

CLAIMS

1. A bispecific antibody comprising

- (i) a first binding arm comprising a first antigen-binding region binding to human CD3ε (epsilon), wherein said first antigen-binding region comprises (a) heavy chain variable (VH) region CDR1, CDR2, and CDR3 having the sequences as set forth in SEQ ID NOs:1, 2, and 3, respectively, and light chain variable (VL) region CDR1, CDR2, and CDR3 having the sequences as set forth in SEQ ID NO:4, the sequence GTN, and the sequence as set forth in SEQ ID NO:5, respectively, and
- (ii) a second binding arm comprising a second antigen-binding region binding to human CD20,

wherein the first binding arm comprises a first heavy chain and the second binding arm comprises a second heavy chain, wherein in both the first and second heavy chain the amino acids in the positions corresponding to positions L234, L235 and D265 in a human IgG1 heavy chain are F, E and A, respectively,

wherein (i) the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in said first heavy chain, and the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in said second heavy chain, or (ii) the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in said first heavy chain, and the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in said second heavy chain, and

wherein the amino acid positions are according to EU-numbering.

2. The bispecific antibody according to claim 1, wherein said first antigen-binding region comprises a first heavy chain variable sequence (VH), and a first light chain variable sequence (VL), and said second antigen-binding region comprises a second heavy chain variable sequence (VH), and a second light chain variable sequence (VL) and wherein said variable sequences each comprises three CDR sequences, CDR1, CDR2 and CDR3, respectively, and four framework sequences, FR1, FR2, FR3 and FR4, respectively.
3. The bispecific antibody according to any one of the preceding claims, wherein said VH sequence of the first antigen-binding region has at least 90%, at least 95%, at least 97%, or at least 99% amino acid sequence identity to the VH sequence set forth in SEQ ID NO:6.

4. The bispecific antibody according to any one of the preceding claims, wherein said VL sequence of the first antigen-binding region has at least 90%, at least 95%, at least 97%, or at least 99% amino acid sequence identity to the VL sequence set forth in SEQ ID NO:10.
5. The bispecific antibody according to any one of the preceding claims, wherein the framework sequences of said VH sequence of the first antigen-binding region has at least 90%, at least 95%, at least 97%, or at least 99% amino acid sequence identity to the framework amino acid sequence as set forth in the VH sequence set forth in SEQ ID NO:6
6. The bispecific antibody according to any one of the preceding claims, wherein the framework sequences of said VL sequence of the first antigen-binding region has at least 90%, at least 95%, at least 97%, or at least 99% amino acid sequence identity to the framework amino acid sequence as set forth in the VL sequence set forth in SEQ ID NO:10.
7. The bispecific antibody according to any one of the preceding claims, wherein said VH sequence of the first antigen-binding region is the VH sequence set forth in SEQ ID NO:6.
8. The bispecific antibody according to any one of the preceding claims, wherein said VL sequence of the first antigen-binding region is the VL sequence set forth in SEQ ID NO:10.
9. The bispecific antibody according to any one of the preceding claims, wherein said VH and VL sequences of the first antigen-binding region are the VH sequence set forth in SEQ ID NO:6, and the VL sequence set forth in SEQ ID NO:10.
10. The bispecific antibody according to any one of the preceding claims, wherein the first binding arm is derived from a full-length IgG1, λ (lambda) antibody.
11. The bispecific antibody according to any of the preceding claims, wherein the second antigen-binding region which binds to human CD20 comprises the VH CDR1 region of SEQ ID NO:32, the VH CDR2 region of SEQ ID NO:33, the VH CDR3 region of SEQ ID NO:34.
12. The bispecific antibody according to any of the preceding claims, wherein the second antigen-binding region, which binds to human CD20, comprises the VL CDR1 region of SEQ ID NO:35, the VL CDR2 region DAS, the VL CDR3 region of SEQ ID NO:36.

13. The bispecific antibody according to any of the preceding claims, wherein the second antigen-binding region, which binds to human CD20, comprises the VH CDR1 region of SEQ ID NO:32, the VH CDR2 region of SEQ ID NO:33, the VH CDR3 region of SEQ ID NO:34, the VL CDR1 region of SEQ ID NO:35, the VL CDR2 region of SEQ ID NO:36, and the VL CDR3 region of SEQ ID NO:36.
14. The bispecific antibody according to any one of the preceding claims, wherein the second antigen-binding region, which binds human CD20, comprises a VH sequence which has at least 90%, at least 95%, at least 97%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO:27, and a VL sequence which has at least 90%, at least 95%, at least 97%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO:28.
15. The bispecific antibody according to any one of the preceding claims, wherein the second antigen-binding region, which binds to human CD20, comprises the VH sequence of SEQ ID NO:27, and the VL sequence of SEQ ID NO:28.
16. The bispecific antibody according to any one of the preceding claims, wherein the second binding arm is derived from a full-length IgG1, κ (kappa) antibody.
17. The bispecific antibody according to any one of the preceding claims, wherein (a) the first antigen-binding region comprises the VH sequence as set forth in SEQ ID NO:6, and a VL sequence as set forth in SEQ ID NO:10, and (b) the second antigen-binding region comprises the VH sequence as set forth in SEQ ID NO:27, and the VL sequence as set forth in SEQ ID NO:28.
18. The bispecific antibody according to claim 40, wherein the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in said first heavy chain, and the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in said second heavy chain.
19. The bispecific antibody according to any one of the preceding claims, wherein the bispecific antibody is of the IgG1 isotype, such as a IgG1m(f).

20. The bispecific antibody according to any of the preceding claims, wherein the first and second heavy chains have at least 95% identity to the human IgG1m(f) heavy chain of SEQ ID NO: 23 and 24, respectively
21. The bispecific antibody according to any of the preceding claims, wherein the bispecific antibody comprises SEQ ID NO: 23 and 24. [TLG1]
22. The bispecific antibody according to any of the preceding claims, wherein the bispecific antibody comprises a lambda light chain constant region and a kappa light chain constant region.
23. The bispecific antibody according to any of the preceding claims, wherein the first binding arm comprises a human lambda light chain constant region.
24. The bispecific antibody according to any of the preceding claims, wherein the second binding arm comprises a human kappa light chain constant region.
25. A nucleic acid construct encoding one or more amino acid sequences of claims 1-24.
26. A nucleic acid construct encoding a bispecific antibody as defined in any of claims 1-24.
27. An expression vector comprising:
- (i) a nucleic acid sequence encoding a heavy chain sequence of a first binding arm as defined in any one of the preceding claims 1-10;
 - (ii) a nucleic acid sequence encoding a light chain sequence of a first binding arm as defined in any one of the preceding claims 1-10;
 - (iii) a nucleic acid sequence encoding a heavy chain sequence of a second binding arm as defined in any one of the preceding claims 11-16;
 - (iv) a nucleic acid sequence encoding a light chain sequence of a second binding arm as defined in any one of the preceding claims 11-16;
 - (v) the nucleic acid set forth in (i) and the nucleic acid set forth in (ii);
 - (vi) the nucleic acid set forth in (iii) and the nucleic acid set forth in (iv).
 - (vii) the nucleic acid set forth in (i), (ii), (iii) and (iv).
28. A host cell comprising an expression vector of claim 27.

29. The host cell according to claim 28, wherein said host cell is a recombinant eukaryotic, recombinant prokaryotic, or recombinant microbial host cell.
30. A composition comprising a bispecific antibody according to any one of the preceding claims 1-24.
31. A pharmaceutical composition comprising the bispecific antibody according to any one of the preceding claims 1-24 and a pharmaceutically acceptable carrier.
32. The bispecific antibody according to any one of the preceding claims 1-24, the composition according to claim 30, or the pharmaceutical composition according to claim 31 for use as a medicament.
33. The bispecific antibody according to any one of the preceding claims 1-24, the composition according to claim 30, or the pharmaceutical composition according to claim 31 for use in the treatment of cancer.
34. The bispecific antibody for use according to claim 33 wherein the use is for treating B cell malignancies.
35. The bispecific antibody for use according to claim 21 or 33 wherein the use is for treating NHL or B cell leukemia.
36. Use of a bispecific antibody according to any one of the preceding claims 1-24 for the manufacture of a medicament.
37. Use of a bispecific antibody according to any one of the preceding claims 1-24 for the manufacture of a medicament for the treatment of cancer.
38. The use according to claim 37 wherein the use is for treating B cell malignancies.
39. The use according to claims 37 or 38 wherein the use is for treating NHL or B cell leukemia.
40. The use according to any of claims 32-39 wherein the method or use of the bispecific antibody of any of claims 1-24 is used in combination with one or more further therapeutic agent, such as a chemotherapeutic agent.

41. A method for producing a bispecific antibody according to any one of the preceding claims 1-24, comprising the steps of
- a) culturing a host cell comprising an expression vector according to claim 27(v) and purifying said antibody from the culture media;
 - b) culturing a host cell comprising an expression vector according to claim 27(vi) and purifying said antibody from the culture media;
 - c) incubating said first antibody together with said second antibody under reducing conditions sufficient to allow the cysteines in the hinge region to undergo disulfide-bond isomerization, and
 - d) obtaining said bispecific antibody.
42. A diagnostic composition comprising a bispecific antibody according to any one of the preceding claims 1-24.
43. A method for detecting whether cross-linking between CD3- and CD20-expressing cells occurs in a sample derived from a patient such as a blood sample, lymph node sample or bone marrow sample, upon administration of a bispecific antibody according to any one of the preceding claims 1-24, comprising the steps of:
- (i) contacting the sample with a bispecific antibody according to any one of the preceding claims 1-24, under conditions that allow for formation of a complex between said bispecific antibody and the CD3- and CD20-expressing cells; and
 - (ii) analyzing whether a complex has been formed.
44. A kit for detecting cross-linking between CD3- and CD20-expressing cells, in a sample derived from a patient such as a blood sample, lymph node sample or bone marrow sample, comprising
- i) a bispecific antibody according to any one of the preceding claims 1-24; and
 - ii) instructions for use of said kit.
45. An anti-idiotypic antibody which binds to the first antigen-binding region as defined in any one of the preceding claims 1-10, or which binds to the second antigen-binding region as defined in any one of the preceding claims 11-15.