Title: ANTIBODIES AGAINST CD19 AND USES THEREOF

Abstract: The invention provides anti-CD 19 antibodies and methods of using the same. An isolated antibody that binds to CD 19, characterized in binding to the same CD 19 epitope to which monoclonal antibody 381 binds, which is characterized by comprising as heavy chain variable region SEQ ID NO: 1 and as light chain variable region SEQ ID NO: 5. The antibodies are useful as a therapeutic agent for treatment of autoimmune, rheumatoid arthritis, lupus, psoriasis, and bone diseases, as well as for tumor treatment.
Antibodies against CD19 and uses thereof

Field of the Invention

The present invention relates to antibodies against human CD19 (CD 19 antibody), such antibodies that do not bind complement factor Clq, methods for their production, pharmaceutical compositions containing said antibodies, and uses thereof.

Background of the Invention


Antibodies with increased affinity to the FcyRIIIa are mentioned in WO 2008/022152. Antibody against CD19 can have inhibitory or stimulating effects on B-cell activation. Binding of CD19 antibodies to mitogen-stimulated B-cells inhibits the subsequent rise in Ca^{2+} and the resulting activation and proliferation of these cells and B-cell proliferation and differentiation can either be inhibited or enhanced by CD19 antibody depending on the mitogenic stimulus used and the degree of crosslinking by the antibody.

Herein are provided antibodies against CD19 which are useful as a therapeutic agent for treatment of autoimmune, rheumatoid arthritis, lupus, psoriasis, and bone diseases and for tumor treatment.

In WO 2004/106381 pharmaceutical compositions comprising bispecific anti-CD3, anti-CD19 antibody constructs for the treatment of B-cell related disorders are reported. Anti-CD19 antibodies are reported in WO 2005/012493. In WO 2006/089133 anti-CD19 antibodies and uses in oncology are reported. Anti-CD^\wedge antibodies and their uses are reported in WO 2007/002223. In WO 2006/133450 anti-CD19 antibody therapy for the transplantation is reported.

**Summary of the invention**

The invention relates to an antibody binding to human CD19 and being characterized in binding to the same CD19 epitope to which monoclonal antibody 381 binds. Antibody 381 is characterized by comprising as heavy chain variable region SEQ ID NO: 1 and as light chain variable region SEQ ID NO: 5. Antibody 381 comprises as CDRs a CDRH1 region of SEQ ID NO: 2, a CDRH2 region of SEQ ID NO: 3 and a CDRH3 region of SEQ ID NO: 4, and a CDRL1 region of SEQ ID NO: 20, a CDRL2 region of SEQ ID NO: 6 and a CDRL3 region of SEQ ID NO: 7. As can be seen from table 1 and example 10, antibody 396 and the other mentioned antibodies, light chains, heavy chains and CDRs according to the invention are humanized variants of antibody 381 and of its light and heavy chains and CDRs.

In one embodiment the antibody according to the invention is characterized in being of human IgGl isotype comprising mutations L234A, which is alanine instead of leucine at amino acid position 234, and L235A, which is alanine instead of leucine at amino acid position 235.
In one embodiment the antibody according to the invention is characterized in being of human IgG4 isotype with or without mutation S228P.

The antibody according to the invention contains in one embodiment an Fc part derived from human origin.

In one embodiment the antibody according to the invention is characterized in that the heavy chain variable domain comprises as CDRs

a) a CDRH1 region of SEQ ID NO: 2, a CDRH2 region of SEQ ID NO: 3 and a CDRH3 region of SEQ ID NO: 4,
b) a CDRH1 region of SEQ ID NO: 9, a CDRH2 region of SEQ ID NO: 10 and a CDRH3 region of SEQ ID NO: 11,
c) a CDRH1 region of SEQ ID NO: 9, a CDRH2 region of SEQ ID NO: 16 and a CDRH3 region of SEQ ID NO: 11, or
d) a CDRH1 region of SEQ ID NO: 9, a CDRH2 region of SEQ ID NO: 17 and a CDRH3 region of SEQ ID NO: 11.

In one embodiment the antibody according to the invention is characterized in that the light chain variable domain comprises as CDRs

a) a CDRL1 region of SEQ ID NO: 20, a CDRL2 region of SEQ ID NO: 6 and a CDRL3 region of SEQ ID NO: 7,
b) a CDRL1 region of SEQ ID NO: 13, a CDRL2 region of SEQ ID NO: 14 and a CDRL3 region of SEQ ID NO: 15,
c) a CDRL1 region of SEQ ID NO: 13, a CDRL2 region of SEQ ID NO: 18 and a CDRL3 region of SEQ ID NO: 15,
d) a CDRL1 region of SEQ ID NO: 19, a CDRL2 region of SEQ ID NO: 14 and a CDRL3 region of SEQ ID NO: 15, or
e) a CDRL1 region of SEQ ID NO: 20, a CDRL2 region of SEQ ID NO: 14 and a CDRL3 region of SEQ ID NO: 15.

In one embodiment the antibody according to the invention is characterized in that the heavy chain variable domain and the light chain variable domain comprises as CDRs

a) a CDRH1 region of SEQ ID NO: 2, a CDRH2 region of SEQ ID NO: 3 and a CDRH3 region of SEQ ID NO: 4, and a CDRL1 region of SEQ ID NO: 20, a CDRL2 region of SEQ ID NO: 6 and a CDRL3 region of SEQ ID NO: 7, or
b) a CDRH1 region of SEQ ID NO: 9, a CDRH2 region of SEQ ID NO: 10 and a CDRH3 region of SEQ ID NO: 11, and a CDRL1 region of SEQ ID NO: 13, a CDRL2 region of SEQ ID NO: 14 a CDRL3 region of SEQ ID NO: 15.

In one embodiment the antibody according to the invention is characterized in that the antibody comprises as heavy chain variable domain

a) the heavy chain variable domain of SEQ ID NO: 1,
b) the heavy chain variable domain of SEQ ID NO: 8,
c) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A,
d) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16 instead of SEQ ID NO: 10,
e) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 17 instead of SEQ ID NO: 10,
f) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A, and mutation VI11L and optionally mutation K12V, or

g) the heavy chain variable domain of SEQ ID NO: 8 including mutations T28A, VI11L, Y91F, and T108S, and optionally mutation K12V.

In one embodiment the antibody according to the invention is characterized in that the antibody comprises as light chain variable domain

a) the light chain variable domain of SEQ ID NO: 5
b) the light chain variable domain of SEQ ID NO: 12,
c) the light chain variable domain of SEQ ID NO: 12 including as CDRL2 SEQ ID NO: 18 instead of SEQ ID NO: 14
d) the light chain variable domain of SEQ ID NO: 12 including as CDRL1 SEQ ID NO: 19 instead of SEQ ID NO: 13,
e) the light chain variable domain of SEQ ID NO: 12 including as CDRL1 SEQ ID NO: 20 instead of SEQ ID NO: 13, or
f) the light chain variable domain of SEQ ID NO: 12 including mutations P80S and F83L and as CDRL1 SEQ ID NO: 20 instead of SEQ ID NO: 13.

In one embodiment the antibody according to the invention is characterized in that the antibody comprises as heavy chain variable domain and as light chain variable domain

a) the heavy chain variable domain of SEQ ID NO: 1 and the light chain variable domain of SEQ ID NO: 5,
b) the heavy chain variable domain of SEQ ID NO: 8 and the light chain variable domain of SEQ ID NO: 12,
c) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and the light chain variable domain of SEQ ID NO: 12,

d) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16 or 17, and the light chain variable domain of SEQ ID NO: 12,
e) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16 or 17, and the light chain variable domain of SEQ ID NO: 12, and as CDRL1 SEQ ID NO: 19 or 20,
f) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16 or 17, and the light chain variable domain of SEQ ID NO: 12, and as CDRL2 SEQ ID NO: 18, or
g) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16 or 17, and the light chain variable domain of SEQ ID NO: 12 including mutations P80S and F83L, and as CDRL1 SEQ ID NO: 20.

In one specific embodiment the antibody according to the invention is characterized in that the heavy chain variable domain comprises in addition to mutation T28A,

a) mutation V11L and optionally mutation K12V, or
b) mutations V11L, Y91F and T108S, and optionally mutation K12V.

In one embodiment the antibody according to the invention is characterized by the above mentioned amino acid sequences or amino acid sequence fragments and properties. The antibody according to the invention in one embodiment comprises an Fc part derived from human origin. In a specific embodiment the antibody according to the invention is of human IgG1 or IgG4 isotype. The antibody according to the invention is in a specific embodiment of IgG1 isotype modified in the hinge region at about amino acid position 216-240, especially at about amino acid position 220-240, between CH1 and CH2 and/or in the second inter-domain region at about amino acid position 327-331 between CH2 and CH3.

In one embodiment the antibody is of human IgG1 isotype comprising mutations L234A (alanine instead of leucine at amino acid position 234) and L235A.
The antibody according to the invention is in one embodiment of human IgG4 isotype with or without mutation S228P.

The antibody is in one embodiment a monoclonal antibody and, in addition, a chimeric antibody (human constant chain), or a humanized antibody. In a specific embodiment the antibody is a humanized antibody.

The antibody is in one embodiment further characterized by an affinity of $10^{-9}$ M ($K_D$) or less, especially of about $10^{-9}$ M to $10^{-13}$ M in binding to human CD19. In one embodiment an antibody according to the invention binds also to mouse CD19 and chimpanzee CD19.

The invention further provides methods for the recombinant production of such antibodies.

The invention further provides a nucleic acid encoding a heavy chain variable domain of an antibody binding to human CD19, characterized in that said nucleic acid encodes

1) the heavy chain variable domain of SEQ ID NO: 1,
2) the heavy chain variable domain of SEQ ID NO: 8,
3) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A,
4) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16
5) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 17,
6) the heavy chain variable domain of SEQ ID NO: 8 including mutations T28A, V11L and optionally mutation K12V, or
7) the heavy chain variable domain of SEQ ID NO: 8 including mutations T28A, V11L, Y91F, and T108S, and optionally mutation K12V.

The invention further provides a nucleic acid encoding a light chain variable domain of an antibody binding to human CD19, characterized in that said nucleic acid encodes

1) the light chain variable domain of SEQ ID NO: 5
2) the light chain variable domain of SEQ ID NO: 12,
3) the light chain variable domain of SEQ ID NO: 12 including as CDRL2 SEQ ID NO: 18 instead of SEQ ID NO: 14
d) the light chain variable domain of SEQ ID NO: 12 including as CDRL1 SEQ ID NO: 19 instead of SEQ ID NO: 13, or
e) the light chain variable domain of SEQ ID NO: 12 including as CDRL1 SEQ ID NO: 20 instead of SEQ ID NO: 13.

f) the light chain variable domain of SEQ ID NO: 12 including mutations P80S and F83L and as CDRL1 SEQ ID NO: 19 and as CDRL2 SEQ ID NO: 14.

The invention further provides a nucleic acid encoding a heavy chain variable domain and a light chain domain of an antibody binding to human CD19, characterized in that said nucleic acid encodes

a) the heavy chain variable domain of SEQ ID NO: 1 and the light chain variable domain of SEQ ID NO: 5,
b) the heavy chain variable domain of SEQ ID NO: 8 and the light chain variable domain of SEQ ID NO: 12,
c) the heavy chain variable domain of SEQ ID NO: 12 including mutation T28A and the light chain variable domain of SEQ ID NO: 12,
d) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16 or 17, and the light chain variable domain of SEQ ID NO: 12,
e) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16 or 17, and the light chain variable domain of SEQ ID NO: 12, and as CDRL1 SEQ ID NO: 19 or 20,
f) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16 or 17, and the light chain variable domain of SEQ ID NO: 12, and as CDRL2 SEQ ID NO: 18, or
g) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16 or 17, and the light chain variable domain of SEQ ID NO: 12 including mutations P80S and F83L, and as CDRL1 SEQ ID NO: 20.

In one embodiment the antibody according to the invention blocks B-cell activation without causing B-cell depletion. Such an antibody is antibody 381. In a further embodiment the antibody according to the invention blocks B-cell activation and causes in addition B-cell depletion in a whole blood B-cell depletion assay. Such an antibody is antibody 396.
The invention further comprises the use of an antibody according to the invention, for the treatment of inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone diseases. The invention further comprises the use of an antibody according to the invention for the manufacture of a medicament for the treatment of diseases, in one embodiment of inflammatory, autoimmune, lupus, psoriasis, or bone diseases. The invention further comprises a method for the manufacture of a medicament for the treatment of diseases, in one embodiment of inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone diseases, characterized in comprising an antibody according to the invention. The antibodies according to the invention have properties causing a benefit for a patient suffering from a disease associated with pathologic increase of B-cells.

Antibodies according to the invention provide for the reduction of tumor growth and a significant prolongation of the time to progression.

The antibodies according to the invention are characterized by the above mentioned properties.

The invention further provides methods for treating inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone diseases, comprising administering to a patient diagnosed as having such disease (and therefore being in need of such a therapy) an antibody against CD19 according to the invention. The antibody may be administered alone, in a pharmaceutical composition, or alternatively in combination with other medicaments for treating inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone diseases. The antibody is administered in a pharmaceutically effective amount.

The invention further comprises the use of an antibody according to the invention for the treatment of inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis or bone diseases, and for the manufacture of a pharmaceutical composition comprising an antibody according to the invention. In addition, the invention comprises a method for the manufacture of a pharmaceutical composition comprising an antibody according to the invention.

The invention further comprises an antibody according to the invention for the treatment of inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone diseases.
The invention further comprises a pharmaceutical composition containing an antibody according to the invention, optionally together with a buffer and/or an adjuvant useful for the formulation of antibodies for pharmaceutical purposes.

The invention further comprises a pharmaceutical composition comprising an antibody according to the invention.

The invention further provides pharmaceutical compositions comprising such antibodies in a pharmaceutically acceptable carrier. In one embodiment, the pharmaceutical composition may be included in an article of manufacture or kit. The invention further provides the use of an antibody according to the invention for the manufacture of a pharmaceutical composition for the treatment of cancer or inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone diseases. The antibody is used in a pharmaceutically effective amount.

The invention further comprises the use of an antibody according to the invention for the manufacture of a pharmaceutical composition for the treatment of inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone diseases. The antibody is used in a pharmaceutically effective amount.

The invention further comprises a method for the production of a recombinant antibody according to the invention, characterized by expressing a nucleic acid encoding an antibody binding to CD19 according to the invention in a CHO cell and recovering said antibody from said cell.

The invention further comprises a process for the production of an antibody against CD19 according to the invention, comprising the steps of transforming a host cell, in one embodiment a CHO cell.

**Description of the figures**

**Figure 1:** CD19 target binding of anti-CD19 antibody Fab fragments using Daudi target cells (geometric mean vs. antibody concentration).

**Figure 2:** CD19 target binding of anti-CD19 antibodies comprising constant domains using Daudi target cells (geometric mean vs. antibody concentration).

**Figure 3:** ADCC assay in vitro with anti-CD19 antibody constant region variants with Raji cells.
Figure 4: B-cell depletion in whole blood assay using anti-CD19 antibodies.

Figure 5: B-cell depletion in whole blood assay using anti-CD19 antibodies.

**Detailed description of embodiments of the invention**

1. Definitions

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. 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 amino acid sequence changes. In some embodiments, the number of 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. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

"Affinity" refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (#K_D#). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

The terms "anti-CD19 antibody" and "an antibody that binds to CD19" refer to an antibody that is capable of binding CD19 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting CD19. In one embodiment, the extent of binding of an anti-CD19 antibody to an unrelated, non-CD19 protein is less than about 10% of the binding of the antibody to CD19 as measured, by Surface Plasmon Resonance. In certain embodiments, an antibody that binds to CD19 has a dissociation constant (#K_D#) of 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).
The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')2; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

The term "without causing B-cell depletion" refers to a B-cell depletion experiment, wherein human whole blood samples (after erythrocyte removal) were incubated for 24 hours with an antibody according to the invention. B-cell depletion was measured by counting the CD19 positive cells in a flow cytometer relative to the CD3 positive cells as a reference and with an antibody concentration of 5 ng/ml. The humanized variant antibody 396 shows a depletion potency of human B-cells of 28% compared to antibody 381, which shows no detectable depletion. Constructs that are comprised of the heavy chain variants HA, HB, or HD show a B-cell depleting behavior, whereas constructs having the chimeric heavy chain (either together with the chimeric light chain, or the humanized light chain LE), or the heavy chain variants HE, and HF show a strongly reduced activity. In both experiments, variants HE and HF show B-cell depletion activity at the same low level as the parental antibody, or even less.

The term "blocking B-cell activation" refers to the inhibitory activity of an antibody according to the invention on the proliferation of human B-cells, measured by FACS and SU-DHL4 cells (DSMZ ACC 495) as cell lines in this assay. Blocking is found, if at an concentration of 1 µg/ml of the antibody 30% and more inhibition of proliferation was found compared to the CD19 negative cell line Plasmacytoma RPMI8226 (ATCC CCL 155).

The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.
The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgGi, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called a, δ, ε, γ, and μ, respectively.

The antibody according to the invention is in one embodiment characterized by being of human subclass IgGi with mutations PVA236, L234A/L235A, and/or GLPSS331, or of subclass IgG4. In a further embodiment of the invention, the antibody is characterized by being of any IgG class, in one embodiment being IgGi or IgG4, containing at least one mutation in E233, L234, L235, G236, D270, N297, E318, K320, K322, A327, A330, P331 and/or P329 (numbering according to EU index). Especially suited are the IgGi mutations PVA236, L234A/L235A and/or GLPSS331 as well as the IgG4 mutation L235E. It is further in one embodiment that the antibody of IgG4 subclass contains the mutation S228P, or the mutation S228P and L235E (Angal, S., et al, Mol. Immunol. 30 (1993) 105-108).

The antibody according to the invention therefore is in one embodiment an antibody of human subclass IgGi, containing one or more mutation(s) selected from PVA236, GLPSS331 and/or L234A/L235A (numbering according to EU index of Kabat).

The present invention refers to an antibody that binds CD19 and does not bind complement factor Clq, and/or Fc receptor. In one embodiment of the invention, these antibodies do not elicit the complement dependent cytotoxicity (CDC) and/or antibody-dependent cellular cytotoxicity (ADCC). In one embodiment this antibody is characterized in that it binds CD19, contains an Fc part derived from human origin and does not bind complement factor Clq. In a specific embodiment this antibody is a human or humanized monoclonal antibody. Such an antibody is useful for the treatment of inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone diseases.

"Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: Clq binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity.
(ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B-cell receptor); and B-cell activation.

The antibodies according to the invention contain as Fc part, in one embodiment an Fc part derived from human origin and preferably all other parts of the human constant regions. The Fc part of an antibody is directly involved in complement activation, Clq binding, C3 activation and Fc receptor binding. While the influence of an antibody on the complement system is dependent on certain conditions, binding to Clq is caused by defined binding sites in the Fc part. Such binding sites are known in the state of the art and described e.g. by Lukas, T.J., et al, J. Immunol. 127 (1981) 2555-2560; Brunhouse, R., and Cebra, J.J., Mol. Immunol. 16 (1979) 907-917; Burton, D.R., et al, Nature 288 (1980) 338-344; Thommesen, J.E., et al, Mol. Immunol. 37 (2000) 995-1004; Idusogie, E.E., et al, J. Immunol. 164 (2000) 4178-4184; Hezareh, M., et al, J. Virol. 75 (2001) 12161-12168; Morgan, A., et al, Immunology 86 (1995) 319-324; and EP 0 307 434. Such binding sites are e.g. L234, L235, D270, N297, E318, K320, K322, P331 and P329 (numbering according to EU index of Kabat, see below). Antibodies of subclass IgG1, IgG2 and IgG3 usually show complement activation, Clq binding and C3 activation, whereas IgG4 do not activate the complement system, do not bind Clq and do not activate C3. As used herein the term "Fc part derived from human origin and does not bind human complement factor Clq and/or human Fey receptor on NK cells" denotes a Fc part which is either a Fc part of a human antibody of the subclass IgG4 or a Fc part of a human antibody of the subclass IgG1, IgG2 or IgG3 which is modified in such a way that no Clq binding, C3 activation and/or FcR binding as defined below can be detected. An "Fc part of an antibody" is a term well known to the skilled artisan and defined on the basis of papain cleavage of antibodies. In one embodiment the Fc part is a human Fc part and especially preferred either from human IgG4 subclass, in a specific embodiment mutated in the hinge region (e.g. S228P and/or L235E) or a mutated Fc part from human IgG1 subclass. Mostly preferred are Fc parts comprising heavy chain constant regions selected from the regions shown in SEQ ID NO: 14 and 15 or included in SEQ ID NO: 58, 59, 60, SEQ ID NO: 14 with mutations L234A and L235A or SEQ ID NO: 15 with mutation S228P or mutations S228P and L235E.

In one embodiment of the invention an antibody according to the present invention binds CD19 and does not bind complement factor Clq and/or Fc receptor. In one embodiment of the invention, these antibodies do not elicit the complement dependent cytotoxicity (CDC) and/or antibody-dependent cellular cytotoxicity
(ADCC). In one embodiment this antibody is characterized in that it binds CD19, contains an Fc part derived from human origin and does not bind complement factor Clq. In a specific embodiment this antibody is a human or humanized monoclonal antibody.

The effector functions mediated by the Fc part of the antibody Fc region refer to effector functions that operate after the binding of an antibody to an antigen (these functions involve the activation of the complement cascade and/or cell activation by an Fc receptor).

The function of the complement cascade can be assessed by the CH50 assay. Sheep red cells sensitized with anti-red cell antibodies (EA) are added to test serum to activate the classical pathway resulting in hemolysis. The volume of serum needed to lyse 50 % of the red cells determines the CH50 unit. The AP-CH50 measures the alternative and the terminal pathways. The procedure is similar except that rabbit red cells are used. The alternative pathway is activated upon addition of test serum.

Clq and two serine proteases, Clr and Cls, form the complex Cl, the first component of the complement dependent cytotoxicity (CDC) pathway. To activate the complement cascade Clq binds to at least two molecules of IgGl or one molecule of IgM, attached to the antigenic target (Ward, E.S. and Ghetie, V., Ther. Immunol. 2 (1995) 77-94). Burton, D.R., (Mol. Immunol. 22 (1985) 161-206) described that the heavy chain region comprising amino acid residues 318 to 337 is being involved in complement fixation. Duncan, A.R. and Winter, G. (Nature 332 (1988) 738-740) reported using site directed mutagenesis that Glu318, Lys320 and Lys322 form the binding site to Clq. The role of Glu318, Lys320 and Lys 322 residues in the binding of Clq was confirmed by the ability of a short synthetic peptide containing these residues to inhibit complement mediated lysis.

The term "complement-dependent cytotoxicity (CDC)" refers to lysis of CD19 expressing human endothelial cells by the antibody according to the invention in the presence of complement. CDC is measured in one embodiment by the treatment of CD19 expressing human endothelial cells with an antibody according to the invention in the presence of complement. The cells are in one embodiment labeled with calcein. CDC is found if the antibody induces lysis of 20 % or more of the target cells at a concentration of 30 µg/ml. Binding to the complement factor Clq can be measured in an ELISA assay. In such an assay in principle an ELISA plate is coated with concentration ranges of the antibody, to which purified human Clq
or human serum is added. Clq binding is detected by an antibody directed against Clq followed by a peroxidase-labeled conjugate. Detection of binding (maximal binding \( B_{\text{max}} \)) is measured as optical density at 405 nm (OD405) for peroxidase substrate ABTS\(^{\circledast} \) (2,2'-azino-di-[3-ethylbenzthiazoline-6-sulfonate (6)]).

Accordingly the present invention in one embodiment refers to an antibody, characterized in that non-binding of the antibody to complement factor Clq refers to such an ELISA assay measurement wherein the maximal binding (\( B_{\text{max}} \)) of Clq to an antibody according to the invention at a concentration of 10 \( \mu \)g/ml of the antibody is 20 % or lower of \( B_{\text{max}} \) observed with antibody 381 comprising human IgG1 constant region of SEQ ID NO: 26 and SEQ ID NO: 27, in one embodiment 10 % or lower.

In one embodiment an antibody according to the invention shows a reduced activation of complement factor C3 in an ELISA assay. The assay is performed in the same manner as the Clq assay. In such an assay in principle an ELISA plate is coated with concentration ranges of the antibody, to which human serum is added. C3 binding is detected by an antibody directed against C3 followed by a peroxidase-labeled conjugate. Detection of binding (maximal binding \( B_{\text{max}} \)) is measured as optical density at 405 nm (OD405) for peroxidase substrate ABTS\(^{\circledast} \).

Accordingly the present invention refers in one embodiment to an antibody, characterized in that non-binding of the antibody to complement factor C3 refers to such an ELISA assay measurement wherein the maximal binding (\( B_{\text{max}} \)) of C3 to the antibody at a concentration of 10 \( \mu \)g/ml of the antibody is 10 % of \( B_{\text{max}} \) of antibody 381 comprising human IgG1 constant region of SEQ ID NO: 26 and SEQ ID NO: 27, in one embodiment 5 % or lower.

The term "antibody-dependent cellular cytotoxicity (ADCC)" is a function mediated by Fc receptor binding and refers to lysis of CD19 expressing target cells by an antibody according to the invention in the presence of effector cells. ADCC is measured in one embodiment by the treatment of a preparation of CD19 expressing erythroid cells (e.g. K562 cells expressing recombinant human CD19) with an antibody according to the invention in the presence of effector cells such as freshly isolated PBMC (peripheral blood mononuclear cells) or purified effector cells from buffy coats, like monocytes or NK (natural killer) cells. Target cells are labeled with \(^{51}\text{Cr} \) and subsequently incubated with the antibodies. The labeled cells are incubated with effector cells and the supernatant is analyzed for released \(^{51}\text{Cr} \).

Controls include the incubation of the target endothelial cells with effector cells but without the antibody. The capacity of the antibodies to induce the initial steps
mediating ADCC was investigated by measuring their binding to Fey receptors expressing cells, such as cells, recombinantly expressing FcyRI and/or FcyRIIA or NK cells (expressing essentially FcyRIIIA). Preferably binding to FcyR on NK cells is measured.

Fc receptor binding effector functions can be mediated by the interaction of the Fc region of an antibody with Fc receptors (FcRs), which are specialized cell surface receptors on hematopoietic cells. Fc receptors belong to the immunoglobulin superfamily, and have been shown to mediate both the removal of antibody-coated pathogens by phagocytosis of immune complexes, and the lysis of erythrocytes and various other cellular targets (e.g. tumor cells) coated with the corresponding antibody, via antibody dependent cell mediated cytotoxicity (ADCC) (Van de Winkel, J.G. and Anderson, C.L., J. Leukoc. Biol. 49 (1991) 511-524). FcRs are defined by their specificity for immunoglobulin isotypes; Fc receptors for IgG antibodies are referred to as FcyR, for IgE as FcyR, for IgA as FcyR and so on. Fc receptor binding is described e.g. in Ravetch, J.V., and Kinet, J.P., Annu. Rev. Immunol. 9 (1991) 457-492; Capel, P.J., et al, Immunomethods 4 (1994) 25-34; de Haas, M., et al, J. Lab. Clin. Med. 126 (1995) 330-341; and Gessner, J.E., et al, Ann. Hematol. 76 (1998) 231-248.

Cross-linking of receptors for the Fc domain of IgG antibodies (FcyR) triggers a wide variety of effector functions including phagocytosis, antibody-dependent cellular cytotoxicity, and release of inflammatory mediators, as well as immune complex clearance and regulation of antibody production. In humans, three classes of FcyR have been characterized, which are:


- FcyRII (CD32) binds complexed IgG with medium to low affinity and is widely expressed. These receptors can be divided into two important types, FcyRIIA and FcyRIIB. FcyRIIA is found on many cells involved in killing (e.g. macrophages, monocytes, neutrophils) and seems able to activate the killing
process. FcγRIIB seems to play a role in inhibitory processes and is found on B-cells, macrophages and on mast cells and eosinophils. On B-cells it seems to function to suppress further immunoglobulin production and isotype switching to, for example, the IgE class. On macrophages, FcγRIIB acts to inhibit phagocytosis as mediated through FcγRIIA. On eosinophils and mast cells the B-form may help to suppress activation of these cells through IgE binding to its separate receptor. Reduced binding for FcγRIIA is found e.g. for IgG mutation of at least one of E233-G236, P238, D265, N297, A327, P329, D270, Q295, A327, R292, and K414.

FcγRIII (CD16) binds IgG with medium to low affinity and exists as two types. FcγRIIIA is found on NK cells, macrophages, eosinophils and some monocytes and T cells and mediates ADCC. FcγRIIB is highly expressed on neutrophils. Reduced binding to FcγRIIIA is found e.g. for mutation of at least one of E233-G236, P238, D265, N297, A327, P329, D270, Q295, A327, S239, E269, E293, Y296, V303, A327, K338 and D376.

Mapping of the binding sites on human IgGl for Fc receptors, the above mentioned mutation sites and methods for measuring binding to FcγRI and FcγRIIA are described in Shields, R.L., et al. J. Biol. Chem. 276 (2001) 6591-6604.

The term "Fc receptor" when used herein refers to activation receptors characterized by the presence of a cytoplasmatic ITAM sequence associated with the receptor (see e.g. Ravetch, J.V., and Bolland, S., Annu. Rev. Immunol. 19 (2001) 275-290). Such receptors are FcγRI, FcγRIIA and FcγRIIIA. The antibodies according to the invention in one embodiment show a reduced binding to Fγ receptors, especially to FcγIIIA. In one embodiment the term "no binding of FcγR" means that in an antibody concentration of 10 μg/ml the binding of an antibody according to the invention to NK cells is 10 % or less of the binding found for anti-OX40L antibody LC.001 as reported in WO 2006/029879.

IgGl or IgG2 subclass and comprises mutation PVA236, GLPSS331, and/or L234A/L235A. An antibody according to the invention of IgG4 subclass comprises in one embodiment mutation L235E. In one embodiment further specific IgG4 mutations are S228P or L235E and S228P.

Cell-mediated effector functions of monoclonal antibodies can be enhanced by engineering their oligosaccharide component as described in Umana, P., et al., Nature Biotechnol. 17 (1999) 176-180; and US 6,602,684. IgGl 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.


Glycosylation of human IgGl or IgG3 occurs at Asn297 as core fucosylated biantennary complex oligosaccharide glycosylation terminated with up to 2 Gal residues. These structures are designated as GO, G1 (a 1,6 or a 1,3) or G2 glycan residues, depending from the amount of terminal Gal residues (Raju, T.S., Bioprocess Int. 1 (2003) 44-53). CHO type glycosylation of antibody Fc parts is e.g. described by Routier, F.H., Glycoconjugate J. 14 (1997) 201-207.

The "partially- or non-fucosylated anti-CD19 antibody" according to the invention can be expressed in a glycomodified host cell engineered to express at least one nucleic acid encoding a polypeptide having GnTIII activity in an amount sufficient
to partially fucosylate the oligosaccharides in the Fc region. In one embodiment, the polypeptide having GnTIII activity is a fusion polypeptide. Alternatively αL,6-fucosyltransferase activity of the host cell can be decreased or eliminated according to US 6,946,292 to generate glycomodified host cells. The amount of antibody fucosylation can be predetermined e.g. either by fermentation conditions (e.g. fermentation time), or by combination of at least two antibodies with different fucosylation amount. Measurement of the fucosylation is performed by Liquid chromatography-mass spectrometry (LC-MS). The term "a partially- or non-fucosylated anti-CD19 antibody" according to the invention when used herein refers to an anti-CD19 antibody showing a fucosylation at Asn297 of 50 % or lower compared with antibody 381 comprising human IgGl constant region of SEQ ID NO:26 and SEQ ID NO:27. Such an antibody is useful for the treatment of cancer diseases.

The partially- or non-fucosylated anti-CD19 antibody according to the invention can be produced in a host cell by a method comprising: (a) culturing a host cell engineered to express at least one polynucleotide encoding a fusion polypeptide having GnTIII activity under conditions which permit the production of said antibody with partial fucosylation of the oligosaccharides present on the Fc region of said antibody; and (b) isolating said antibody. In one embodiment, the polypeptide having GnTIII activity is a fusion polypeptide, especially comprising the catalytic domain of GnTIII and the Golgi localization domain of a heterologous Golgi resident polypeptide selected from the group consisting of the localization domain of mannosidase II, the localization domain of B(1,2)-N-acetylglucosaminyltransferase I ("GnTI"), the localization domain of mannosidase I, the localization domain of B(1,2)-N-acetylglucosaminyltransferase II ("GnTII"), and the localization domain of al-6 core fucosyltransferase. In one embodiment the Golgi localization domain is from mannosidase II or GnTI. In a further aspect, the invention is directed to a method for modifying the glycosylation profile of an anti-CD\(^\wedge^\) antibody by using such method.

An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region.

The term includes native sequence Fc regions and variant Fc regions. In one
embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991).

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the CDR and FR sequences generally appear in the following sequence in VH (or VL): FR1-CDR1(L1)-FR2-CDR2(L2)-FR3-CDR3(L3)-FR4.

The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

A "human consensus framework" is a framework which 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, E.A., et al., Sequences of Proteins of Immunological Interest, fifth edition, NIH Publication 91-3242, Bethesda, MD (1991), Vol. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat, E.A., et al., supra. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat, E.A., et al., supra.
A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human CDRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDRs correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

The term "complementarity determining regions" or "CDR," as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops) (Kabat, E.A., et al, Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991).) With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. Unless otherwise indicated, CDR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat, E.A., et al, supra.

An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s).

An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95 % or 99 % purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman, S., et al, J. Chromatogr. B 848 (2007) 79-87.

An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid
molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

"Isolated nucleic acid encoding an anti-CD19 antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

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 and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, 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 but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety. The naked antibody may be present in a pharmaceutical formulation.

"Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 Daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region
(VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU5 10087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid
sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \times \frac{X}{Y}$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject, a pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

The term "CD19," as used herein, refers to human B-lymphocyte antigen CD19 (alternative name(s) are: Differentiation antigen CD19, B-lymphocyte surface antigen B4, T-cell surface antigen Leu-12; UniProtKB P15391).

As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, decreasing the rate of disease progression, amelioration or palliation of the disease state. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.
The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt, et al, Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007)). A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Potolano, S., et al, J. Immunol. 150 (1993) 880-887; Clackson, T., et al, Nature 352 (1991) 624-628.

The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

II. Compositions and methods

In one aspect, the invention is based on antibodies that bind to CD19. Antibodies of the invention are useful, e.g., for the diagnosis or treatment of inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone diseases.

A. Exemplary Anti-CD19 Antibodies

In a further aspect of the invention, an anti-CD19 antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric or humanized antibody. In one embodiment, an anti-CD19 antibody is an antibody fragment, e.g., an Fv, Fab, Fab', scFv, diabody, or F(ab')2 fragment. In another embodiment, the antibody is a full length antibody, e.g., an intact IgGl antibody or other antibody class or isotype as defined herein.

In a further aspect, an anti-CD19 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in the sections below:
1. Antibody Affinity

In certain embodiments, an antibody provided herein has a dissociation constant \( K_D \) of \( 10^{-8} \) M or less, e.g. from \( 10^{-8} \) M to \( 10^{-13} \) M, e.g., from \( 10^{-9} \) M to \( 10^{-13} \) M.

According to another embodiment, \( K_D \) is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIacore, Inc., Piscataway, NJ) at 25 °C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, 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 sensograms. The equilibrium dissociation constant \( K_D \) is calculated as the ratio \( k_{off}/k_{on} \). See, e.g., Chen, Y., et al, J. Mol. Biol. 293 (1999) 865-881. If the on-rate exceeds \( 10^4 \) 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 25 °C 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 spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

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 (2003) 129-134. For a review of scFv fragments, see, e.g., Pluckthun, in The Pharmacology of
Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and US 5,571,894 and US 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 US 5,869,046.


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., US 6,248,516 Bl).

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. Multispecific Antibodies

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 CD19 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of CD19. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

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, C., and Cuello, Nature 305 (1983) 537-540), WO 93/08829, and Traunecker, A., et al, EMBO J. 10 (1991) 3655-3659), and "knob-in-hole" engineering (see, e.g., US 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004 A1); cross-linking two or more

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

The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to CD19 as well as another, different antigen (see, US 2008/0069820, for example).


4. Antibody Variants

a) Glycosylation variants

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.

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 (1997) 26-32. 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.


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 2003/01 1878, US 6,602,684, and US 2005/0123546. 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, WO 1998/58964, and WO 1999/22764.
b) Fc region variants

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 IgGl, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g., a substitution) at one or more amino acid positions.

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 FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcγR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch, J.V., and Kinet, J.P., Annu. Rev. Immunol. 9 (1991) 457-492. Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest is described in US 5,500,362 (see, e.g. Hellstrom, I., et al. Proc. Natl. Acad. Sci. USA 83 (1986) 7059-7063) and Hellstrom, I., et al, Proc. Natl. Acad. Sci. USA 82 (1985) 1499-1502; US 5,821,337 (see Bruggemann, M. et al, J. Exp. Med. 166 (1987) 1351-1361).

Alternatively, non-radioactive assays methods may be employed (see, for example, ACTITM 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, R., et al., Proc. Natl. Acad. Sci. USA 95 (1998) 652-656. Clq binding assays may also be carried out to confirm that the antibody is unable to bind Clq and hence lacks CDC activity. See, e.g., Clq 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, H., et al, J. Immunol. Methods 202 (1997) 163-171; Cragg, M.S., et al, Blood 101 (2003) 1045-1052; and Cragg, M.S., and M.J., Glennie, Blood 103 (2004) 2738-2743). 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. Immunol. 18 (2006) 1759-1769).

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 (US 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 7,332,581).


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).

In some embodiments, alterations are made in the Fc region that result in altered (i.e., either improved or diminished) Clq binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US 6,194,551, WO 99/51642, and Idusogie E.E., et al. J. Immunol. 164 (2000) 4178-4184.

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, R.L., et al, J. Immunol. 117 (1976) 587-593) and Kim, J.K., et al, J. European J of Immunol. 24 (1994) 2429-2434), are described in US 2005/0014934A1 (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 7,371,826).


c) Cysteine engineered antibody variants

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 US 7,521,541.

d) Antibody Derivatives

In certain embodiments, an antibody provided herein may be further modified to contain additional non-proteinaceous 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)poly ethylene glycol, propylene 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 is 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.

In another embodiment, conjugates of an antibody and non-proteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the non-proteinaceous moiety is a carbon nanotube (Kam, N.W., et al, Proc. Natl. Acad. Sci. USA 102 (2005) 11600-1 1605). 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 non-proteinaceous moiety to a temperature at which cells proximal to the antibody-non-proteinaceous moiety are killed.

**B. Recombinant Methods and Compositions**

Antibodies may be produced using recombinant methods and compositions, e.g., as described in US 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-CD 19 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, NSO, Sp20 cell). In one embodiment, a method of making an anti-CD 19 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).

For recombinant production of an anti-CD 19 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).

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., US 5,648,237, US 5,789,199, and US 5,840,523. (See also Charlton, K.A., Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2004), 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.

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 (2004) 1409-1414, and Li et al, Nat. Biotech. 24 (2006) 210-215.

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 *Spodopterafrugiperda* cells.

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

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. F.L., et al, J. Gen Virol. 36 (1977) 59-74; baby hamster kidney cells (BHK); mouse Sertoli cells (TM4 cells as described, e.g., in Mather, J.P., Biol. Reprod. 23 (1980) 243-252); monkey kidney cells (CVI); 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, J.P., et al, Annals N.Y. Acad. Sci. 383 (1982) 44-68; MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR™ CHO cells (Urlaub, G., et al, Proc. Natl. Acad. Sci. USA 77 (1980) 4216-4220); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable

C. Methods and Compositions for Diagnostics and Detection

In certain embodiments, any of the anti-CD19 antibodies provided herein is useful for detecting the presence of CD19 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as tumor tissue.

In one embodiment, an anti-CD19 antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of CD19 in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-CD19 antibody as described herein under conditions permissive for binding of the anti-CD19 antibody to CD19, and detecting whether a complex is formed between the anti-CD19 antibody and CD19. Such method may be an in vitro or in vivo method. In one embodiment, an anti-CD19 antibody is used to select subjects eligible for therapy with an anti-CD19 antibody, e.g., where CD19 is a biomarker for selection of patients.

Exemplary disorders that may be diagnosed using an antibody of the invention are inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone diseases.

In certain embodiments, labeled anti-CD19 antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes $^{32}$P, $^{14}$C, $^{125}$I, $^{3}$H, and $^{131}$I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (US 4,737,456), luciferin, 2,3-dihydropthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or
microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

**D. Pharmaceutical Formulations**

Pharmaceutical formulations of an anti-CD19 antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyl dimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinyl pyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rhuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rhuPH20, are described in US 2005/0260186 and US 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycansases such as chondroitinases.

Exemplary lyophilized antibody formulations are described in US 6,267,958. Aqueous antibody formulations include those described in US 6,171,586 and WO 2006/044908, the latter formulations including a histidine-acetate buffer.

The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with
complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methyl methacrylate) microcapsules, respectively, in colloidial drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed.), (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

**E. Therapeutic Methods and Compositions**

Any of the anti-CD19 antibodies provided herein may be used in therapeutic methods.

In one aspect, an anti-CD19 antibody as reported herein for use as a medicament is provided. In further aspects, an anti-CD19 antibody as reported herein for use in treating inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone diseases is provided. In certain embodiments, an anti-CD19 antibody as reported herein for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-CD19 antibody for use in a method of treating an individual having inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone disease comprising administering to the individual an effective amount of the anti-CD19 antibody as reported herein. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In further embodiments, the invention provides an anti-CD19 antibody for use in blocking B-cell activation without causing B-cell depletion. In certain embodiments, the
invention provides an anti-CD19 antibody for use in a method of blocking B-cell activation without causing B-cell depletion in an individual comprising administering to the individual an effective amount of the anti-CD19 antibody as reported herein. An "individual" according to any of the above embodiments is in one embodiment a human.

In a further aspect, the invention provides for the use of an anti-CD19 antibody as reported herein in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone disease. In a further embodiment, the medicament is for use in a method of treating inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone disease an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In a further embodiment, the medicament is for blocking B-cell activation without causing B-cell depletion. In a further embodiment, the medicament is for use in a method blocking B-cell activation without causing B-cell depletion in an individual comprising administering to the individual an amount effective of the medicament. An "individual" according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for treating an inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone disease. In one embodiment, the method comprises administering to an individual having such inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone disease an effective amount of an anti-CD19 antibody as reported herein. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An "individual" according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for blocking B-cell activation without causing B-cell depletion in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-CD19 antibody as reported herein. In one embodiment, an "individual" is a human.
In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-CD19 antibodies provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-CD19 antibodies provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-CD19 antibodies provided herein and at least one additional therapeutic agent, e.g., as described below.

Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies of the invention can also be used in combination with radiation therapy.

An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder
or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1 mg/kg - 10 mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. An exemplary dosing regimen comprises administering about 4 to 10 mg/kg. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-CD19 antibody.

F. Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described
above is provided. The article of manufacture 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 of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture 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.

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-CD19 antibody.

III. Description of the Sequence Listing

SEQ ID NO: 1  heavy chain variable region (VH) of Mab381
SEQ ID NO: 2  heavy chain CDRH1 Mab381
SEQ ID NO: 3  heavy chain CDRH2 Mab381
SEQ ID NO: 4  heavy chain CDRH3 Mab381
SEQ ID NO: 5  light chain variable region (VL) of Mab381
SEQ ID NO: 6  light chain CDRL2 Mab381
SEQ ID NO: 7  light chain CDRL3 Mab381
SEQ ID NO: 8  heavy chain variable region (VH) of Mab396 (CD HA)
SEQ ID NO: 9  heavy chain CDRH1 Mab396
IV. Antibody Nomenclature

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<tr>
<th>Antibody or antibody variable region</th>
<th>Sequence Number</th>
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<tbody>
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<td>Mab381</td>
<td>1  5  2  3  4  20  6  7</td>
</tr>
<tr>
<td>Mab396</td>
<td>8  12(LB) 9  10  11  13  14  15</td>
</tr>
<tr>
<td>CD HA</td>
<td>8  9  10  11</td>
</tr>
<tr>
<td>CD HB&lt;sup&gt;1)&lt;/sup&gt;</td>
<td>T28A 9 10 11</td>
</tr>
<tr>
<td>CD HC</td>
<td>T28A 9 16 11</td>
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Sequence Number

<table>
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<th>17</th>
<th>11</th>
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<td>9</td>
<td>10</td>
<td>11</td>
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<tr>
<td></td>
<td>V11L</td>
<td></td>
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<tr>
<td></td>
<td>K12V</td>
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<tr>
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<td>T108S</td>
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<td></td>
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<tr>
<td>CD LA</td>
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<tr>
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<td>P80S</td>
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<tr>
<td></td>
<td>F83L</td>
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</table>

1) CD HB is a heavy chain variable region comprising T28A mutation in SEQ ID NO: 8; T28A means that amino acid T of position 28 of SEQ ID NO: 8 is replaced by amino acid A; etc. CD HB comprises CDRHI of SEQ ID NO: 9, CDRH2 of SEQ ID NO: 10, and CDRH3 of SEQ ID NO: 11.

2) CD LE is a light chain variable region comprising P80S and F83L mutation in SEQ ID NO: 12. P80S means that amino acid P of position 80 of SEQ ID NO: 12 is replaced by amino acid S; etc. CD LE comprises CDRL1 of SEQ ID NO: 20, CDRL2 of SEQ ID NO: 14, and CDRL3 of SEQ ID NO: 15.

Mab396 and all CD HX and CD LX regions are humanized versions of Mab381 with analog nomenclature as CD HB and CD LE.

V. EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.
Example 1

Immunization and generation of mouse anti-hCD19 antibodies (hybridomas)

Balb/c mice were immunized six times and boosted with CD19-transfected HEK293 cells (mean receptor density 35,000 per cell). The immune response was monitored by testing serum samples with a CD19 - cell-ELISA on CD19-transfected NIH-3T3 cells. Spleen cells from mice with sufficient titers of anti-CD\(^\wedge\) immunoglobulin were used for immortalization by fusion with mouse myeloma cell line P3X63 Ag8.653. Three fusions were carried out and hybridoma supernatants screened by cell-ELISA on CD19-transfected NIH-3T3 cells and FACS binding assay using Daudi (CD19\(^+\)) and CD19\(^-\) cells for anti-CD19 specific antibodies.

The positive hybridoma 8C3, producing Mab381, was selected for the further evaluation based on the combination of the assays and properties. Mab381 was selected as preferred antibody due to its biochemical and functional properties described in the following. The functional properties of the antibody in biochemical and cellular assays are described in examples 2-8. A property of the hybridoma 8C3 and antibody Mab381, derived from this hybridoma, as well as for the humanized version Mab396 and the other humanized light and heavy chain versions, for the selection was their inhibitory activity on activated primary B-cells.

Purified antibody from in-vitro production of the 8C3 hybridoma was used for characterization in different biochemical and cellular assays (see below). The hybridoma 8C3 was cloned by single cell FACS sorting.

Example 2

Hybridoma screening and cell biological functional evaluation of anti-CD19 antibody

Cell-ELISA for screening antibodies against hCD19

A cell ELISA was applied for screening of hybridomas, and to identify those hybridomas that secrete antibodies against human-CD19. NIH3T3 cells transfected with human-CD19 were used as positive cells; untransfected NIH3T3 cells were used as negative control cells. For the assessment of the positive hybridomas the OD ratio between transfected and untransfected NIH3T3 cells was quantified.
Culture Medium: DMEM high glucose (4.5 mg/ml), 10 % FCS, Na-Pyruvate, NEAA, Glutamin
- Antibodies positive control: anti CD19 monoclonal antibody (IgG1) Pharmingen Cat# 555409 c = 1 mg/ml
- Detection antibody: Goat anti-Mouse IgG (H+L) HRP Conjugate Bio-Rad Cat# 170-06516
- Dilution 1:2000 in 1x ELISA Blocking Reagent
- Other reagents: Fibronectin Roche Cat# 838039 c = 1 mg/ml
- Glutardialdehyde: 25 % stock solution // Grade Agar Scientific #R102 final concentration: 0.05 % in PBS
- ELISA Blocking Reagent: 10x stock solution // Roche Cat# 1112589
- TMB substrate: Roche Cat# 11432559
- Stop Solution: 1 M H₂SO₄
- BioRad Cat# 170-6516 Dilution 1:2000 in 1x ELISA Blocking Reagent

Day 1:
- Fibronectin coating: 5 µg/cm² in PBS; 96well plate = 32 cm²; 160 µg/plate in 6 ml
- PBS, 50 µl/well
- incubate 45 min at RT, aspirate coating solution
- Seed 1.25 x 10⁴ cells/well in 50 µl culture medium in a 96well plate
- incubate 40 hours at 37 °C
- add to upper half of the plate: NIH3T3 cells expressing CD19
- add to lower half of the plate: non-transfected NIH3T3 cells

Day 3:
- Addition of positive control antibody or samples (supernatant or mouse serum) in 50 µl culture medium
- incubate for 2 h at 4 °C
- Remove medium, fix cells with 100 µl Glutardialdehyde (0.05 % in PBS)
- Wash two times with 200 µl PBS
- Addition of detection antibody 1:2000, 50 µl/well
- incubate 2 h at RT
- wash three times with 200 µl PBS
- add 50 µl TMB, incubate for 30 min. at RT,
- stop by addition of 25 µl 1 M H₂SO₄; read extinction at 450nm/620nm
- Calculation of results: ratio OD NIH3T3 CD19 : OD NIH3T3 untransfected
Results:
The antibody Mab381 demonstrates specific binding to CD19 transfected NIH3T3 cells as compared to untransfected NIH3T3 cells (ratio OD NIH3T3 CD19 to OD NIH3T3 untransfected = 11.4).

Example 3
FACS Binding of anti-CD19 antibodies on DAUDI cells

To evaluate the binding potency of and antibody according to the invention, e.g. Mab381, to human CD19 expressed on the cell surface the human B-cell line Daudi was used. Daudi cells were incubated with different concentrations of the purified Mab381 and in a second step with a goat-anti-mouse labeled antibody. Quantification of the binding was performed by FACS analysis; the EC50 value for the binding potency was calculated.

Material & Method

- Cell line: Daudi (Human Burkitt Lymphoma) ATCC-Nr. CCL-213
- Culture Medium: RPMI1640 supplemented with 10% FCS, 2 mM L-Glutamine, 100 µM NEAA, 1 mM Na-Pyruvate
- Positive Control: anti-CD19 antibody SJ25-C1 c = 0.31 mg/ml
- Isotype Control: mlgGl kappa Sigma Cat# M5284 Lot#023K4855 c = 0.2 mg/ml
- Detection antibody: goat anti mouse RPE F(ab')2 IgG (H+L) Caltag Cat# M355-004-3 Lot#26020107 c = 0.2 mg/ml
- Sample: purified antibody Mab381.
- 3-5×10⁵ cells per sample in 12 x 75 mm FACS-tube were stained with 100 µι antibody solution
  - incubate in PBS/10 % FCS 20 min. on ice
  - Antibody concentrations: Samples and positive control: from 30 to 0.04 µg/ml in 1:3 dilutions
  - Isotype control: 1 µg/100 µι
- Wash one with 3 ml PBS/10 % FCS
- Addition of 100 µι detection antibody (1 µg/100 µι); incubation 15 min. on ice
- Wash once with 3 ml PBS/10 % FCS
- Resuspend pellet in 500 µι PBS/10 % FCS and analyze in FACS-Calibur.
Antibody Mab381 shows binding to Daudi cells as determined by FACS analysis (EC$_{50}$ mean = 1.45 nM). Results are shown in table 1.

### Table 1: Comparison of Mab381 and humanized Mab396

<table>
<thead>
<tr>
<th>Antibody</th>
<th>IC$_{50}$ µg/ml</th>
<th>MFI at saturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mab381</td>
<td>0.15</td>
<td>65.8</td>
</tr>
<tr>
<td>Mab396</td>
<td>0.2</td>
<td>36.9</td>
</tr>
</tbody>
</table>

#### Example 4

**CD19 phosphorylation inhibiting assay / Western blot analysis of phospho-CD19 (Tyr531) with human RAMOS cells**

CD19 is a part of the B-cell co-receptor. On the cellular level stimulation of the B-cell coreceptor is possible with an anti-human-IgM antibody. The activated CD19 can be detected with an anti-phospho-CD19 (Tyr531) antibody. The Ramos cell line (human B-cell lymphoma) was used and the quantification of the phosphorylated CD19 was done in the cell extracts by Western-Blot-Analysis with a Lumi-Imager™.

### Material & Method:

- Cell line: RAMOS (ATCC CRL 1596, human, Caucasian, lymphoma, Burkitt)
- Pre-Incubation with anti-CD19 antibody
- Stimulation: anti-human IgM (Pharmingen, Cat-Nr.: 555780) 12 µg/ml for 2 minutes
- Extracts: 2 x 10$^6$ cells in modified RIPA-buffer (+ 2 mM Na-Orthovanadate)
- Gel: 7.5 % Tris-HCl Ready Gel, Biorad
- Nitrocellulose membrane: Trans-Blot$^R$ Transfer Medium (7 x 8.4 cm), Biorad
- Blotting: 30 min. in SEMI-Dry Blotting Apparatus (Biorad), 20 V const.
- Primary antibody: Anti-Phospho-CD19 (Tyr531), polyclonal rabbit, Cell Signaling #3571
- Secondary antibody: Anti-Rabbit-POD (Roche, Chemiluminescence Western Blotting Kit) 1:1000
- Detection: Lumi-Light$^PLUS$ Western Blotting Substrate (Roche)
Results:
Antibody Mab381 (1 µg/µl and 10 µg/µl) induces inhibition of anti-IgM stimulated phospho Tyr531-CD19 levels. Level of inhibition reaches non-stimulated control.

Example 5
Internalization / Down-modulation of CD19 in RAMOS cells

Goal was to test whether Mab381 causes internalization/down-modulation of the CD19 receptor from the cell surface after binding. Ramos cells were incubated for 3 hours with antibodies, and a PE labeled goat-anti-mouse-antibody was used as detection antibody. The cell surface staining was analyzed by FACSscan flow cytometer. The internalization was calculated applying a specific formula (see below) under consideration of the non-modulated probe (cells with antibody incubated by 0°C).

Material & Method

Modulation of CD19 induced by anti-CD19 antibodies was evaluated by flow cytometry.

Cells: RAMOS (ATCC CRL 1596, human, Caucasian, lymphoma, Burkitt) cell line.

After centrifugation at 200 g 5 x 10^5 cells in RPMI1640 medium supplemented with 10 % FCS were mixed at 0 °C with 2.5 µg/ml anti-CD19 antibodies for 45 min. (100 µl/5 x 10^5 cells).

The suspensions were then incubated at 37 °C for 15 min. up to 18 hours in the continuous presence of antibody, so that the dissociation rate was negligible, and that the antigen was always saturated. Other samples treated with antibody were kept at 0 °C to evaluate the level of antigen expression on non-modulated cells.

After two washings with cold medium cells were stained with Goat F(ab')2 anti-mouse IgG (H+L) PE (see example 3) for 30 min. at 0 °C, washed twice again and kept in 1 ml cold medium on ice to measure the amount of surface-bound antibody on the cell. Ten thousand events were analyzed on a FACSscan™ flow cytometer (Becton Dickinson). The extent of modulation at 37 °C was calculated for each sample as the percentage of MFI in relation to non-modulated cells according to the equation (calculation formula):
Modulation (%) = 100 - 100 x (MFI [sample] - MFI [neg. control]/MFI [non-modulated cells]- MFI [neg. control]).

Results:
Down modulation was calculated after 3 hours incubation of Ramos cells with the antibodies (table 2).

Table 2:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>MFI (mean fluorescence) 0 °C</th>
<th>MFI (mean fluorescence) 37 °C</th>
<th>Modulation [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse IgG1, control</td>
<td>2.26</td>
<td>2.72</td>
<td>No binding</td>
</tr>
<tr>
<td>Mab381</td>
<td>15.04</td>
<td>8.23</td>
<td>56.9</td>
</tr>
</tbody>
</table>

Mab381 shows an internalization rate of 57% after 3 hours.

Example 6
Proliferation assay by the B-cell lines SU-DH4 and RCHACV

CD19 as a member of the B-cell-co-receptor may play a role for proliferation of B-cells. The inhibitory activity of Mab381 is determined for the proliferation of human B-cell lines with different expression levels of CD19 and CD20 (see below). Cells were incubated with antibodies for 3 days. Viable cells after this incubation period were analyzed and calculated by FACS (FACSAarray™ BD Biosciences).

Material & Method

- Cell lines: human B-cell lymphoma SU-DHL4 (CD19+ / CD20+) (DSMZ # ACC 495),
- human B-cell precursor leukemia RCHACV (CD19+ / CD20−) (DSMZ #ACC 548)
- human Plasmacytoma (B lymphocyte) RPMI 8226 (CD19− / CD20+) (ATCC, CCL 155)
- Medium: RPMI 1640 with 10% FBS and 2 mM L-Glutamin
- Plates: 96-well plates (Costar # 3799)
Antibodies

Anti CD20 recombinant human monoclonal antibody $c = 10 \text{ mg/ml}$

Mouse Anti-human CD19 Antibody IgG1 (PharMingen) $c = 1.05 \text{ mg/ml}$

Clone HD37 (Chemicon) CD19_HD37; $c = 0.56 \text{ mg/ml}$

Mouse Anti-human CD19 Antibody IgG1 (Cymbus) CD19_SJ25-C1 $c = 0.31 \text{ mg/ml}$

Mouse Anti-human CD19 Antibody IgG1 Mab381 $c = 1.22 \text{ mg/ml}$

Day 1:

Seed cells with $2 \times 10^4$ cells/well in 100 µl culture medium in 96-well round bottom plate. Add antibodies diluted in culture medium 2-fold of the desired concentrations in 100 µl/well. Incubate for 72 h at 37 °C, 5 % CO$_2$, 95 % rH.

Day 3:

Analyze viable cell number by FACS (FACSArray™ BD Biosciences) and calculate % inhibition of proliferation. Maximal inhibition of proliferation achieved with the antibody 381 was 25-39 % at a concentration of 2.5 - 10 µg/ml (values at an antibody concentration of 10 µg/ml: SU-DHL4: 37 %; RCHACV 25 %; RPMI 8226: 0 %). The antibody shows no inhibition of proliferation of a CD19 negative cell line.

**Example 7**

**Proliferation assay on B-cells isolated from Leucapheresis**

Proliferation-inhibitory activity of Mab381 was tested using primary human B-cells after stimulation with a combination of an anti-IgM-antibody and h-IL4. The primary B-cells were isolated from Leucapheresis material from different healthy donors applying a negative selection process (depletion of non-B-cells); purity of the B-cells used in these experiments was >90 %. The cells were incubated with antibodies for 7 days; proliferation rate was measured using a BrdU assay.

**Material & Method**

B-cells isolated from Leucapheresis Material of healthy donors isolated by depletion of non-B-cells (negative selection) with Magnetobeads (Dynal® B-cell Negative Isolation Kit [CD2,14,16,36,43,235a]).
B-cells; purity 92% - 96%; thawed, viability 90-96%. 2 x wash and seeded ~2-3 x 10^5 cells/well in 100 µl RPMI/2% FBS/NEAA/pen-strep in 96 well plate. Anti-hCD19 antibodies were added in concentration from 0.08 µg/ml to 10 µg/ml dilution 1:5 (positive control - SJ25-C1 and MabThera). One hour preincubation with anti-CD19 antibodies in incubator at 37°C/5% CO₂. Cells were stimulated with 10 µg/ml anti-IgM/20 ng/ml IL-4; end Volume = 200 µl/well; incubation for 7 days at 37°C/5% CO₂; read out BrdU Assay.

Materials:
1. Medium: RPMI +1% L-Glutamin, PAN Biotech, Cat. No P04-17500; Lot No 030905; Exp: 09/2007 with 10% FBS, ultra low IgG, Gibco, Lot No 3102090A; NEAA, PAN Biotech, Cat. No 08-32100; Lot No 420506; Pen/Strep, Roche, 50,000 U/ml/50 mg/ml
2. Read out: BrdU, Roche, Cat No: 1 647 229;
3. Plate: NUNC 96 MicroWell™ Plate, Flatbottom, Cat No 167008,
4. for Stimulation: Anti IgM (AffiniPure F(ab')₂ Fragment Goat Anti-human IgA + IgG + IgM (H+L), Dianova, code 109-006-064, Lot. 67679) 1.3 mg/ml Human, recombinant IL-4

Results:
The antibody Mab381 shows a significant inhibition of proliferation of primary B-cells from three donors stimulated with anti-IgM and IL-4 (table 4).

Table 4:

<table>
<thead>
<tr>
<th>Antibody [µg/ml]</th>
<th>% Inhibition of proliferation; B-cells from Leukapheresis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donor 1</td>
</tr>
<tr>
<td>2 (Mab381)</td>
<td>34%</td>
</tr>
<tr>
<td>10 (Mab381)</td>
<td>45%</td>
</tr>
<tr>
<td>2 ((Mab396 = 381LBHA))</td>
<td>83%</td>
</tr>
<tr>
<td>10 (Mab396 = 381LBHA)</td>
<td>91%</td>
</tr>
</tbody>
</table>
Example 8
Ca(2+) Influx Assay with the Ramos cell line

B-cells show changes of intracellular Ca^{2+} levels upon activation. Goal here was to show the inhibitory activity of Mab381 on activation of B-cells with Ca^{2+} Influx as read out. The Ramos cell line was used for these studies. Modulation of the Ca^{2+} Influx (changes in intracellular calcium) was measured using fluorescent Ca^{2+} indicators. After loading cells with the calcium sensitive fluorescent dye, cells were incubated with antibodies for 2.5-3.0 hours. Quantification of fluorescence was done by using the Flex Station workstation.

Material & Method

Modulation of Ca^{2+} Influx induced by anti-CD19 antibodies in Ramos cells was evaluated by FLIPR Calcium Kit/Flex Station™ (Molecular Devices)-Fluorescence-based assay for changes detecting in intracellular calcium. After centrifugation at 200 g about 2 x 10^{5} cells in HBSS medium (50 µl/well) were seeded in 96 well plate (black with clear bottom). The antibodies were added - 25 µl/ well in end concentrations - from 10 µg/ml to 0.001 µg/ml (positive control-SJ25-C1). The cells were then incubated in the incubator at 37 °C/5 % CO_{2} for 1 hour in the continuous presence of antibody. The Loading puffer added (loading the cells with calcium sensitive fluorescent dye) - 100 µl/well. The antibodies were added - 25 µl/ well. The cells were then incubated in the incubator at 37 °C/5 % CO_{2} for 1 hour in the continuous presence of antibody. And 30 to 60 min at RT for a total time of incubation with anti-CD19 antibodies 2.5-3 hours. Fluorescence monitored in Flex Station™ after addition 3 µg/ml of anti-IgM antibodies to the cells. Flex Station™ is benchtop scanning fluorometer with integrated fluid transfer workstation; the machine monitors the wells over a time course, during which compounds can be added robotically. Responses were measured as peak fluorescence intensity minus basal fluorescent intensity.

Result:

In Ramos cells after activation, the antibody Mab381 inhibits Ca^{2+} Influx by an EC_{50} of 0.37 nM (56 ng/ml, with a maximal inhibition of 50 %) and the antibody Mab396 inhibits Ca^{2+} Influx by an EC_{50} of 0.20 nM (30 ng/ml, with a maximal inhibition of 50 %).
Example 9
Sequencing of anti-human CD19 mouse hybridoma monoclonal antibody Mab381

The murine monoclonal antibody Mab381 expressed by a hybridoma cell line was cloned to determine the protein sequence of the antibody. The isotype of the murine antibody was immunologically identified as IgGl and kappa.

Example 10
Humanization of anti-CD19 antibody Mab381

The CD19 binding specificity of the murine antibody Mab381 was transferred onto a human acceptor framework to eliminate potential immunogenicity issues arising from sequence stretches that the human body will recognize as foreign. This was done by engrafting the entire complementary determining regions (CDR) of the murine (donor) antibody onto a human (acceptor) antibody framework, and is called CDR-grafting or antibody humanization.

The murine amino acid sequence was aligned with a collection of human germ-line antibody V genes, and sorted according to sequence identity and homology. Before selecting one particular acceptor sequence, the so-called canonical loop structures of the donor antibody have to be determined (Morea, V., et al, Methods, Vol 20, Issue 3 (2000) 267-279). These canonical loop structures are determined by the type of residues present at the so-called canonical positions. These positions lie (partially) outside of the CDR regions, and have to be kept functionally equivalent in the final construct in order to retain the CDR conformation of the parental (donor) antibody. The human germ-line sequence IGHVI-69*06 (IMGT Acc No L22583; old nomenclature: DP88) was chosen as the acceptor for the heavy chain and sequence IGKV1-9 (IMGT Acc No. Z00013) was chosen for the light chain.

Table 5: Determination of the canonical loop structures of Mab381

<table>
<thead>
<tr>
<th>Determination of canonical classes of CDR loops</th>
<th>CDR residues</th>
<th>CDR length</th>
<th>Canonical Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDR-L1</td>
<td>L2=I; L25=A; L29=V; L30=G; L33=V; L71=F.</td>
<td>11 aa</td>
<td>Class 2A</td>
</tr>
<tr>
<td>CDR-L2</td>
<td>L34=A</td>
<td>7 aa</td>
<td>Class 1</td>
</tr>
</tbody>
</table>
Determination of canonical classes of CDR loops CDR residues CDR length

<table>
<thead>
<tr>
<th>Canonical Structure</th>
<th>(L48=I; L64=G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDR-L3</td>
<td>L90=Q; L94=Y; L95=P; L97=T</td>
</tr>
<tr>
<td>CDR-H1</td>
<td>H24=A; H26=G; (H27=Y); H29=F; H34=M; H94=R</td>
</tr>
<tr>
<td>CDR-H2</td>
<td>H52a=P; [H54=D]; H55=G; H71=A;</td>
</tr>
</tbody>
</table>

The canonical structure class is: 1-2-2-1-1. (H1-H2-L1-L2-L3)

The potential acceptor sequence was selected based on high overall homology and the presence of the right canonical residues already in the acceptor sequence. The humanized constructs are labeled CD LA, CD LB, CD LC, CD LD, and CD LE for the light chain, and CD HA, CD HB, CD HC, CD HD, CD HE, and CD HF for the heavy chain.

Example 11

Binding of humanized antibodies compared to murine antibody

Daudi target cells were incubated with the antibodies and binding was quantified using a fluorescently labeled secondary antibody and subsequent analysis via flow cytometry (FACS). The EC50 values of the parental murine antibody and the humanized variants are essentially unchanged. The different signal amplitude of the murine antibody compared to the humanized ones is due to a different secondary antibody used for detection of human or murine Fc.

Comparison of binding affinity of different humanized and chimeric Mab381 variants, as determined with flow cytometry is shown in Figure 1 and Figure 2.

Example 12

Binding to Clq (competence for CDC)

The binding property of the antibody to the Clq protein of the human complement system is an indicator for its CDC potency. Different variants of the humanized Mab381 (such as Mab396) produced in HEK293 or CHO cells were tested by Clq-ELISA. Result: The humanized antibody Mab396 did not show any binding
activity to the human Clq protein; therefore the expectation is that it will not trigger CDC.

**Example 13**

Glycoengineering of Mab396 (Mab396-g2 (an antibody with low fucosylation, see WO 99/54342))

The expression system comprises the MPSV promoter system for transient expression and is described in tables 6 and 7.

**Table 6: pETR 4078 / pETR3388 (antibody expression vectors)**

<table>
<thead>
<tr>
<th>Element</th>
<th>Length [bp]</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mab396 HC (pETR4078)</td>
<td>1413</td>
<td>encoding heavy chain of Mab396</td>
</tr>
<tr>
<td>Mab396 LC (pETR3388)</td>
<td>711</td>
<td>encoding light chain of Mab396</td>
</tr>
<tr>
<td>MPSV</td>
<td>640</td>
<td>promoter</td>
</tr>
<tr>
<td>oriP</td>
<td>1976</td>
<td>origin of replication</td>
</tr>
</tbody>
</table>

**Table 7: pETR1519 / pCLF9 (glycosylation vectors)**

<table>
<thead>
<tr>
<th>Element</th>
<th>Length [bp]</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPSV</td>
<td>640</td>
<td>promoter</td>
</tr>
<tr>
<td>GnTIII (ManII stem region)</td>
<td>1716</td>
<td>encoding N-acetylglucosaminyl-transferase III</td>
</tr>
<tr>
<td>ManII</td>
<td>3435</td>
<td>Encoding mannosidase II</td>
</tr>
<tr>
<td>oriP</td>
<td>1976</td>
<td>origin of replication</td>
</tr>
</tbody>
</table>

All four plasmids were transfected in HEK293 EBNA host cells using a Ca\(^{2+}\)-phosphate dependent co-transfection procedure. For antibody expression transfected cells were incubated in DMEM/10 % FCS at 37 °C in humidified incubators with a 5 % CO\(_2\) atmosphere for seven days. Mab396-g2 was isolated from the supernant and purified by chromatographic methods.
Example 14
ADCC assay

The ADCC potency of the humanized antibody 396 was investigated. The ADCC was performed using the Raji cell line and a high affinity Fcgamma-Receptor expressing cell line as effector cells. The read out for killing of Raji cells was LDH release. It was found, that the antibody does not show any ADCC. Mab396 (wt Fc region) and Mab396 with mutations L234A/L235A (LALA-Fc region) did not mediate significant amount of ADCC. Only by using very high concentrations of up to 300 ng/ml a moderate ADCC effect (15%) has been observed. In contrast, Mab396 with low fucosylation (Mab396-g2, see WO 99/54342 and example 13) shows already ADCC mediated cell killing at lower concentrations. Results are shown in Figure 3.

Example 15
Whole blood (B-cells) depletion assays

Goal was to evaluate the depletion potency of the antibody 381 and its humanized variants in human B-cells in-vitro. Whole Blood samples (after erythrocyte removal) from different healthy donors were incubated for 24 hours with the antibodies; B-cell depletion was calculated by the ratio: B-cells (CD19 positive cells): T-cells (CD3 positive cells) with FACS analysis.

A human/mouse chimeric variant of Mab381 antibody, the construct with chimeric heavy chain and light chain LB, as well as light chain LE combined with heavy chain HF do not mediate significant B-cell depletion. The other humanized variants do induce B-cell depletion.

Figures 4 and 5 show the results of a B-cell depletion experiment, where human whole blood samples were incubated for 24 hours with the indicated antibody samples (ch: chimeric; LBHch: LB and heavy chain chimeric 8C3 Mab381)). B-cell depletion was measured by counting the CD19 positive cells stained with a labeled anti-CD20 antibody in a flow cytometer (after erythrocyte removal by cell lysis) relative to the CD3 positive cells as a reference. Constructs that are comprised of the heavy chain variants HA, HB, or HD show a B-cell depleting behavior, whereas constructs having the chimeric heavy chain (either together with the chimeric light chain, or the humanized light chain LE), or the heavy chain variants HE, and HF show a strongly reduced activity. In both experiments, variants HE and HF show B-cell depletion activity at the same low level as the
parental antibody, or even less. The humanized variant antibody 396 shows a depletion potency of human B-cells of 28%. This depletion potency is achieved at a concentration of 5 ng/ml and remains with higher concentrations.

**Example 16**

**Cross-reactivity of anti-CD19 antibodies**

Cross-reactivity was measured by FACS using B-cells of the respective species. It was found that Mab381 and its humanized variants show cross-reactivity with human and chimpanzee CD19 only, but not with cynomolgus, dog, rabbit, hamster, mouse and other species investigated.
Patent Claims

1. An isolated antibody that binds to CD19, characterized in binding to the same CD19 epitope to which monoclonal antibody 381 binds, wherein antibody 381 is characterized by comprising as heavy chain variable region SEQ ID NO:1 and as light chain variable region SEQ ID NO:5.

2. The antibody of claim 1, characterized in being a human, humanized, or chimeric antibody.

3. The antibody according to any one of claims 1 to 2, characterized in that the heavy chain variable domain comprises as CDRs:

   a) a CDRH1 region of SEQ ID NO: 2, a CDRH2 region of SEQ ID NO: 3 and a CDRH3 region of SEQ ID NO: 4,
   b) a CDRH1 region of SEQ ID NO: 9, a CDRH2 region of SEQ ID NO: 10 and a CDRH3 region of SEQ ID NO: 11,
   c) a CDRH1 region of SEQ ID NO: 9, a CDRH2 region of SEQ ID NO: 16 and a CDRH3 region of SEQ ID NO: 11, or
   d) a CDRH1 region of SEQ ID NO: 9, a CDRH2 region of SEQ ID NO: 17 and a CDRH3 region of SEQ ID NO: 11.

4. The antibody according to any one of claims 1 to 3, characterized in that the light chain variable domain comprises as CDRs:

   a) a CDRL1 region of SEQ ID NO: 20, a CDRL2 region of SEQ ID NO: 6 and a CDRL3 region of SEQ ID NO: 7,
   b) a CDRL1 region of SEQ ID NO: 13, a CDRL2 region of SEQ ID NO: 14 a CDRL3 region of SEQ ID NO: 15,
   c) a CDRL1 region of SEQ ID NO: 13, a CDRL2 region of SEQ ID NO: 18 and a CDRL3 region of SEQ ID NO: 15,
   d) a CDRL1 region of SEQ ID NO: 19, a CDRL2 region of SEQ ID NO: 14 and a CDRL3 region of SEQ ID NO: 15, or
   e) a CDRL1 region of SEQ ID NO: 20, a CDRL2 region of SEQ ID NO: 14 and a CDRL3 region of SEQ ID NO: 15.

5. The antibody according to any one of claims 1 to 4, characterized in that the heavy chain variable domain and the light chain variable domain comprises as CDRs.
a) a CDRH1 region of SEQ ID NO: 2, a CDRH2 region of SEQ ID NO: 3 and a CDRH3 region of SEQ ID NO: 4, and a CDRL1 region of SEQ ID NO: 20, a CDRL2 region of SEQ ID NO: 6 and a CDRL3 region of SEQ ID NO: 7, or

b) a CDRH1 region of SEQ ID NO: 9, a CDRH2 region of SEQ ID NO: 10 and a CDRH3 region of SEQ ID NO: 11, and a CDRL1 region of SEQ ID NO: 13, a CDRL2 region of SEQ ID NO: 14 a CDRL3 region of SEQ ID NO: 15.

6. The antibody according to any one of claims 1 to 5, characterized in that the antibody comprises as heavy chain variable domain

a) the heavy chain variable domain of SEQ ID NO: 1,
b) the heavy chain variable domain of SEQ ID NO: 8,
c) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A,
d) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16,
e) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 17,
f) the heavy chain variable domain of SEQ ID NO: 8 including mutations T28A, V11L and K12V, or
g) the heavy chain variable domain of SEQ ID NO: 8 including mutations T28A, V11L, K12V, Y91F, and T108S.

7. The antibody according to any one of claims 1 to 6, characterized in that the antibody comprises as light chain variable domain

a) the light chain variable domain of SEQ ID NO: 5,
b) the light chain variable domain of SEQ ID NO: 12,
c) the light chain variable domain of SEQ ID NO: 12 including as CDRL2 SEQ ID NO: 18 instead of SEQ ID NO: 14,
d) the light chain variable domain of SEQ ID NO: 12 including as CDRL1 SEQ ID NO: 19 instead of SEQ ID NO: 13,
e) the light chain variable domain of SEQ ID NO: 12 including as CDRL1 SEQ ID NO: 20 instead of SEQ ID NO: 13, or
f) the light chain variable domain of SEQ ID NO: 12 including mutations P80S and F83L and as CDRL1 SEQ ID NO: 20 instead of SEQ ID NO: 13.

8. The antibody according to any one of claims 1 to 7, characterized in that the antibody comprises as heavy chain variable domain and as light chain variable domain

a) the heavy chain variable domain of SEQ ID NO:1 and the light chain variable domain of SEQ ID NO:5,

b) the heavy chain variable domain of SEQ ID NO: 8 and the light chain variable domain of SEQ ID NO: 12,

c) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and the light chain variable domain of SEQ ID NO: 12,

d) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16 or 17, and the light chain variable domain of SEQ ID NO: 12,

e) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16 or 17, and the light chain variable domain of SEQ ID NO: 12, and as CDR1 SEQ ID NO: 19 or 20,

f) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16 or 17, and the light chain variable domain of SEQ ID NO: 12, and as CDR2 SEQ ID NO: 18, or

g) the heavy chain variable domain of SEQ ID NO: 8 including mutation T28A and as CDRH2 SEQ ID NO: 16 or 17, and the light chain variable domain of SEQ ID NO: 12 including mutations P80S and F83L, and as CDR1 SEQ ID NO: 20.

9. The antibody according to any one of claims 1 to 8, characterized in that the heavy chain variable domain comprises in addition to mutation T28A,

a) mutations V11L and K12V, or

b) mutations V11L, K12V, Y91F and T108S.

10. The antibody according to any one of claims 1 to 9, characterized in being of human IgGl isotype comprising mutations L234A, which is alanine instead of leucine at amino acid position 234, and L235A, which is alanine instead of leucine at amino acid position 235.
11. The antibody according to any one of claims 1 to 9, characterized in being of human IgG4 isotype with or without mutation S228P.

12. Isolated nucleic acid encoding the antibody according to any one of claims 1 to 11.

13. A host cell comprising the nucleic acid of claim 12.

14. Method for the production of a recombinant antibody binding to CD19, characterized by expressing a nucleic acid according to claim 12 in a prokaryotic or eukaryotic host cell and recovering said antibody from said cell or the cell culture supernatent.

15. A pharmaceutical formulation comprising the antibody according to any one of claims 1 to 11 and a pharmaceutically acceptable carrier.

16. The antibody of according to any one of claims 1 to 11 for use as a medicament.

17. The antibody of according to any one of claims 1 to 11 for use in treating inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone diseases.

18. Use of the antibody according to any one of claims 1 to 11 in the manufacture of a medicament.

19. The use of claim 18, wherein the medicament is for treating inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone diseases.

20. The use of claim 18, wherein the medicament is for blocking B-cell activation.

21. A method of treating an individual having inflammatory, autoimmune, rheumatoid arthritis, lupus, psoriasis, or bone disease comprising administering to the individual an effective amount of the antibody of any one of claims 1 to 11.

22. The antibody according to anyone of claims 1 to 9, characterized in being partially-fucosylated or non-fucosylated.
23. A pharmaceutical formulation comprising the antibody according to claim 22 and a pharmaceutically acceptable carrier.

24. The antibody of according to claim 22 for use as a medicament.

25. The antibody of according to claim 22 for use in treating cancer diseases.

26. Use of the antibody according to claim 22 in the manufacture of a medicament.

27. The use of claim 26, wherein the medicament is for treating cancer diseases.

28. A method of treating an individual having a cancer disease comprising administering to the individual an effective amount of the antibody of claim 22.
Fig. 3

Antibody Concentration [ng/ml]

Killing % Ab dependent

- control
- CD19 g2
- CD19 wt
- CD19 LALA
**INTERNATIONAL SEARCH REPORT**

**PCT/EP2011/058482**

**A. CLASSIFICATION OF SUBJECT MATTER**

Inv. C07K16/28 A61K39/395

**ADD.**

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K

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 practical, search terms used)

EPO-Internal, BIOSIS, EMBASE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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Date of the actual completion of the international search: 19 September 2011

Date of mailing of the international search report: 23/09/2011

Name and mailing address of the ISA:
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Authorized officer:
Hi x, Rebecca
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