SUMMARY

The disclosure provides anti-human CD19 antibodies with high affinity.

In one aspect, provided is an antibody, that specifically binds to human CD19 with a higher affinity than an antibody comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO:113 and a variable light chain comprising an amino acid sequence of SEQ ID NO:114.

The disclosure provides an antibody that specifically binds to human CD19, wherein the antibody comprises

(a) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 43,

(b) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 44,

(c) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 45,

(d) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 46,

(e) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 47, and

(f) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 48.

In an embodiment, the antibody is a monoclonal antibody. In another embodiment, the antibody is a human, humanized or chimeric antibody. In another embodiment, the antibody is an antibody fragment that specifically binds to human CD19.

In a particular embodiment, provided is an antibody, wherein the antibody comprises a VH domain comprising an amino acid sequence of SEQ ID NO: 99 and a VL domain comprising an amino acid sequence of SEQ ID NO: 100.

In a further embodiment, provided is an antibody as described herein before, which is a full length IgG1 antibody.

In a particular embodiment, provided is an antibody as described herein, which is a full length IgG1 antibody with mutations L234A, L235A and P329G (numbering according to the EU index of Kabat).

In a particular embodiment, provided is an antibody as described herein, which is a full length IgG1 antibody with mutations L234A, L235A and P329G (numbering according to the EU index of Kabat).

In a further embodiment, provided is an antibody as described herein, which is cross reactive for human and cynomolgus CD19.

In another aspect, provided is an antibody that is bispecific, wherein said antibody specifically binds to human CD19 and a second antigen binding moiety, wherein the antibody comprises

(a) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 43,

(b) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 44,

(c) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 45,

(d) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 46,

(e) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 47, and

(f) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 48.

According to the disclosure, there is provided an isolated polynucleotide encoding an antibody as defined herein before. According to another aspect, there is provided a polynucleotide encoding the antibody as defined herein before. The disclosure further provides a vector, particularly an expression vector, comprising the isolated polynucleotide of the disclosure and a host cell comprising the isolated polynucleotide or the vector of the disclosure. According to another aspect, there is provided a vector, particularly an expression vector, comprising the polynucleotide as defined herein before. According to another aspect, there is provided a host cell comprising the polynucleotide as defined herein before or the vector as defined herein before. In some embodiments the host cell is a eukaryotic cell, particularly a mammalian cell.

There is also provided a method for producing the antibody of the disclosure, comprising the steps of (i) culturing the host cell of the disclosure under conditions suitable for expression of the antigen binding molecule, and (ii) recovering the antigen binding molecule. In another aspect, provided is a method of producing the antibody as defined herein before, comprising the steps of (i) culturing the host cell as defined herein under conditions suitable for expression of the antibody, and (ii) recovering the antibody. The disclosure also encompasses an antibody produced by the method of the disclosure.

The disclosure further provides a pharmaceutical composition comprising the antibody of the disclosure and at least one pharmaceutically acceptable excipient. In another aspect, provided is a pharmaceutical formulation comprising the antibody as defined herein and a pharmaceutically acceptable carrier.

Also encompassed by the disclosure is the antibody of the disclosure, or the pharmaceutical composition of the disclosure, for use as a medicament. In another aspect, provided is the antibody as defined herein before for use as a medicament. There is provided the antibody of the disclosure, or the pharmaceutical composition of the disclosure, for use in the treatment of a disease in an individual in need thereof. There is provided the antibody of the disclosure, or the pharmaceutical composition of the disclosure, for use in the treatment of cancer. In another aspect, provided is the antibody as defined herein before for use in treating a B-cell cancer. In another aspect, provided is the antibody as defined herein before for use in depleting B-cells. There is provided the antibody of the disclosure, or the pharmaceutical composition of the disclosure, for use in the treatment autoimmune diseases, rheumatoid arthritis, lupus, psoriasis, or a bone disease. In another aspect, provided is the antibody as defined herein before for use in the treatment of autoimmune diseases, rheumatoid arthritis, lupus, psoriasis, or a bone disease.

Also provided is the use of the antibody of the disclosure for the manufacture of a medicament for the treatment of a disease in an individual in need thereof, in particular for the manufacture of a medicament for the treatment of cancer, as well as a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the antibody in a pharmaceutically acceptable form. In another aspect, provided is use of the antibody as defined herein before in the manufacture of a medicament, particularly in a medicament for the treatment of B cell cancer. In an

embodiment, the disease is cancer. In another embodiment, the disease is selected from the group consisting of autoimmune diseases, rheumatoid arthritis, lupus, psoriasis, or a bone disease. In any of the above embodiments the individual is preferably a mammal, particularly a human.

BRIEF DESCRIPTION OF THE DRAWINGS

In **Figure 1** is illustrated the randomization strategy for the CDR regions of the parental clone 8B8. Shown are the variable domains of the parental clone 8B8 and the CDR regions (boxed) according to the numbering of Kabat. The VL sequence corresponds to SEQ ID NO:114 and the VH sequence corresponds to SEQ ID NO:113. (X) represents the randomized positions.

Figure 2 shows the schematic description of the library generation strategies. Shown is the PCR amplification and cloning strategy used for the generation of the 8B8-based library with A) randomized CDR1 and CDR2 regions in the light and heavy chain or B) randomized CDR1 and CDR3 regions in the light and CDR3 region in the heavy chain. Respective enzymes used for cloning into the phagemide are indicated.

Figure 3 shows the alignment of the parental anti-CD19 clone 8B8 with the selected affinity-matured binders. Shown are the sequences of clone 8B8 and all selected affinity-matured binders. CDRs of both heavy and light chains are framed.

Figures 4A to 4H relate to the SPR analysis of the parental 8B8 clone and its affinity-matured variants. Shown are the sensorgrams of clone 8B8 and its affinity-matured derivatives that are devoid of the LCDR1 N27d and N28 hotspots.

Figure 5 shows the binding of different CD19 IgG1 clones to human CD19-expressing tumor cells (WSU-DLCL2 cells). Binding was detected with PE-conjugated AffiniPure anti-human IgG F(ab`)2-fragment-specific goat F(ab`)2 fragment. Shown is the median of fluorescence intensity (MFI) versus the concentration of tested clones.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Unless defined otherwise, technical and scientific terms used herein have the same meaning as generally used in the art to which this invention belongs. For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa.