单抗(RAPTTVA®)或α4整联蛋白抗体如可从Biogen获得的那他珠单抗(ANTEGREN®)或二氮杂环苯丙氨酸衍生物、苯丙氨酸衍生物、苯丙酸衍生物、烯胺衍生物、丙酸衍生物、链烷酸衍生物、取代的苯基衍生物、芳族胺衍生物、ADAM解聚素结构域多肽、抗αV3β整联蛋白、氮杂桥连双环氨基酸衍生物等。可以在施用本公开的II型抗CD20抗体之前、期间或之后施用整联蛋白拮抗剂，例如作为对狼疮的一种或多种症状的治疗。

[0293] 在一些实施方案中，本公开的方法进一步包括施用有效量的细胞因子拮抗剂(例如，结合本文所述的II型抗-CD20抗体)。可以使用的细胞因子拮抗剂的实例包括但不限于针对IL-1、IL-1α、IL-2、IL-3、IL-4、IL-5、IL-6、IL-7、IL-8、IL-9、IL-11、IL-12、IL-15的拮抗剂(例如拮抗剂抗体)；肿瘤坏死因子如TNF-α或TNF-β；和其他多肽因子，包括LIF和kit配体(KL)。在一些实施方案中，细胞因子拮抗剂可以在施用本公开的II型抗CD20抗体之前、期间或之后施用，例如作为对狼疮的一种或多种症状的治疗。

[0294] 在一些实施方案中，本公开的方法进一步包括施用有效量的激素(例如，与本文所述的II型抗CD20抗体结合)。在一些实施方案中，可以在施用本公开的II型抗-CD20抗体之前、期间或之后施用激素(例如，用于激素替代疗法)，例如用于患有狼疮的妇女的药物治疗。

[0295] 在一些实施方案中，本公开的方法进一步包括施用护理标准治疗(例如，结合本文所述的II型抗-CD20抗体)。在一些实施方案中，可以在施用本公开的II型抗CD20抗体之前、期间或之后施用护理标准治疗，例如用于治疗或预防狼疮的一种或多种症状。在某些实施方案中，可以在本公开的第二次抗体暴露后施用护理标准治疗。例如，本公开的II型抗CD20抗体可以如本文所述施用于患者作为诱导疗法，然后根据护理标准治疗患者作为维持治疗。用于狼疮的护理标准治疗是本领域公知的，包括但不限于血管紧张肽转化酶(ACE)抑制剂、血管紧张肽受体阻断剂、环磷酰胺、霉酚酸酯(例如，在本文所述的剂量下，例如2.0-2.5g/天)、硫唑嘌呤和糖皮质激素或皮质类固醇(例如强的松，如强的松逐渐减量)。

[0296] 在一些实施方案中，本公开的方法还包括施用抗高血压剂(例如，与本文所述的II型抗-CD20抗体结合)。在一些实施方案中，可以在施用本公开的II型抗CD20抗体之前、期间或之后施用抗高血压剂，例如用于治疗或预防高血压。在一些实施方案中，抗高血压剂包括但不限于ACE抑制剂和血管紧张肽受体阻断剂。在一些实施方案中，表5中列出的抗高血压剂例如以表5所述范围内的剂量施用。

[0297] 在一些实施方案中，本公开的方法导致个体中的完全肾反应(CRR)。在一些实施方案中，CRR包括以下所有因素：血清肌酐标准化，无活性尿沉渣和尿蛋白与肌酸酐比<0.5。在一些实施方案中，血清肌酐的标准化的特征在于血清肌酐小于或等于中心实验室值的正常(ULN)范围的上限，和/或血清肌酐比基线高≤15%，且如果基线血清肌酐(例如，第1天)在中心实验室值的正常范围内，则小于或等于中心实验室值的ULN范围。在一些实施方案中，非活性尿沉渣的特征在于<10RBC/高功率场(HPF)和/或不存在红细胞管型。关于LN中CRR和部分肾反应(PRR)的更详细的讨论，参见例如Chen，Y.E.等人，人，(2008)Clin.J.Am.SOC.Nephrol.3:46-53。

[0298] 在一些实施方案中，本公开的方法导致个体中的完全肾反应(CRR)或部分肾反应

(PRR)。在一些实施方案中,PRR包括以下一个或多个:血清肌酸酐的标准化,无活性的尿沉渣和尿蛋白与肌酸酐比<0.5。在一些实施方案中,PRR包括以下一个或多个:缓解一种或多种症状,包括但不限于血清肌酐减少、尿沉渣减少、蛋白尿减少以及肾功能的任何其它改善。在一些实施方案中,CRR或PRR包括狼疮活性的一种或多种生物标志物的降低,包括但不限于抗dsDNA抗体、抗核抗体/ENA、抗补体抗体、降低的补体C3和/或C4的水平,以及降低的补体活性(例如,通过CH50测定法测定)。

[0299] 在一些实施方案中,本公开的方法导致个体中循环的外周B细胞的消耗。在一些实施方案中,在施用本公开的II型抗CD20抗体(例如,根据本文所述的任何方法)后,循环的外周B细胞以约10个细胞/μL或更少、约9个细胞/μL或更少、约8个细胞/μL或更少、约7个细胞/μL或更少、约6个细胞/μL或更少、约5个细胞/μL或更少、约4个细胞/μL或更少、约3个细胞/μL或更少、约2个细胞/μL或更少、或约1个细胞/μL或更少存在于外周血中。在一些实施方案中,个体中循环的外周B细胞消耗至少约10%、至少约20%、至少约30%、至少约40%、至少约50%、至少约60%、至少约70%、至少约80%、至少约90%或约100%。在一些实施方案中,循环外周B细胞的消耗是指例如,与治疗前相同个体的相应测量相比,或者与对照个体(例如,未接受治疗的个体)的相应测量相比,在第一次抗体暴露(例如,包括1或2剂量的本文所述的抗CD20抗体)后,在第二次抗体暴露(例如,包括1或2剂量的本文所述的抗CD20抗体)后,治疗后3个月(例如,在接受本文所述的第一和/或第二次抗体暴露后),治疗后6个月(例如,在接受后如本文所述的第一和/或第二次抗体暴露),治疗后9个月(例如,在接受本文所述的第一和/或第二次抗体暴露后)或治疗后12个月(例如,在接受第一和第/或如本文所述的第二次抗体暴露),取样的循环的外周B细胞的测量。

[0300] 用于测定个体中循环的外周B细胞的消耗的方法是本领域已知的,例如使用识别B细胞标记的一种或多抗体的流式细胞术。在一些实施方案中,高灵敏度流式细胞术(HSFC)可用于测定循环外周B细胞的耗竭(参见例如Vital,E.M.等人,(2011)Arthritis Rheum.63:3038-3047)。在一些实施方案中,B细胞是CD19+ B细胞。在一些实施方案中,B细胞是初始B细胞(例如CD19+ CD27B细胞)、记忆B细胞(例如CD19+ CD27+ B细胞)或成浆细胞(例如,CD19+ CD27+ CD38++ B细胞)。

[0301] IV.制品或药盒

[0302] 在本发明的另一方面,提供了可用于治疗、预防和/或诊断上述病症的制品或药盒。制品或药盒包括容器和在容器上或与容器相关联的标签或包装插页。合适的容器包括例如瓶、小瓶、注射器、IV溶液袋等。容器可以由各种材料如玻璃或塑料形成。容器容纳组合物,所述组合物自身或者与有效治疗、预防和/或诊断病症的另一种组合物组合,并且所述容器可以具有无菌进入口(例如,该容器可以是静脉内溶液袋或具有通过皮下注射针可刺破的小瓶)。组合物中至少一种活性剂是本文所述的抗体(例如本公开的II型抗-CD20抗体)。标签或包装插页指示组合物用于治疗选择的病症,例如根据本文所述的任何方法。或者或另外,制品或药盒还可包括第二(或第三)容器,其包括药学上可接受的缓冲液,例如用于注射的抑菌水(BWFI)、磷酸盐缓冲盐水、林格氏溶液和右旋糖溶液。它还可以包括从商业和用户的观点所需的其它材料,包括其它缓冲液、稀释剂、过滤器、针和注射器。

[0303] 在一些实施方案中,本文提供了一种药盒,其包括:包括本公开的II型抗-CD20抗体和任选的药学上可接受的载体的容器,以及任选的包括治疗或延缓狼疮进展的说明书的

包装插页，例如，其中说明书指示向个体施用对于II型抗CD20抗体的至少第一次抗体暴露和对于II型抗CD20抗体的第二次抗体暴露，第二次抗体暴露在第一次抗体暴露后约18周至约26周才提供；其中所述第一次抗体暴露包括一个或两个剂量的II型抗CD20抗体，所述第一次抗体暴露包括约1800mg至约2200mg所述II型抗-CD20抗体的总暴露；并且其中所述第二次抗体暴露包括一个或两个剂量的II型抗CD20抗体，所述第二次抗体暴露包括约1800mg至约2200mg所述II型抗-CD20抗体的总暴露。在一些实施方案中，本文提供的药盒药盒：包括本公开的II型抗CD20抗体和任选的药学上可接受的载体的容器，以及任选的包括用于治疗或延缓个体中III类或类IV狼疮性肾炎的说明书的包装插页。在任何上述实施方案的一些实施方案中，II型抗CD20抗体包含：包含SEQ ID NO:1的HVR-H1序列，SEQ ID NO:2的HVR-H2序列和SEQ ID NO:3的HVR-H3序列的重链，以及包含SEQ ID NO:4的HVR-L1序列，SEQ ID NO:5的HVR-L2序列和SEQ ID NO:6的HVR-L3序列的轻链。在任何上述实施方案的一些实施方案中，II型抗CD20抗体是奥比妥珠单抗。

[0304] 制品可以进一步包括：包括第二药物的第二或第三容器，其中抗CD20抗体(例如，本公开的II型抗-CD20抗体)是第一药物，其中所述制品还包括用于用第二药物治疗受试者的包装插页上的说明。示例性的第二药物包括化学治疗剂、免疫抑制剂、抗疟剂、细胞毒剂、整联蛋白拮抗剂、细胞因子拮抗剂、激素以及可与本文所述的II型抗-CD20抗体联合的任何治疗。这些实施方案中的制品可进一步包括指示组合物可用于治疗特定病症的包装插页。

[0305] 应当理解，代替抗CD20抗体或除抗CD20抗体之外，上述任何制品可以包括本发明的免疫缀合物。

[0306] 本说明书被认为足以使本领域技术人员能够实施本发明。除了本文所示和所述之外，本发明的各种修改对于本领域技术人员将从前述描述中变得显而易见，并且落在所附权利要求的范围内。出于所有目的，本文引用的所有出版物、专利和专利申请的全部内容通过引用并入本文。

实施例

[0307] 通过参考以下实施例将更全面地理解本发明。然而，它们不应被解释为限制本发明的范围。应当理解，本文描述的实施例和实施方案仅用于说明目的，并且将对本领域技术人员建议进行其各种修改或改变，并且将包括在本申请的精神和所附权利要求的范围内。

[0308] 实施例1：在患有III/IV类狼疮性肾炎患者中与霉酚酸酯一起施用的奥比妥珠单抗的药理学研究

[0309] 研究设计

[0310] II期研究旨在评估奥比妥珠单抗(即，II型抗CD20抗体)作为霉酚酸酯(MMF)的添加治疗用于患有活性ISN/RPS III/IV类狼疮性肾炎(LN)的安全性和疗效。II期研究是平行组、双盲、随机、安慰剂对照研究，比较奥比妥珠单抗加MMF与安慰剂加MMF对III类和IV类增殖性LN患者的疗效和安全性(图1)。

[0311] 该研究也是前瞻性的多中心研究。在遍布全世界的中心募集被诊断为患有ISN/RPS III或IV类LN的患者(在一些实施方案中根据当前ACR标准诊断为SLE(必须存在至少4个标准，其中一个必须是阳性抗核抗体)。该研究包括用血管紧张肽转化酶(ACEI)抑制剂/血管紧张肽II受体阻断剂，MMF(2.0-2.5g/天给药)和强的松递减量的护理标准疗法。

[0312] 如以下更详细描述的那样，患者年龄18-75岁并具有ISN/RPS 2003 III或IV类增殖性LN(参见Weening，JJ(2004) J.Am.Soc.Nephrol.15:241-250)，如筛选前6个月内进行的肾活检证明，并可伴有V类疾病(例如III/V类或IV/V类)。排除具有III(C)或IV(C)类疾病的患者，因为这些类别的反应可能性较小。

[0313] 研究的纳入标准包括：

[0314] (a)签署知情同意书；

[0315] (b)年龄18-75岁；

[0316] (c)遵守研究方案的能力；

[0317] (d)根据现行ACR标准诊断系统性红斑狼疮(SLE)(必须至少存在4个标准，其中之一必须是阳性抗核抗体)；

[0318] (e)在筛选前6个月内进行的肾活检证明的ISN/RPS 2003 III类或IV型LN的诊断(除了III类或IV类疾病之外，患者也可共同表现出V类疾病)；

[0319] (f)≥10RBCs/HPF或红细胞管型存在证明活性尿沉渣；和

[0320] (g)蛋白尿(尿蛋白与肌酐比>1.0，基于24小时尿液收集)。

[0321] 主要排除标准包括：

[0322] (a)目前活跃且由SLE引起的视网膜炎、控制不良的癫痫发作障碍、急性混乱状态、脊髓炎、中风或中风综合征、小脑共济失调或痴呆症；

[0323] (b)存在快速进行性肾小球肾炎(由肾脏活检中评估的≥50％肾小球中存在月牙体形成或筛选后12周内血清肌酐倍增而定义)；

[0324] (c)由估计的GFR<30mL/min或对透析或肾移植的需要所限定的严重肾损伤；

[0325] (d)在肾活检时具有大于50％的肾小球硬化；

[0326] (e)在随机化前3个月内用环磷酰胺或钙调神经磷酸酶抑制剂进行治疗；和

[0327] (f)具有血小板减少症的不稳定疾病或发展临床显著出血或器官功能障碍的高风险，需要治疗，如血浆置换术或急性血液或血小板输注。

[0328] 患者在筛选之前或筛选期间静脉内(IV)接受初始1000mg甲泼尼龙，并且可以在根据这些患者的常规护理指南对严重的临床活动随机分组之前静脉内接受高达3000mg的甲泼尼龙。患者在奥比妥珠单抗/安慰剂输注当天静脉内接受80mg甲泼尼龙(或甲泼尼龙安慰剂)以减少输注相关事件。口服强的松递减量为0.5mg/kg，并在12周中减少。这种修改的递减量是由认识到10mg/天以上的强的松剂量与重大不良事件(包括增加的心血管事件风险)相关联所启动的(Bichile，T.和Petri，M.(2014) Presse Med.43:e187-195)。用利妥昔单抗的先前经验表明，在没有口服强的松或强的松递减量的情况下，它可以潜在地实现完全和部分肾反应，从而允许使用较低剂量的皮质类固醇(Condon，MB等人，(2013) Ann.Rheum.Dis 72:1280-1286)。

[0329] 患者遵循12个月直到主要终点评估，并且进行在6个月时的中间分析以评估CRR的早期差异。所有患者均有肾活检组织病理学资料的中央阅读，并可根据临床状态和当地实践接受重复肾活检。所有患者均通过高灵敏度流式细胞术(HSFC)进行评估，以评估奥比妥珠单抗消耗循环周围B细胞的能力，并进行中间PD分析，以评估患者是否未按预期完全消耗外周CD19+ B细胞。

[0330] 给药和非调查药用产品

[0331] 研究的给药方案是通过静脉内输注施用，在第1、15、168和182天（试验组）以1000mg的剂量施用奥比妥珠单抗；或在第1、15、168和182天通过IV输注施用奥比妥珠单抗安慰剂（例如，对应于奥比妥珠单抗1000mg剂量的盐水IV）。奥比妥珠单抗/安慰剂在全部复苏设施立即可用的医院或临床环境中施用并在调查员或指定人员的密切监督下进行。在输注结束后，IV线保持在适当位置至少1小时，以便在必要时能够施用IV药物。如果在这段时间内没有发生不良事件，可除去IV线。

[0332] 筛选后，尚未接受MMF的患者以分剂量（2-3次/天）接受1500mg/天的MMF，并且将所有患者剂量按照耐受，在第4周以分剂量（2-3次/天）逐步增高剂量至2.0-2.5g/天的目标剂量。如果需要减少剂量，允许以250-500mg减量来减少。在筛选期间或随机分组时，如果临床指示，则患者可以每天静脉内接受750-1000mg甲泼尼龙长达三天，以治疗潜在的LN临床活动。患者在筛选期间或随机化时接受0.5mg/kg的口服强的松，按照方案从第16天起递减这一强的松剂量，并在第12周将强的松剂量降至7.5mg/天。这些治疗方法在下面进一步描述。

[0333] 伴随治疗和临床实践

[0334] 尚未服用维生素D（400IU/天）和钙补充剂（1200mg/天柠檬酸钙或1500mg/天碳酸钙）的患者随机化开始服用这些补充剂。所有患者均服用血管紧张肽转化酶抑制剂或血管紧张肽受体阻断剂，其逐渐增加剂量至足够的血压控制，如National Kidney Foundation为慢性肾脏疾病所推荐。在研究期间不允许开始影响蛋白尿的其它试剂，包括但不限于非二氢吡啶钙拮抗剂、二氢吡啶钙拮抗剂、醛固酮拮抗剂和直接肾素拮抗剂。

[0335] 霉酚酸酯（MMF）

[0336] 所有患者在筛选期间或不晚于第1天继续或开始使用MMF。初始剂量为口服1500mg/天，以两次或三次分剂量给药，并以分剂量向上增加剂量，在第4周至2.0-2.5g/天。按耐受，MMF可以增加至500mg/周，至2.5g/天的最大剂量。由于副作用，则允许降低。

[0337] 推荐不预先暴露于MMF的新诊断的LN患者，以启动诱导剂（MMF或环磷酰胺），然后重新评估其资格。最初用MMF或环磷酰胺治疗的患者的比例达到CRR，因此对添加的免疫抑制的需求最小（Dall'Era，M.等人，（2011）Arthritis Care Res.63:351-357）。

[0338] 对于进入研究已经接受高于1500mg/天的MMF剂量的那些患者，MMG将按照耐受以分剂量向上增加剂量，在第4周至2.5g/天的目标，患者目前的MMF剂量以2或3次分剂量给药，并且按耐受增加至500mg/周。

[0339] 皮质类固醇给药

[0340] 所有患者接受IV和口服皮质类固醇的组合，作为其对LN的初始治疗的一部分。为两个目的实施甲泼尼龙（例如**Solu-Medrol®**）：作为用于III类或IV类LN的患者的常规护理一部分，并且还在奥比妥珠单抗/安慰剂输注日减少输注相关反应（IRR）。根据调查员的判断和当地实践，给予高达三剂量IV甲泼尼龙1000mg。在筛选之前或筛选间隔期间，可能已经开始了高达三次1000mg输注。

[0341] 在第1、15、168和186天，在研究药物输注之前30-60分钟患者接受80mg IV甲泼尼龙或安慰剂以预防IRR。另外，口服强的松可以在筛选间隔之前或期间开始，并且递减量在第2天开始。从第2天到第16天，给予0.50mg/kg/天口服强的松（最大剂量60mg），除了在IV甲泼尼龙/安慰剂输注那天，并将持续至第16天。从第16天起，开始强的松递减量。

[0342] 所有患者在第16天开始计划的皮质类固醇递减量。患者在12周内分次地减少强的

松剂量，直到第12周剂量为7.5mg/天。在14周递减后，患者继续服用强的松7.5mg/天或更少。在患者疾病的临床活性太强以至于进行其强的松递减量的第一步的患者中，如通过活性尿沉渣，血清肌酐升高或中度至重度肾外症状证明，这些患者可能继续接受初始强的松剂量达额外的28天。

[0343] 为了保持肾脏复发治疗的一致性，如果由调查员判断临床适宜，并且如果患者满足肾脏复发的标准，则允许用较高剂量的皮质类固醇进行再治疗。患者可以用强的松(高达0.5mg/kg；不超过60mg/天)治疗2周。初始皮质类固醇增加后，强的松递减以在6周达到10mg/天。如果临床上有必要，允许患者接受皮质类固醇用于紧急疾病(创伤、严重哮喘)或手术；如果可能，皮质类固醇使用限制在总共≤7天。在患者剂量递减至10mg/天后，调查员然后可以将强的松剂量增加≤2.5mg/天，以治疗肾上腺功能不全或皮质类固醇戒断症状。

[0344] 如果由调查员判断临床适宜，经历严重的肾外SLE复发的患者可以接受另外的口服皮质类固醇的治疗。这些患者可以根据疾病和器官系统受累的严重程度，用强的松(高达1.0mg/kg)再治疗达2周，并且剂量递减至7.5mg/天。如果由调查员判断临床适宜，经过轻度或中度肾外复发的患者可将强的松剂量每天临时增加达20mg，并且在4周内递减该剂量。如果胃肠道受累暂时阻止用口服皮质类固醇治疗，则允许等效剂量的IV皮质类固醇。

[0345] 抗疟药

[0346] 在研究进入期间服用抗疟疾药物的患者在整个研究中保持恒定的剂量。以前没有使用抗疟疾药物的患者可被招募到研究中，但不应启动抗疟药，除非经历对皮质类固醇无反应的疾病复发。表4列出了预期在研究过程中使用的抗疟药物和剂量范围。

[0347] 表4.抗疟药物

[0348]

抗疟药	剂量范围(口服)
羟氯喹	每天 200-400 mg
氯喹	每天或每隔一天 500 mg
奎纳克林	每天 100 mg

[0349]

[0350] 抗高血压治疗

[0351] 所有目前未服用ACE抑制剂或血管紧张肽受体阻断剂的患者均应在筛选时开始服用。患者在随机分组前至少10天，或者服用ACE抑制剂或者服用血管紧张肽受体阻断剂。不允许用两种药剂联合治疗。

[0352] 在筛选期间，尽一切努力充分控制患者的血压。ACE抑制剂或血管紧张肽受体阻断剂的剂量可以向上渐增到当前包装内的最大推荐剂量，以实现足够的血压控制，如Eighth Report of the Joint National Committee on the Prevention,Detection, Evaluation,and Treatment of High Blood Pressure(James,P.A.等人，(2014)JAMA 311:507-520)推荐。如果没有达到足够的血压控制，患者可以服用额外的抗高血压剂，但不能服用影响蛋白尿的药剂(例如，非二氢吡啶钙通道阻断剂、醛固酮拮抗剂、直接肾素拮抗剂)。在研究期间不会启动特异性靶向肾素-血管紧张肽系统的其他药剂。特异性ACE抑制剂和血管紧张肽受体阻断剂的建议剂量范围列于表5。如果患者对ACE抑制剂和血管紧张肽受体阻断剂不耐受，则它们可以使用直接肾素抑制剂或醛固酮拮抗剂，但不能组合使用。

[0353] 表5.ACE抑制剂和血管紧张肽受体阻断剂的建议剂量范围

AACE 抑制剂或血管紧张肽受体阻断剂	剂量范围(口服)，mg/天
ACE 抑制剂	
贝那普利	10-80
雷米普利	2.5-10
赖诺普利	10-80
依那普利	10-40
喹那普利	10-80
卡托普利	75-450
培哚普利	4-16
群多普利	1-8
莫昔普利	7.5-30
血管紧张肽受体阻断剂	
依普罗沙坦	400-600
缬沙坦	80-320
奥美沙坦	20-40
坎地沙坦	8-32
替米沙坦	20-80
氯沙坦	25-100
厄贝沙坦	75-300

[0356] 研究目标和结果措施

[0357] 这种概念验证研究的主要目标是在施用奥比妥珠单抗52周时测量完全肾反应(CRR)。将奥比妥珠单抗加MMF在第52周达到CRR的能力与安慰剂加MMF进行比较，并通过肾功能、尿沉渣和蛋白尿的改善进行评估。次要目标包括对该患者群体中奥比妥珠单抗的安全性的评估，在第52周时，奥比妥珠单抗诱导总体反应(CRR+ PRR)的能力，奥比妥珠单抗在52周的过程中改善反应时间(CRR+ PRR)的能力，以及奥比妥珠单抗改善LN疾病活动的生物标志物的能力(例如，降低的抗dsDNA抗体水平，增加的C3和C4水平；参见Tew，GW等人(2010) Lupus 19:146-157)。

[0358] 主要疗效结果测量是在52周评估达到CRR的受试者的比例。在本研究中，CRR通过实现以下所有来定义的：

[0359] (a)血清肌酐正常化，如下所证明：

[0360] (i)如果基线(第1天)不在中心实验室值的正常范围内，血清肌酐小于或等于中心

实验室值的正常(ULN)范围的上限范围;和

[0361] (ii)如果基线(第1天)血清肌酐在中心实验室值的正常范围内,血清肌酐比基线高≤15%,且小于或等于中心实验室值的ULN范围;

[0362] (b)非活性尿沉渣(由<10个红细胞/高功率场(HPF)证明,并且不存在红细胞管型);和

[0363] (c)尿蛋白与肌酐比<0.5。

[0364] 在第52周之前转换到救援药物的任何患者被认为是无反应者。使用Cochrane-Mantel-Haenzel(CMH)测试比较各治疗组达到CRR的患者比例,用种族(非洲裔加勒比/非裔美国人及其他)和区域(美国与非美国)作为分层因素。如果测试结果在α<0.1级(单侧)有利于奥比妥珠单抗组,则总结与奥比妥珠单抗组相关的更好的肾反应的转变。

[0365] 次要疗效结果测量如下:

[0366] (a)在第52周达到总反应的患者的比例分析(CRR+PRR);

[0367] (b)52周过程内总反应的时间(CRR+PRR);

[0368] (c)从基线和LN疾病活动生物标志物的平均值和中值评估的减少或增加的百分比(例如抗dsDNA抗体水平的降低,增加的C3和C4水平);

[0369] (d)如通过达到以下所有来限定的在第52周实现PRR的患者比例:

[0370] (i)血清肌酐比基线水平高≤15%

[0371] (ii)红细胞/HPF比基线高50%,且无红细胞管型;

[0372] (iii)尿蛋白与肌酸酐比率提高50%,满足以下条件之一:

[0373] (A)如果基线尿蛋白与肌酐比≤3.0,则尿蛋白与肌酸比<1.0;

[0374] (B)如果基线蛋白与肌酐比>3.0,则尿蛋白与肌酐比<3.0;

[0375] (e)在第24周达到CRR的患者的比例;

[0376] (f)在52周过程内达到CRR的时间;

[0377] (g)在第52周采用主要疗效测量定义并去除尿沉渣分析标准获得改良CRR(mCRR1)的患者的比例

[0378] mCRR1指达到血清肌酐正常化,如下所证明:

[0379] (i)血清肌酐小于或等于中心实验室值的ULN范围;

[0380] (ii)如果基线(第1天)血清肌酐在中心实验室值的正常范围内,血清肌酐比基线高≤15%,且小于或等于中心实验室值的ULN范围;

[0381] (iii)尿蛋白与肌酐比<0.5;

[0382] (h)如达到以下所限定的第52周实现第二CRR(mCRR2)的患者比例:

[0383] (i)血清肌酐正常化,如下所证明:

[0384] (A)血清肌酐≤中心实验室值的ULN范围;

[0385] (B)如果基线(第1天)血清肌酐高于中心实验室值的正常范围,则血清肌酐≤基线水平15%或者如果基线(第1天)血肌酐值在中心实验室值的正常范围内,S≤中心实验室值的ULN范围;

[0386] (ii)非活性尿沉渣(由<10RBCs/HPF证明,不存在红细胞管型)。

[0387] (iii)尿蛋白与肌酐比<0.5。

[0388] 药效学(PD)的目标是比较奥比妥珠单抗对安慰剂治疗后在外周血中CD19+ B细胞

的变化。在筛选和第15、28、84、168、364和728天测量循环CD19+ B细胞的水平。

[0389] 药代动力学(PK)目标是表征奥利珠单抗在LN群体中的药代动力学，并评估奥比妥珠单抗与伴随药物(包括霉酚酸酯(MMF))之间的潜在PK相互作用。使用非线性混合效应建模(使用软件NONMEM)分析奥比妥珠单抗的剂量浓度-时间数据。PK谱数据用于进一步开发PK模型，包括主要协变量(例如性别、种族/种族、体重、基线时的生物化学和血液学参数、潜在疾病程度)对主要参数(例如，清除率)的影响。单独测量暴露量的推导，如浓度-时间曲线下面积(AUC)和观察到的最大浓度(Cmax)取决于所使用的最终PK模型。总结血清奥比妥珠单抗(平均值、最小值、最大值、SD和几何平均值)并报告。

[0390] 研究的探索性目标包括评估探索性生物标志物的剂量前水平(包括但不限于B细胞亚群和血清、血液和尿液中的蛋白质和/或mRNA水平)以及与结果的潜在关联，评估用奥比妥珠单抗与安慰剂给药的患者中随时间推移的探索性生物标志物的变化(包括但不限于B细胞亚群和血清、血液和尿液中的蛋白质和/或mRNA水平)，评估肾脏外复发的发生情况，评估治疗对患者和医师报告的结果的影响，以及评估肾活检组织病理学(例如，在筛选和/或随后的活组织检查中CD19+ B细胞的存在)。探索性结果测量包括：

[0391] (a)筛选和第15、28、84、168、364和728天的循环B细胞亚群的水平；

[0392] (b)筛选和第1、15、28、84、168、252、364、532和728天的探索性生物标志物(包括但不限于B细胞亚群和血清、血液和尿液中的蛋白质和/或mRNA水平)的水平；

[0393] (c)经历系统性红斑狼疮病活动指数(SLEDAI)-2K复发的患者比例；

[0394] (d)经历52周和104周肾复发的患者比例；

[0395] (e)在额外时间点(包括第12周和第36周)达到CRR、mCRR1、mCRR2的患者比例；

[0396] (f)医师的全局评估(在筛选，基线访视以及在学习过程中的几个时间点捕获的视觉模拟量表)；和

[0397] (g)肾活检评估。

[0398] 实验室、生物标志物和其他生物样品

[0399] 在研究期间记录以下实验室评估：

[0400] (a)血液学：血红蛋白、血细胞比容、RBC、平均红细胞体积、平均红细胞血红蛋白、WBC(绝对和差异)和定量血小板计数；

[0401] (b)血液化学：AST/SGOT、ALT/SGPT、碱性磷酸酶、淀粉酶、脂肪酶、总蛋白、白蛋白、胆固醇、总胆红素、尿素、尿酸、肌酐、随机葡萄糖、钾、钠、氯、钙、磷酸盐、乳酸脱氢酶、CPK和甘油三酯；

[0402] (c)尿液分析：血液、硝酸盐、蛋白质、葡萄糖和尿液显微镜的浸渍片；

[0403] (d)在随机分组和第3、6、9和12月进行24小时尿液收集(分析总蛋白、总肌酐和肌酐清除率)；

[0404] (e)流式细胞术：B细胞(包括CD19、CD27、CD38和IgD)，T细胞(CD3、CD4、CD8)和NK细胞(CD16、CD56)；

[0405] (f)自身抗体谱：抗核抗体(ANA)、抗dsDNA、抗Sm、抗RNP、抗Ro、抗La和抗C1q；

[0406] (g)抗dsDNA抗体：通过ELISA在所有访问中作为SLEDAI-2K评估的一部分测量；

[0407] (h)定量免疫球蛋白：总Ig水平，包括IgG、IgM和IgA同种型；

[0408] (i)补体：C3、C4和CH50；

[0409] (j)抗体滴度:对常见抗原(风疹、破伤风、流感、肺炎链球菌)的抗体滴度;和

[0410] (k)妊娠试验:在筛选时和每次研究药物输注之前进行尿妊娠试验。除非测试为阴性,否则不进行输注。在所有其他时间点,尿妊娠试验是根据月经史和怀孕风险进行的。

[0411] 发送以下样品用于分析:用于B细胞和狼疮相关生物标志物(包括但不限于CD19+B细胞和与B细胞活性相关的mRNA)的来自血液和尿液的细胞,用于B-细胞和狼疮相关的生物标志物(包括但不限于B细胞激活因子或BAFF)的血清和尿液和用于免疫组织病理学评估的肾活检片。

[0412] 输注

[0413] 在每次输注研究药物或安慰剂之前,在输注期开始前,患者通过口服接受对乙酰氨基酚(650-1000mg)和苯海拉明(50mg;或相当剂量的相似药剂)的预防性治疗,给予30-60分钟。在开始奥比妥珠单抗/安慰剂输注前30-60分钟,接受奥比妥珠单抗的患者接受80mg甲泼尼龙IV并且接受安慰剂的患者接受安慰剂-甲泼尼龙IV。如果患者经历被调查员认为具有临床重要性的轻度输注相关反应(IRR),则应将输注速率降低至初始输注速率的一半(符合非霍奇金淋巴瘤方案输注速率和时间表)。反应结束后,输注应以降低的速度保持额外的30分钟。如果降低的速率是可忍受的,则输注速率可以增加到输注计划表的下一个最接近的速率。经历严重IRR的患者应立即中止输注,并应进行积极的对症治疗。在所有症状消失前不应重新启动输注。在重新启动输注时,速率应该是使反应下降的速度的一半。下列表6提供了施用奥比妥珠单抗输注的说明。

[0414] 表6.奥比妥珠单抗输注的施用。

[0415]

第一次输注(第1天)	后续输注
[0100] 以50mg/hr的初始速率开始输注。 如果没有发生输注反应,则以每30分钟50mg/hr递增将输注速度增加到最大400mg/hr。 如果发生输注反应,则停止或减慢输注。根据制度方案管理输注反应药物和支持治疗。 如果反应已经结束,以速率(在超敏反应或与输注有关的反应发生时使用的速率)50%的降低恢复输注。	如果患者在先前输注期间经历了输注反应,则以与第一次输注(50mg/hr)相同的速率开始,并遵循如上所述的那些指示。 如果患者对以前的输注耐受良好,开始以100mg/hr的速度输注。 如果没有发生输注反应,则以每30分钟100mg/hr的增量将输注速度增加到最大400mg/hr。 如果发生输注反应,则停止或减慢输注。根据制度方案管理输注反应药物和支持治疗。如果反应已经结束,以速率(在超敏反应或与输注有关的反应发生时使用的速率)50%的降低恢复输注。

[0416] 对作为研究条目的一部分获得的所有肾活检和报告进行显微拍照并发送到在线中央阅读门户，用于监督由局部肾组织病理学家进行的组织病理学评估。专家组对这些活检进行评估，并对最终解释作出裁决。在患者进行筛选时，尽一切努力完成此过程，但不是完成筛选活动的强制性措施。在筛选期间或在研究期间获得的所有新的活组织检查样品以能够对B细胞的存在进行肾小管间质的免疫组织化学染色的方式进行处理。该研究鼓励但并不要求对没有达到CRR的患者进行重复肾活检，并寻求丰富用于执行重复肾活检的研究中心。

[0417] 实施例2：在类风湿关节炎和系统性红斑狼疮患者样品中通过Fcγ受体依赖和非依赖的效应机制诱导B细胞细胞毒性方面，奥比妥珠单抗优于利妥昔单抗

[0418] 用标准剂量的利妥昔单抗(RTX)治疗的类风湿关节炎(RA)和系统性红斑狼疮(SLE)患者的比例显示出低效的B细胞消耗和差的临床反应，其可以通过递送更高的剂量来增强，表明标准剂量RTX是这些患者的次优疗法。为了调查是否可以用其他抗CD20 mAb实现更好的反应，在测量在SLE和RA样品中的B细胞毒性的一系列体外测定中，对RTX与奥比妥珠单抗(OBZ)(新一代，糖改造的II型抗CD20mAb)进行比较。已经发现OBZ在体外全血测定中诱导B细胞毒性比RTX至少有效2倍。剖析这个差异，发现RTX比OBZ引发更强的补体依赖性细胞毒性(CDC)。相比之下，OBZ在诱导Fcγ受体(FcγR)介导的效应机制(包括NK细胞和嗜中性粒细胞的激活)方面更有效。OBZ在诱导直接细胞死亡也更有效。这对于作为整体的所有CD19+ B细胞和初始(IgD+ CD27-)都是正确的；并且特异性地转换(IgD-CD27+)记忆B细胞，其较高的频率与RTX后的差的临床反应相关。

[0419] 材料和方法

[0420] 患者

[0421] 本研究的所有参与者根据当地研究伦理委员会批准的赫尔辛基宣言提供同意。所有RA患者均符合美国风湿病学会(ACR)/欧洲抗风湿病联盟分类标准(Aletaha D.等人，2010 Ann Rheum Dis.2010;69(9):1680-8)，所有SLE患者均符合ACR分类标准(Petri M.等人，Arthritis Rheum.2002;64(8):2677-86)。

[0422] 抗体和试剂

[0423] 研究中使用的抗CD20 mAb包括RTX、OBZ和非糖改造的野生型糖基化OBZ(OBZ_{G1y})，并且在一些实验中，具有不参与任何Fc相关效应子功能的突变Fc部分(P329G LALA)的OBZ(Herter S.等人，Cancer Research.2015;75(15Supplement):2460)，OBZ-PG LALA。瑞士苏黎世的罗氏创新中心产生除RTX以外的所有抗CD20 mAb，RTX是一个由University Hospital，U.K.药厂的馈赠。FcγRII拮抗剂AT10(Greenman J.等人，Mol Immunol.1991;28(11):1243-54)，是内部生产的。

[0424] 流式细胞术和B细胞分离

[0425] 从Becton Dickinson biosciences或Biolegend，UK购买荧光缀合的mAb：CD3(藻红蛋白[PE]-Cy 7)，CD15(异硫氰酸荧光素，FITC)：CD16(Allophycoyanin，APC)，CD19(Alexa Fluor 700)，CD45(PE)，CD56(PE)，CD107a(Brilliant Violet 421)，CD11b(PE)，CD62L(APC)，碘化丙锭和膜联蛋白V(FITC)。使用Becton Dickinson LSR Fortessa细胞分析仪进行流式细胞术。基于前向和侧向散射特征鉴定淋巴细胞。B细胞被鉴定为CD19+或CD20+，T细胞被鉴定为CD3+以及NK细胞鉴定为CD3-56+。基于前向和侧向散射特征和CD15阳

性鉴定嗜中性粒细胞。将用mAb温育的样品中的CD11b和CD62L的平均荧光强度(MFI)与没有抗体温育的样品中的平均荧光强度(MFI)进行比较。

[0426] 在所有实验中,通过Ficoll-Hypaque密度梯度从全血样品中分离外周血单核细胞(PBMC),并使用EasySep™ Human B Cell Enrichment Kit(Cambridge,U.K.)从PBMC分离B细胞。

[0427] 全血B细胞消耗测定

[0428] 简言之,在37℃和5%CO_2下,用或不用mAb以1μg/mL温育300μl用肝素抗凝的新鲜全血24小时。然后如先前所述(Reddy V.等人,Arthritis&rheumatology.2005;67(8):2046-55),将样品用抗CD3、抗CD19和抗-CD45染色,然后裂解红细胞并在流式细胞仪上分析。B细胞消耗%由在治疗后剩余的B细胞与T细胞比例计算并如前所述定义为细胞毒性指数(CTI)(Mossner E.等人,Blood.2010;115(22):4393-402和Reddy V.等人,Arthritis&rheumatology,2015;67(8):2046-55)。

[0429] 表面荧光猝灭试验

[0430] 如前所述(Beers SA等人,Blood.2008;112(10):4170-7)和Reddy V.等人,Arthritis&rheumatology.215;67(8):2046-55)进行表面荧光猝灭测定以评估B细胞的mAb内化。将分离的B细胞用浓度为5μg/mL的Alexa-488缀合的mAb温育6小时,然后通过流式细胞术分析。

[0431] 补体依赖性细胞毒性测定

[0432] 如前所述(Cragg M.S.等人,Blood.2004;103(7):2738-43)进行CDC测定。在37℃和5%CO_2下,将分离的B细胞用浓度为10μg/mL的mAb温育30分钟。样品用荧光缀合的抗CD19抗体、膜联蛋白V(Av)和碘化丙锭(PI)染色,并通过流式细胞术评估CD19+ Av+ PI+细胞的频率。使用新鲜收集的正常健康人血清作为补体来源。为了定义与补体相关的活性,部分血清在56℃下热灭活(HIS)30分钟。通过或者用正常健康血清或者用HIS温育的样品中CD19+ Av+ PI+细胞的相对频率来评估mAb激活补体并裂解靶细胞的能力。

[0433] 直接细胞死亡

[0434] 在37℃和5%CO_2下,将分离的B细胞在补充有10%热灭活的胎牛血清的RPMI中,在具有或不具有10μg/mL浓度的mAb下温育6小时。具有mAb的样品中CD19+ Av+细胞的频率与没有mAb的样品相比,表示mAb诱导直接细胞死亡的能力。

[0435] NK细胞脱粒测定

[0436] 使用来自全血B细胞消耗测定的样品,通过测量CD107a或LAMP-1(溶酶体相关膜蛋白1)的表达来评估NK细胞脱粒,LAMP-1在NK细胞活化后上调,并与NK细胞介导的ADCC相关联(Alter G.等人,J Immunol Methods.2004;294(1-2):15-22和Aktas E.等人,Cell Immunol.2009;254(2):149-54)。因此,将具有mAb的样品中CD3-56+ 107a+ NK细胞的频率与没有mAb温育的样品中的频率进行比较。NK细胞的活化与增加的金属蛋白酶活性相关,金属蛋白酶在NK细胞活化时切割CD16,降低其表达(Romee R.等人,Blood.313;121(18):3599-608)。因此,CD16损失的程度也被用作NK细胞活化的间接测量(Grzywacz B.等人,Leukemia.2007;21(2):356-9;作者回复9,和Bowles J.A.等人,Blood.2006;108(8):2648-54)。

[0437] 嗜中性粒细胞活化测定

[0438] 在全血测定中通过流式细胞术测量CD15+嗜中性粒细胞上的CD11b增加或CD62L降低来评估嗜中性粒细胞的活化(Golay J.等人,Blood.313;122(20):3482-91,以及Wittmann S.等人,Cytometry A.2004;57(1):53-62)。通过比较用或不用mAb温育的样品中CD15+嗜中性粒细胞上CD11b和CD62L的平均荧光强度(MFI)来评估mAb诱导嗜中性粒细胞活化的能力。

[0439] 统计分析

[0440] 使用Graph Pad Prism Software 5.0版分析数据。使用Mann Whitney检验或Wilcoxon匹配对签名秩检验来适当比较各组之间。Spearman相关系数用于分析相关性。

[0441] 结果

[0442] 在诱导B细胞毒性方面II型mAb比I型更有效

[0443] 为了评估I型和II型mAb对RA和SLE样品中B细胞细胞毒性的影响,如先前所述进行全血B细胞消耗测定(Reddy V.等人,Arthritis&rheumatology.2005;67(8):2046-55)。在所有测试的样品中,OBZ在从具有RA(n=31)和SLE(n=34)的患者消耗B细胞的效率比RTX高>2倍,两种非糖基化的OBZ_{Gly}和OBZ都比RTX更有效(图2A)。在RA和SLE两者中,OBZ的中位数CTI显著大于OBZGly和RTX的CTI,在RA和SLE两者中,OBZ_{Gly}的CTI显著高于RTX的CTI。在RA中,RTX、OBZGly和OBZ的中位数(四分位数范围)CTI分别为29(13-50)、60(47-70)和67(60-77),SLE中分别为19(11-39)、40(31-53)和59(52-70)。因此,在RA和SLE两者中,存在mAb诱导的B细胞消耗的层级:RTX<OBZ_{Gly}<OBZ。还注意到B细胞消耗的显著样品间变异性,特别是RTX。OBZ_{Gly}(具有类似于RTX的非糖基化Fc)的优越效率表明,其II型性质是全血测定中B细胞消耗效率的两种类型的mAb之间的差异;而与OBZ_{Gly}相比,OBZ的增加的效率归因于Fc部分的无岩藻糖基化。

[0444] B细胞比OBZ更快地内化RTX

[0445] 接下来,调查了II型mAb在B细胞消耗中的优越效率是否与其II型性质一致,并且因此评估来自具有RA和SLE的患者的B细胞是否比OBZ更大程度地内化RTX。发现RTX在温育6小时后比OBZ更广泛地内化,表面可及性RTX与OBZ的中值(范围)百分比在RA(n=5)中分别为:55(51-57)对83(81-84),以及在SLE(n=8)中分别为:60(49-77)对76(70-80)(图2B)。为了评估FcγRIIb在该内化中的作用,在存在FcγRII阻断单克隆抗体AT10的情况下进行实验,其部分抑制RTX的内化并且较小程度地抑制OBZ的内化(图2B),类似于先前使用OBZ的非糖基修饰的II型抗体变体的观察结果(Reddy V.等人,Arthritis&rheumatology.2005;67(8):2046-55)。

[0446] 在诱导补体依赖性细胞毒性方面RTX比OBZ更有效

[0447] 还调查了这些mAb引发CDC的能力。已经发现,与热灭活血清(HIS)相比,在正常健康血清(NHS)存在下,用RTX温育的样品中裂解的B细胞(CD19+ Av+ PI+)的频率显著更大,具有中值(范围)差为10.9%(8.1-21),而对于OBZ的差为4.8%(0.9-6.5)(图2C)。在用NHS对HIS温育的样品中的裂解细胞的平均值±SD倍数增加对于RTX和OBZ分别为1.9±0.5和1.2±0.2(图2D)。因此,数据表明在引发CDC方面RTX优于OBZ。

[0448] 在激活NK细胞方面OBZ比RTX更有效

[0449] 这些CDC结果与mAb的I型和II型性质一致,但与全血测定中II型mAb的优异效率不同。接下来,调查了mAb引发FcγR介导的效应子机制的能力;首先评估全血B细胞消耗测定

中的NK活化。如图3E所示的门控，相对于CD16的表达，允许评估NK脱粒（CD107a增加）。在CD56+ CD16-级分中观察到最高比例的CD107a+ NK（CD3-CD56+）细胞（图3A-3G），表明脱粒NK细胞如先前报道的那样下调CD16（Grzywacz B.等人，Leukemia.2007；21（2）：356-9；作者回复9）。

[0450] 建立了这些参数后，进行了比较RTX和OBZ的等效测定。在不存在mAb的情况下温育24小时后，RA（n＝18）和SLE（n＝23）患者之间的NK细胞、CD107a+ NK细胞、CD16+ NK细胞或B细胞的频率没有显著差异（图4A）。然而，在OBZ温育的样品中，CD3-CD56+ CD107a+激活的NK细胞的中值（范围）频率比RTX显著更高，分别是5.1％（1.9-22）对2.8％（0.3-14）和5.5％（0.6-12）对4.3％（1.2-8.9）），但在RA和SLE中CD16+ NK细胞的中值（范围）频率显著降低，分别是69（36-94）对89（83-97）和66（42-91）对84（61-95）（图4B）。此外，在SLE中，与用RTX温育相比，用OBZ温育的样品中CD3-CD56+ CD107a+ NK细胞的频率具有显著更高的倍数增加（图4B）。此外，发现如通过CD107a的增益或CD16的损失或通过CD3-CD56+ CD107a+ NK细胞的频率倍数增加评估的，NK细胞活化在RA中比在SLE中更大（图4B）。如通过RTX和OBZ的CD3-CD56+ CD107a+ NK细胞的频率评估的，NK细胞活化在不具有mAb温育的样品中显著相关，在RA中分别是$r^2=0.89$，$p<0.05$；$r^2=0.78$，$p<0.05$（图4C），以及在SLE中分别是$r^2=0.52$，$p<0.05$；$r^2=0.36$，$p<0.05$（图4D）。然而，与SLE相比，RA中的相关性更强。总之，这些数据表明SLE中NK细胞活化中的疾病相关缺陷可能导致全血消耗测定中注意到的低效B细胞消耗（图2A），并且NK细胞的基线激活状态可能影响RA和SLE中对mAb激活的反应（图4B和4C）。

[0451] 接下来调查了RTX和OBZ的NK细胞的差异激活是由于I型和II型特征和/或由于使用具有野生型糖基化（OBZ_{G1y}）的OBZ或完全缺乏FcγR接合的Fc改造的作用（OBZ-PG LALA），因此在诱导ADCC和CDC时的效率较低（Hessell A.J.等人，Nature.2007；449（7168）：101-4）。在RA（n＝6）和SLE（n＝12）两者中与用OBZ-PG LALA温育的样品相比，在没有mAb温育的样品中，CD3-CD56+ CD107a+或CD3-CD56+ CD16+ NK细胞的频率没有发现显著差异，表明FcγR-接合是必要的。在这些样品中，在RA（n＝18）和SLE（n＝23）（图5A）两者中，OBZ在全血测定中消耗B细胞比OBZ_{G1y}和RTX比更有效，但是在CD3-CD56+ CD107a+ NK细胞的频率和倍数增加方面观察到增加的分级如下：无mAbs＝OBZ-PG LALA<RTX<OBZ（图5B和5C）。与其他样品相比，用OBZ温育的样品中，CD3-CD56+ CD16+ NK细胞的频率显著降低（图5D）。与RA而不是SLE中RTX相比，用OBZ_{G1y}温育的样品中CD3-CD56+ CD16+ NK细胞的频率也较低（图5D）。

[0452] 因此，与SLE相比，RA中mAb上调在CD3-CD56+ NK细胞上的CD107a表达的能力较高，使得用RTX、OBZ-PG LALA、OBZ_{G1y}和OBZ温育的样品与无mAb温育的样品相比时，平均倍数差异在RA中分别是1.2、1.5、1.9和3.1，在SLE中分别是1.5、0.8、1.4和1.8（图5C）。尽管RA和SLE中由mAb实现的B细胞消耗的模式（图5A）与mAb的NK细胞活化模式相似（图5B和5D），但mAb实现的B细胞消耗％和单个样品中的CD3-CD56+ CD107a+ NK细胞的频率之间没有直接的相关性（数据未示出）。

[0453] 在激活嗜中性粒细胞方面OBZ比RTX更有效

[0454] 除了NK细胞之外，也提出嗜中性粒细胞为mAb效应子细胞（Golay J.等人，Blood.313；122（20）：3482-91）。因此，接下来，如前所述，通过测量CD11b和CD62L的表达来评估mAb诱导嗜中性粒细胞活化的能力（Wittmann S.等人，Cytometry A.2004；57（1）：53-62），并显示于图9中。CD11b构成β整联蛋白（Mac-1）复合物的一部分，并且该复合物的几种

遗传变体已经与狼疮相关的吞噬缺陷相关(Bologna L.等人,J Immunol.2011;186(6):3762-9)。在嗜中性粒细胞激活后,CD11b的表面表达上调,而粘附分子CD62L的表达下调(Golay J.等人,Blood.313;122(20):3482-91,以及Wittmann S.等人,Cytometry A.2004;57(1):53-62)。发现与无mAb温育的样品相比,在RA(n=10)和SLE(n=22)中,用mAb温育的样品中的CD11b的MFI显著更高(图6A)。在RA和SLE两者中,在不存在mAb时温育的样品中的CD11b的MFI与在RTX温育的样品之间的CD11b的MFI之间存在显著的相关性(分别为r^2=0.81,0.82),而在SLE不是RA中注意到OBZ的显著相关性(为r^2=0.81)(图6B)。还注意到mAb上调CD11b的能力的分级,使得CD11b的MFI在用RTX<OBZ_{Gly}<OBZ温育的样品中更低,如在NK细胞激活的情况下。此外,CD62L的MFI在用RTX>OBZ_{Gly}>OBZ温育的样品中更大(图6C)。在RA和SLE中,在不存在mAb时温育的样品中,注意到CD62L的MFI与RTX(分别为r^2=0.93,0.91)和OBZ(分别为r^2=0.64,0.71)温育的样品中的显著相关性(图6D)。因此,mAb在其激活嗜中性粒细胞的能力方面的分级是OBZ>OBZ_{Gly}>RTX。总之,这些数据表明,在RA和SLE样品的全血测定中,II型mAb在激活嗜中性粒细胞方面优于RTX。与不存在mAb温育的样品相比,OBZ-PG LALA没有引起RA(n=7)和SLE(n=12)两者中的各标记物的显著变化。

[0455] 在诱导直接细胞死亡方面OBZ比RTX更有效

[0456] 接下来,使用如图10所示的膜联蛋白V测定来评估直接细胞死亡(DCD)。对于作为整体的CD19+细胞以及B细胞亚群;IgD+ CD27-初始细胞和IgD-CD27+转换记忆细胞,OBZ诱导直接细胞死亡的能力大于RTX,图7A(RA,n=5,SLE,n=4)。即使在没有mAb温育的样品中,膜联蛋白V+细胞的比例也高于DN细胞>IgD+ CD27+未转换记忆细胞>IgD-CD27+转换记忆细胞>IgD+ CD27-初始细胞。尽管如此,OBZ在诱导DCD方面优于RTX。

[0457] B细胞亚群:CD20、Fcγ RIIb的表达和mAb的内化

[0458] 接下来调查B细胞亚群在CD20、Fcγ RIIb的表达和/或其内化mAb的能力之间的差异是否提供了对mAb诱导的DCD的差异敏感性的解释。B细胞亚群显示不同的内化mAb的能力,使得IgD-CD27+转换记忆细胞内化mAbs比其他B细胞亚群少;并且IgD+ CD27+未转换记忆细胞内化mAb比其他B细胞亚群达更大程度。用AT10拮抗Fcγ RIIb的作用在两种情况下均显著降低内化。当与初始和IgD-CD27+转换记忆细胞相比时,IgD+ CD27+未转换记忆细胞具有显著更高的CD20和Fcγ RIIb表达,并且显示出显著更大的内化mAb的能力,而初始和IgD-CD27+转换记忆细胞具有显著较低的CD20表达和Fcγ RIIb并显示出显著较低的内化水平。DN细胞具有CD20和Fcγ RIIb表达的显著变化水平,但内化的RTX比IgD-CD27+转换记忆细胞达到显著更大程度。来自RA和SLE样品的B细胞一致地显示低水平的OBZ内化。将这些数据结合在一起,B细胞亚群对mAb诱导的DCD的易感性和内化mAb或表达CD20或Fcγ RIIb的能力之间没有明确的关系。

[0459] 这里,显示与RTX相比,具有糖修饰的Fc的II型抗CD20mAb——奥比妥珠单抗在RA和SLE患者的全血样品缺失B细胞方面表现出至少2倍的效力。OBZ的这种增加的活性主要通过Fcγ受体(Fcγ R)介导的效应机制和DCD而实现。相比之下,RTX招募对CDC更有效的补体,但是在诱发ADCC和DCD时迅速内化并且效率显著降低。随后的分析揭示,CD20靶分子的表达在IgD-CD27+转换记忆和DN细胞上较少;也许是由于它们对RTX去除的相对抗性。

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<120>治疗狼疮性肾炎的组合物和方法

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<220>
<223> 合成构建体

[0002]

<400> 7
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Tyr Ser
20 25 30
Trp Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 8
<211> 115
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 8
Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30
Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Val Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala Gln Asn
85 90 95
Leu Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr Val

115

<210> 9
<211> 448
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 9

Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ser
1				5					10					15	
Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Tyr	Ser
			20					25					30		
Trp	Ile	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met
		35					40					45			
Gly	Arg	Ile	Phe	Pro	Gly	Asp	Gly	Asp	Thr	Asp	Tyr	Asn	Gly	Lys	Phe
	50					55					60				
Lys	Gly	Arg	Val	Thr	Ile	Thr	Ala	Asp	Lys	Ser	Thr	Ser	Thr	Ala	Tyr
65					70					75					80
Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	
Ala	Arg	Asn	Val	Phe	Asp	Gly	Tyr	Trp	Leu	Val	Tyr	Trp	Gly	Gln	Gly
			100					105					110		
Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe
		115					120					125			
Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu
	130					135					140				
Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp
145					150					155					160
Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu
				165					170					175	
Glu	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser
			180					185					190		
Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro
		195					200					205			
Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys
	210					215					220				
Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro
225					230					235					240
Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser
				245					250					255	
Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp
			260					265					270		
Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn
		275					280					285			
Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val
	290					295					300				
Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu
305					310					315					320
Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys
				325					330					335	
Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr
			340					345					350		
Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr
		355					360					365			
Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu
	370					375					380				
Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu
385					390					395					400
Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys
				405					410					415	
Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu
			420					425					430		
Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly
		435					440					445			

[0003]

<210> 10
<211> 219
<212> PRT
<213> 人工序列

<220>

<223> 合成构建体

<400> 10
Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30
Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Val Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala Gln Asn
85 90 95
Leu Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115 120 125
Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140
Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145 150 155 160
Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165 170 175
Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
180 185 190
Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
195 200 205
Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 11
<211> 112
<212> PRT
<213> 小鼠

[0004]

<400> 11
Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys
1 5 10 15
Ala Ser Gly Tyr Ala Phe Ser Tyr Ser Trp Met Asn Trp Val Lys Leu
20 25 30
Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Arg Ile Phe Pro Gly Asp
35 40 45
Gly Asp Thr Asp Tyr Asn Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr
50 55 60
Ala Asp Lys Ser Ser Asn Thr Ala Tyr Met Gln Leu Thr Ser Leu Thr
65 70 75 80
Ser Val Asp Ser Ala Val Tyr Leu Cys Ala Arg Asn Val Phe Asp Gly
85 90 95
Tyr Trp Leu Val Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
100 105 110

<210> 12
<211> 103
<212> PRT
<213> 小鼠

<400> 12
Asn Pro Val Thr Leu Gly Thr Ser Ala Ser Ile Ser Cys Arg Ser Ser
1 5 10 15
Lys Ser Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu
20 25 30
Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn
35 40 45
Leu Val Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr
50 55 60
Asp Phe Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val
65 70 75 80
Tyr Tyr Cys Ala Gln Asn Leu Glu Leu Pro Tyr Thr Phe Gly Gly Gly
85 90 95
Thr Lys Leu Glu Ile Lys Arg
100

<210> 13
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 13
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Tyr Ser
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Ala Gln Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 14
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

[0005]

<400> 14
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Tyr Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 15
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 15
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Tyr Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Leu Cys
85 90 95

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 16
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 16
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Ala Phe Ser Tyr Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 17
<211> 119
<212> PRT
<213> 人工序列

[0006]

<220>
<223> 合成构建体

<400> 17
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Tyr Ser
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 18
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 18
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Tyr Ser
20 25 30
Trp Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe

50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 19
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 19
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Tyr Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

[0007]

<210> 20
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Tyr Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 21
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 21
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Tyr Ser
20 25 30
Trp Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Ala Gln Lys Phe
50 55 60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 22
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 22
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Val Ser Gly Tyr Thr Phe Thr Tyr Ser
20 25 30
Trp Met His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Ala Glu Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
[0008] Ala Thr Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 23
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 23
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Val Ser Gly Tyr Thr Phe Thr Tyr Ser
20 25 30
Trp Met Asn Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Thr Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 24
<211> 119
<212> PRT
<213> 人工序列

<220>

<223> 合成构建体

<400> 24
Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Thr Gly Ser
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Tyr Ser
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Ala Gln Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 25
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 25
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
[0009] Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 26
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 26
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Tyr Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 27

<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 27
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 28
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 28
Glu Val Gln Leu Val Glu Ser Gly Ala Gly Leu Val Lys Pro Gly Gly
1 5 10 15
[0010] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 29
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 29
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 30
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 30
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Lys Lys Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Glu Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 31
<211> 119
<212> PRT
<213> 人工序列

<220>
[0011] <223> 合成构建体

<400> 31
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Ser
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 32
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 32
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Arg Val Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr

65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 33
<211> 119
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 33
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Tyr Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

[0012]

<210> 34
<211> 19
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 34
Met Asp Trp Thr Trp Arg Ile Leu Phe Leu Val Ala Ala Ala Thr Gly
1 5 10 15
Ala His Ser

<210> 35
<211> 25
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 35
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser
20 25

<210> 36
<211> 13
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 36
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

1 5 10

<210> 37
<211> 32
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 37
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln
1 5 10 15
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 38
<211> 11
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 38
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
1 5 10

<210> 39
<211> 23
<212> PRT
<213> 人工序列

<220>
[0013] <223> 合成构建体

<400> 39
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys
20

<210> 40
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 40
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
1 5 10 15

<210> 41
<211> 32
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 41
Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15
Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys
20 25 30

<210> 42
<211> 11

<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 42
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
1 5 10

[0014]

<210> 43
<211> 23
<212> PRT
<213> 人工序列

<220>
<223> 合成构建体

<400> 43
Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15
Phe Pro Gly Ala Arg Cys
20

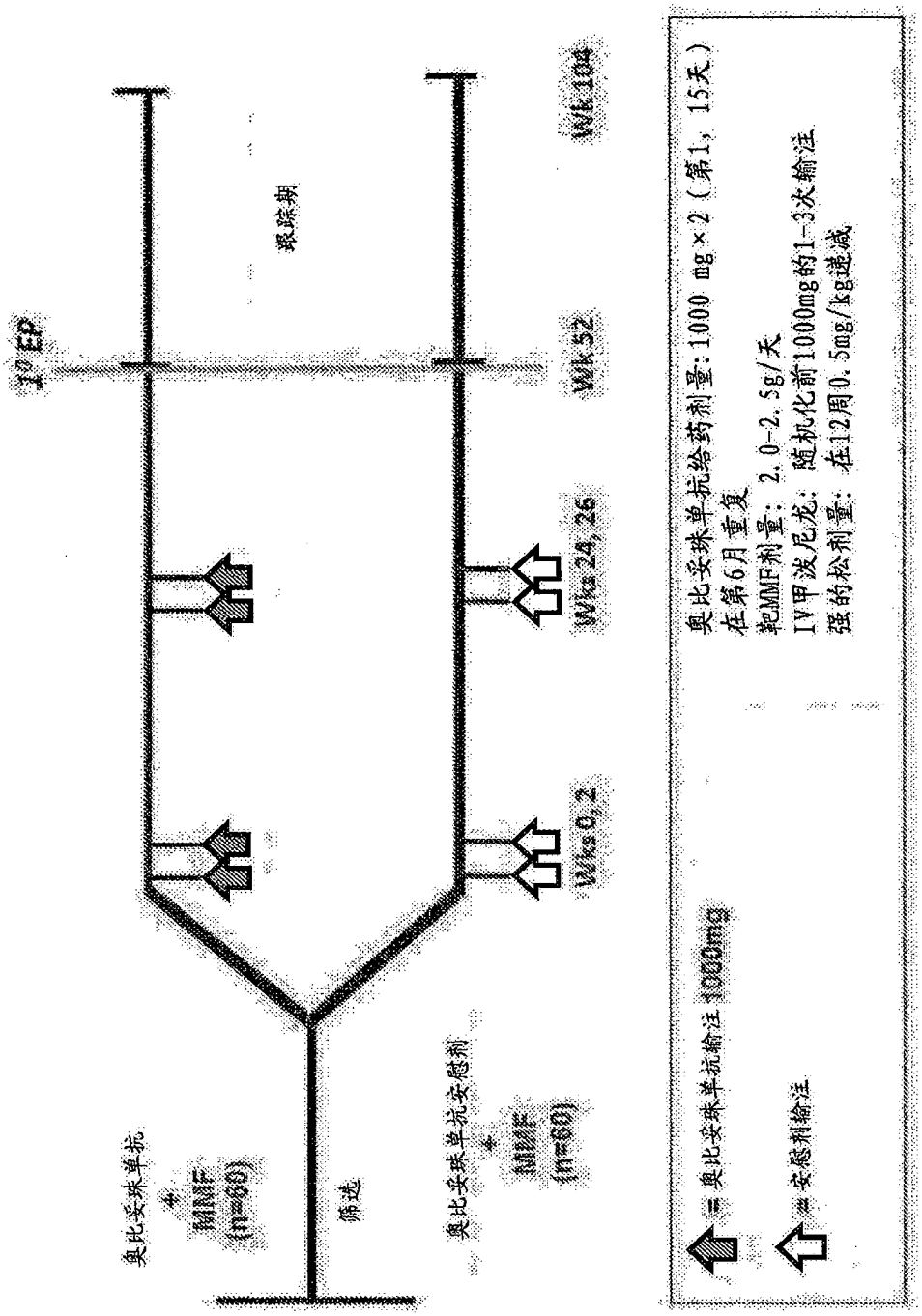
筛选
奥比安珠单抗
+
MMF
(n=60)
奥比安珠单抗安慰剂
+
MMF
(n=60)
Wks 0, 2
Wks 24, 26
Wk 52
Wk 104
IV EP
跟踪期
= 奥比安珠单抗输注 1000mg
= 安慰剂输注
奥比安珠单抗给药剂量: 1000 mg×2（第1，15天）
在第6月重复
起MMF剂量： 2.0-2.5g/天
IV甲泼尼龙： 随机化前1000mg的1-3次输注
强的松剂量： 在12周0.5mg/kg递减

图1

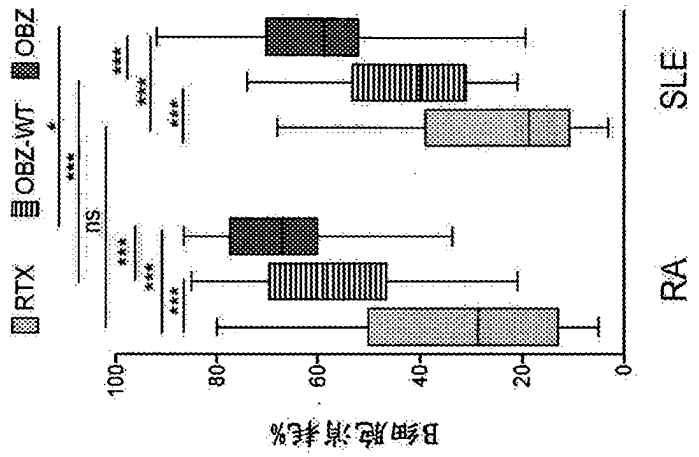
RTX
OBZ-WT
OBZ
SLE
RA
B细胞消耗%
100
80
60
40
20
0
ns
***
*

图2A

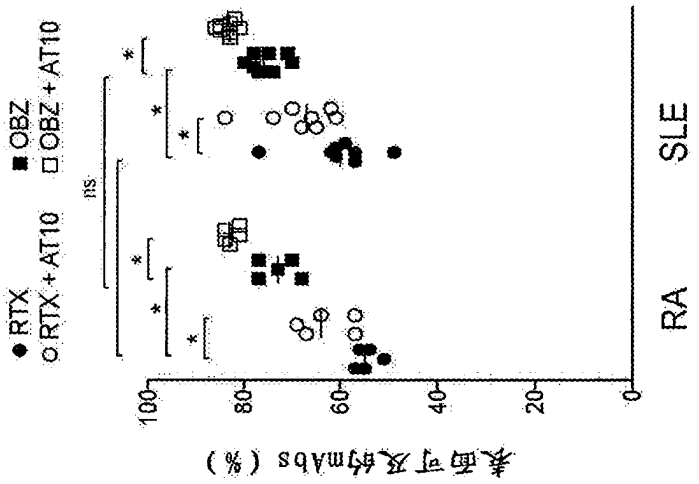
RTX
RTX + AT10
OBZ
OBZ + AT10
SLE
RA
表面可及的mAbs（%）
100
80
60
40
20
0
ns
*

图2B

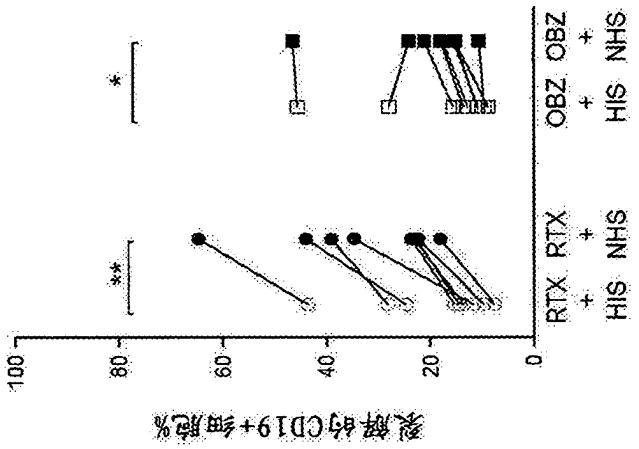
裂解的CD19+细胞%
100
80
60
40
20
0
RTX + HIS
RTX + NHS
OBZ + HIS
OBZ + NHS
**
*

图2C

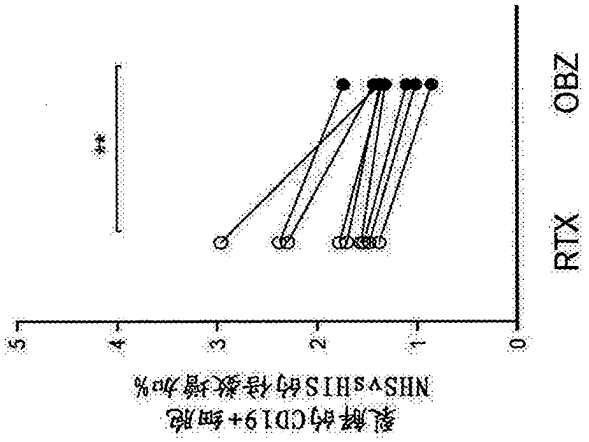
裂解的CD19+细胞
NHSvsHIS的倍数增加%
5
4
3
2
1
0
RTX
OBZ
**

图2D

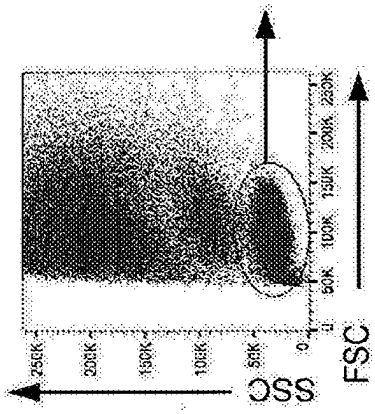
SSC
FSC
250K
200K
150K
100K
50K
0
200K
150K
100K
50K
0

图3A

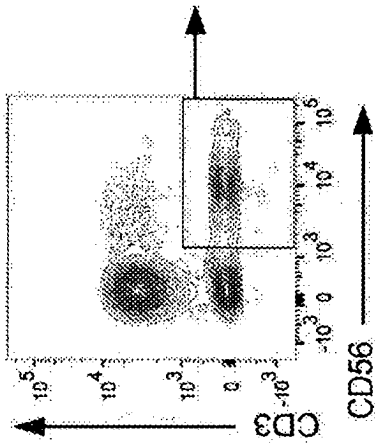

图3B

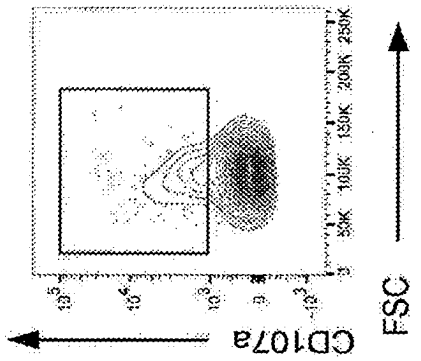

图3C

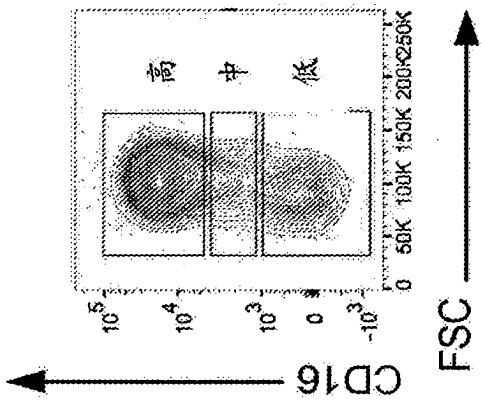

图3D

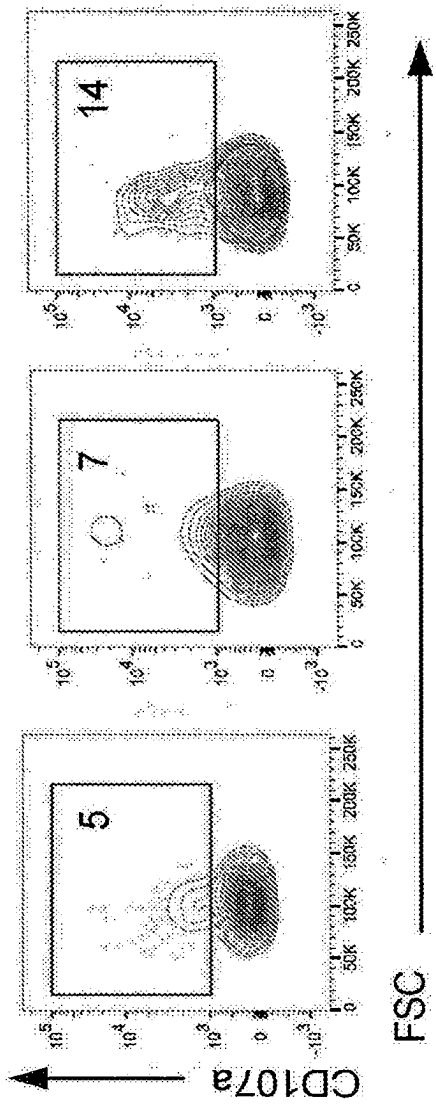

图3E 图3F 图3G

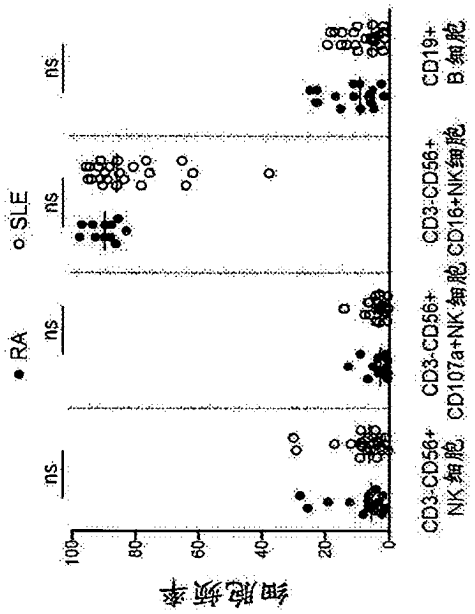
SLE
RA
ns
细胞频率
CD3-CD56+ NK细胞
CD3-CD56+ CD107a+NK细胞
CD3-CD56+ CD16+NK细胞
CD19+ B细胞

图4A

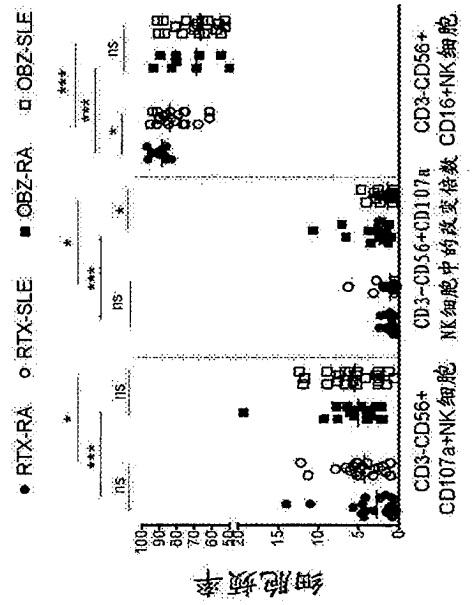
RTX-RA
RTX-SLE
OBZ-RA
OBZ-SLE
细胞频率
CD3-CD56+ CD107a+NK细胞
CD3-CD56+CD107a NK细胞中的改变倍数
CD3-CD56+ CD16+NK细胞

图4B

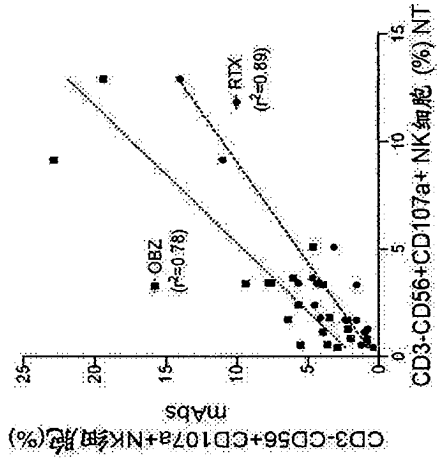
CD3-CD56+CD107a+ NK细胞 (%) NT
RTX
(r^2=0.89)
OBZ
(r^2=0.78)
CD3-CD56+CD107a+NK细胞(%)
mAbs

图4C

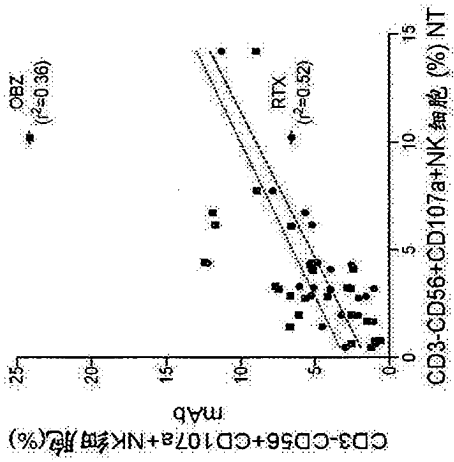
CD3-CD56+CD107a+NK 细胞 (%) NT
RTX
(r^2=0.52)
OBZ
(r^2=0.36)
CD3-CD56+CD107a+NK细胞(%)
mAb

图4D

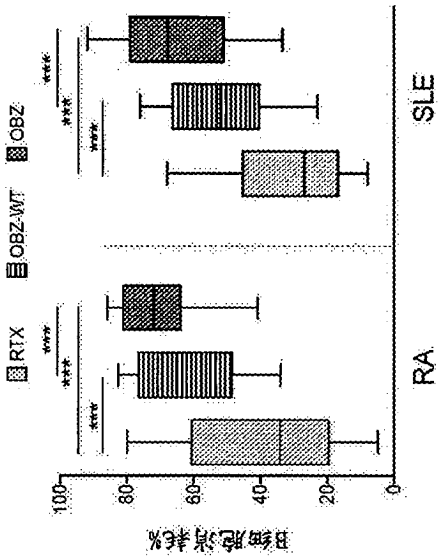
SLE
RA
OBZ
OBZ-WT
RTX
***
***
***
***
***
***
100
80
60
40
20
0

图5A

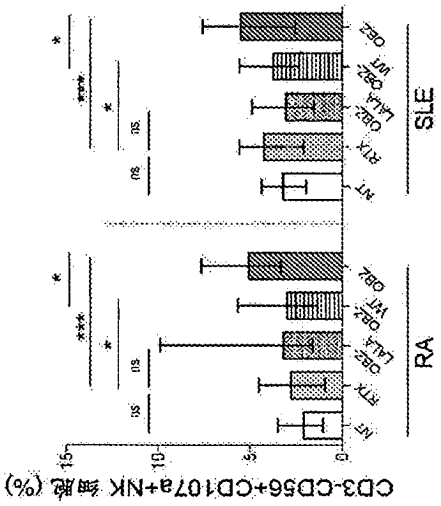
SLE
RA
NT
RTX
OBZ
OBZ-LALA
OBZ-WT
OBZ
*
***
*
ns
ns
15
10
5
0

图5B

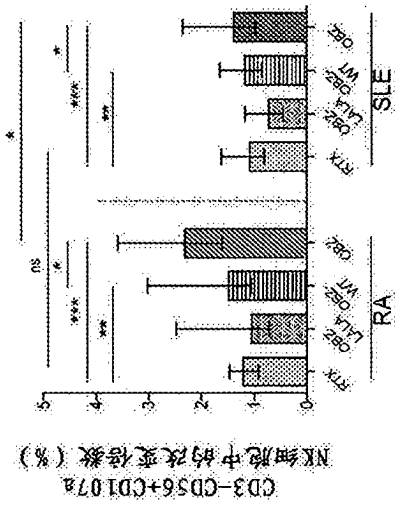
CD3-CD56+CD107a
NK细胞中的改变倍数（%）
SLE
RA
NT
RTX
OBZ
LEA
OBZ
WT
OBZ

图5C

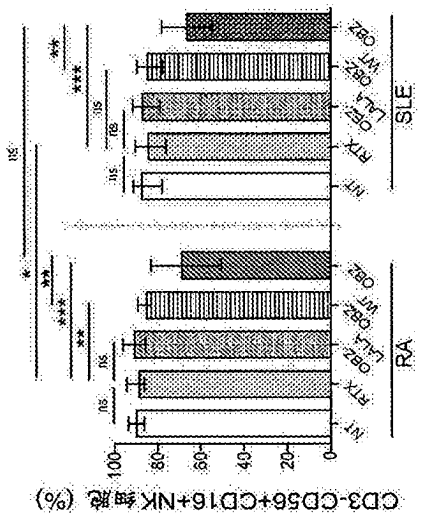
CD3-CD56+CD16+NK细胞（%）
SLE
RA
NT
RTX
OBZ
LEA
OBZ
WT
OBZ

图5D

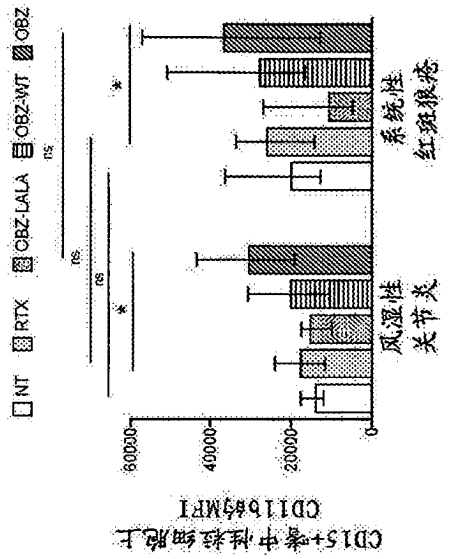
NT
RTX
OBZ-LALA
OBZ-WT
OBZ
ns
ns
*
*
风湿性
关节炎
系统性
红斑狼疮
60000
40000
20000
0
CD15+嗜中性粒细胞上
CD11b的MFI

图6A

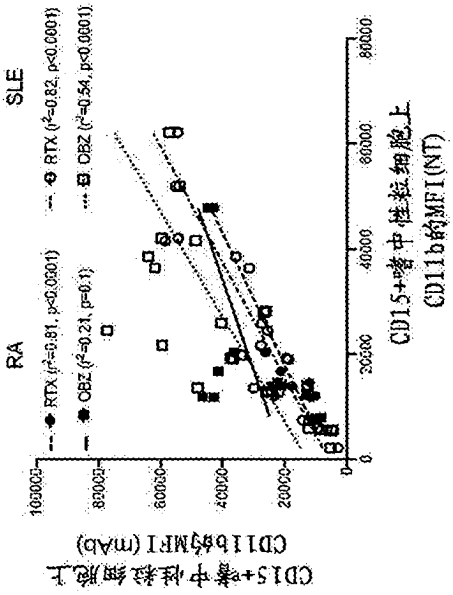
SLE
RA
RTX (r^2=0.82, p<0.0001)
OBZ (r^2=0.54, p<0.0001)
RTX (r^2=0.81, p<0.0001)
OBZ (r^2=0.21, p=0.1)
100000
80000
60000
40000
20000
0
20000
40000
60000
80000
CD15+嗜中性粒细胞上
CD11b的MFI (mAb)
CD15+嗜中性粒细胞上
CD11b的MFI(NT)

图6B

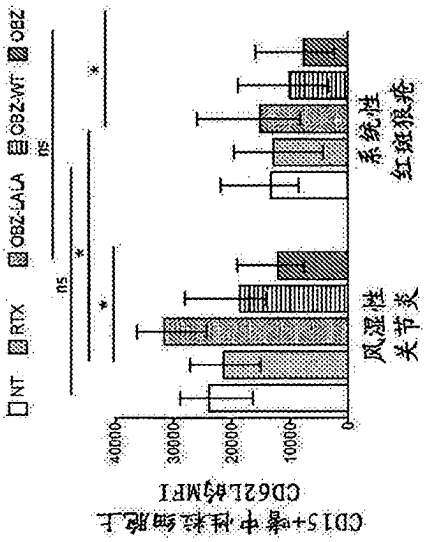
NT
RTX
OBZ-LALA
OBZ-WT
OBZ
ns
*
*
*
风湿性关节炎
系统性红斑狼疮
CD15+嗜中性粒细胞上CD62L的MFI
40000
30000
20000
10000
0

图6C

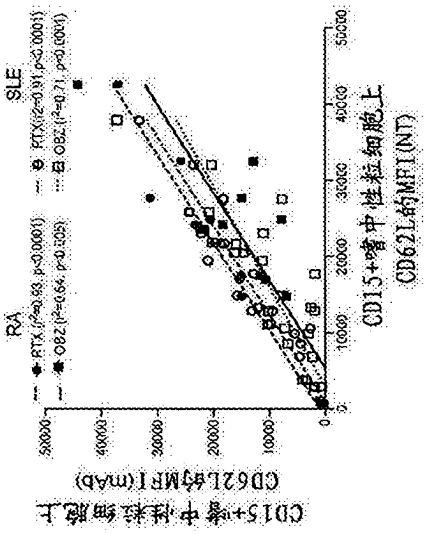
SLE
RA
RTX
OBZ
CD15+嗜中性粒细胞上CD62L的MFI(mAb)
CD15+嗜中性粒细胞上CD62L的MFI(NT)
50000
40000
30000
20000
10000
0

图6D

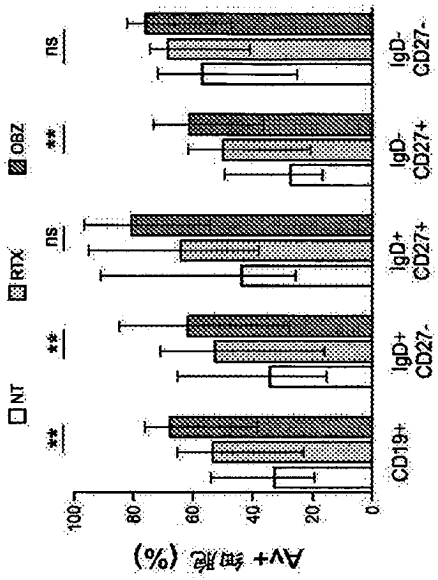
NT
RTX
OBZ
ns
**
IgD- CD27-
IgD- CD27+
IgD+ CD27+
IgD+ CD27-
CD19+
AV+ 细胞 (%)
100
80
60
40
20
0

图7A

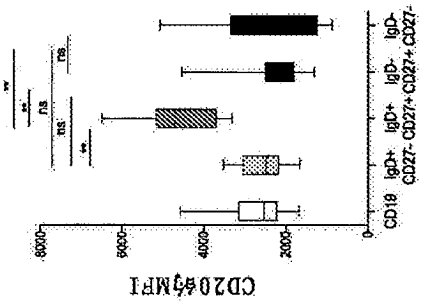
CD20的MFI
6000
4000
2000
0
CD19
IgD+ CD27-
IgD+ CD27+
IgD- CD27+
IgD- CD27-
ns
**

图7B

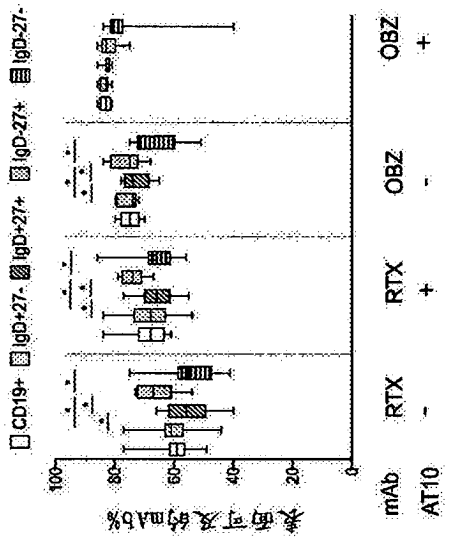
CD19+
IgD+27-
IgD+27+
IgD-27+
IgD-27-
浆母细胞
mAb
RTX
RTX
OBZ
OBZ
AT10
-
+
-
+
100
80
60
40
20
0

图7C

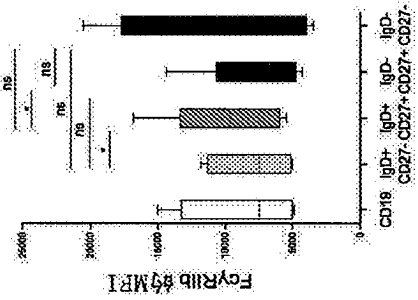
FcγRIIb 的MFI
CD19+
IgD+ CD27-
IgD+ CD27+
IgD- CD27+
IgD- CD27-
ns
*
0
5000
10000
15000
20000
25000

图7D

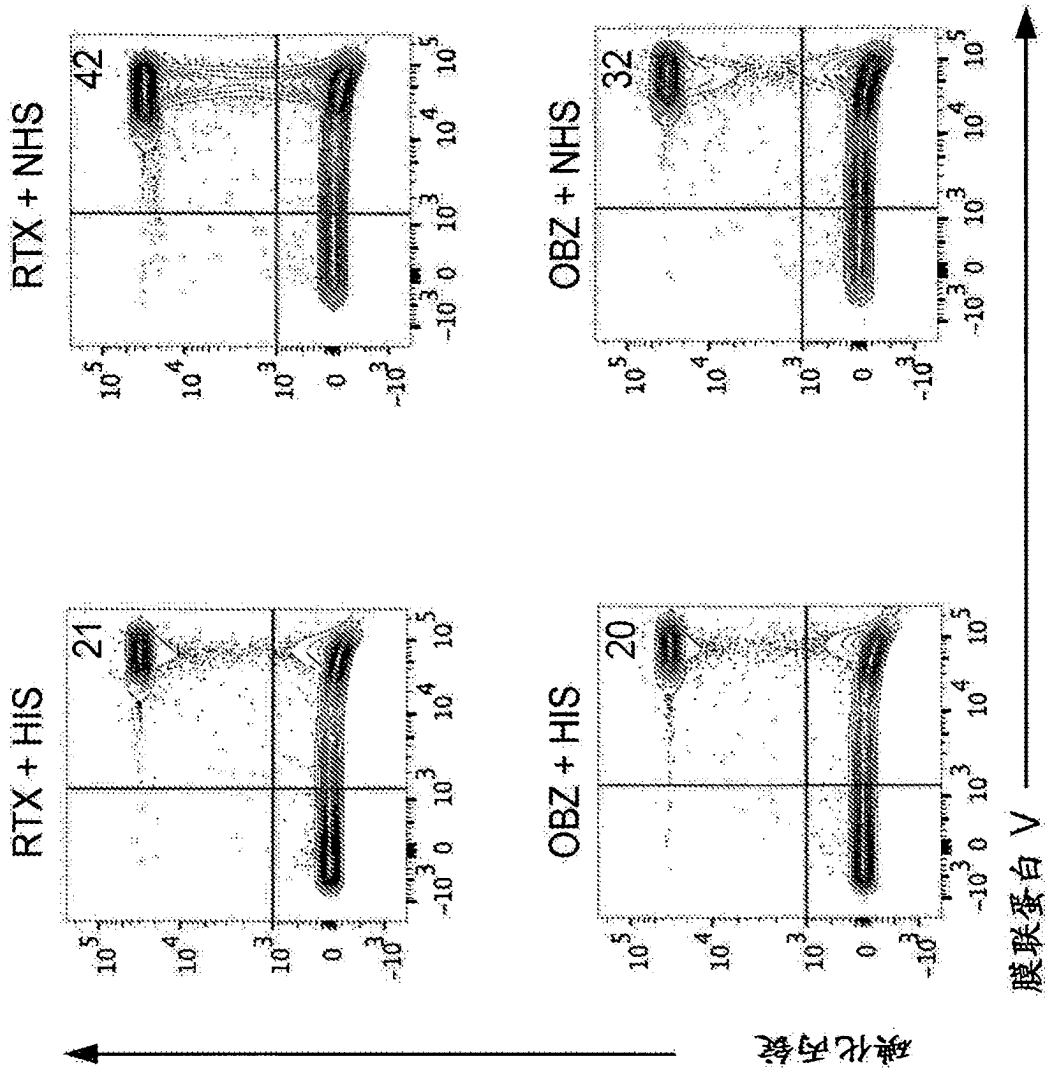

图8

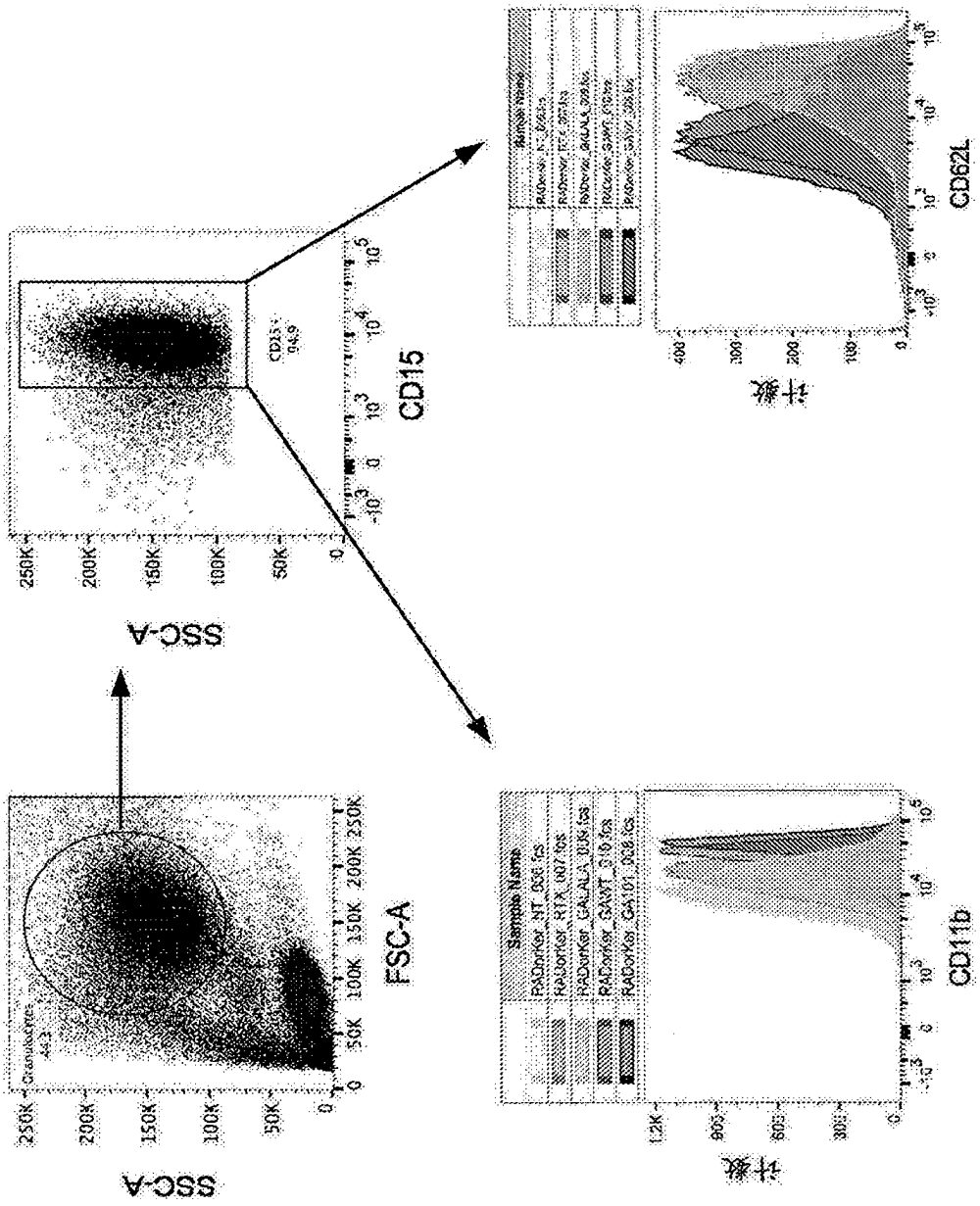

图9

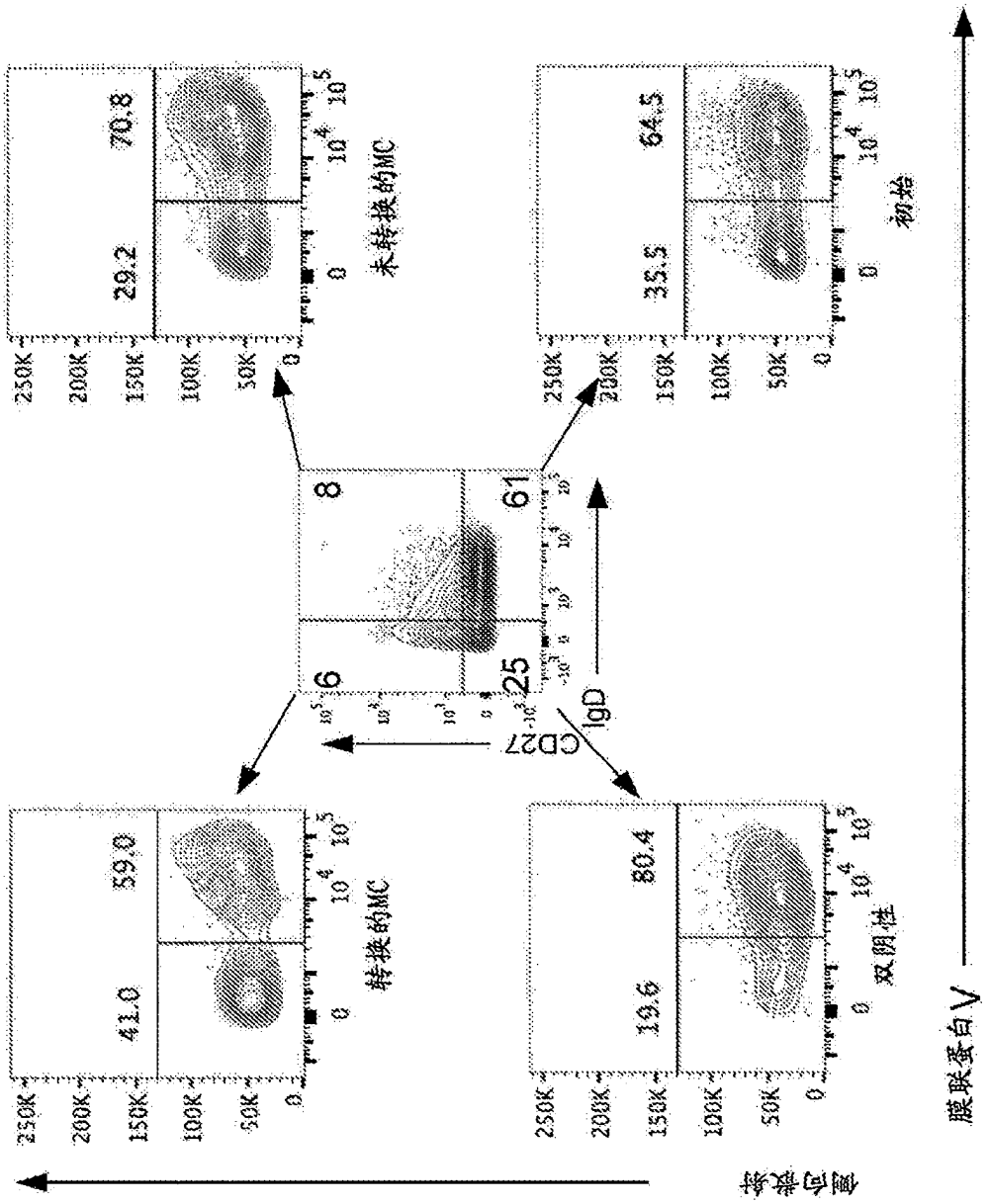
未转换的MC
初始
转换的MC
双阴性
侧向散射
膜联蛋白V
CD27
IgD
70.8
29.2
64.5
35.5
59.0
41.0
80.4
19.6
8
61
6
25

图10

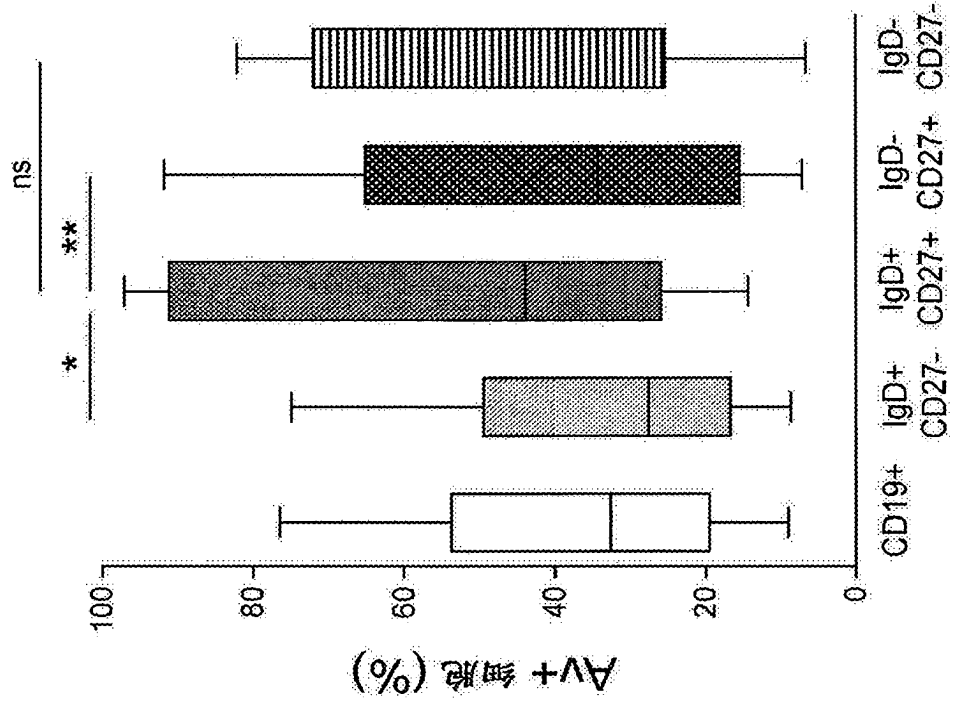
ns
**
*
CD19+
IgD+ CD27-
IgD+ CD27+
IgD- CD27+
IgD- CD27-
0
20
40
60
80
100
AV+ 细胞 (%)

图11

Abstract

The invention provides methods for treating or delaying progression of lupus nephritis in an individual that has lupus. In some embodiments, the methods comprise administering to the individual an effective amount of a type II anti-CD20 antibody. The invention also provides methods for treating or delaying progression of rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) in an individual. In some embodiments, the methods comprise administering an effective amount of an anti-CD20 antibody.