

SUMMARY OF THE INVENTION

The present ~~invention~~disclosure provides antibodies, including multispecific (e.g. bispecific) antibodies, that bind to CD3 and are resistant to degradation by e.g. asparagine deamidation and thus particularly stable as required for therapeutic purposes. The (multispecific) antibodies
5 provided further combine good efficacy and produceability with low toxicity and favorable pharmacokinetic properties.

As is shown herein, the antibodies, including multispecific antibodies, that bind to CD3, provided by the present ~~invention~~disclosure, retain more than about 90% binding activity to CD3 after 2 weeks at pH 7.4, 37°C, relative to the binding activity after 2 weeks at pH 6, -80°C, as determined
10 by surface plasmon resonance (SPR).

In one aspect, the ~~invention~~disclosure provides an antibody that binds to CD3 and CD19, wherein the antibody comprises (a) a first antigen binding domain that binds to CD3, comprising a heavy chain variable region (VH) comprising a heavy chain ~~complementary~~complementarity determining region (HCDR) 1 of SEQ ID NO: 2, a HCDR 2 of SEQ ID NO: 3, and a HCDR 3 of
15 SEQ ID NO: 5, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 8, a LCDR 2 of SEQ ID NO: 9 and a LCDR 3 of SEQ ID NO: 10; and (b) a second and optionally a third antigen binding domain that binds to CD19. In one ~~aspect~~example, the VH of the first antigen binding domain comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid
20 sequence of SEQ ID NO: 7, and/or the VL of the first antigen binding domain comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 11.

In a further aspect, the ~~invention~~disclosure provides an antibody that binds to CD3 and CD19, wherein the antibody comprises (a) a first antigen binding domain that binds to CD3 comprising a
25 VH sequence of SEQ ID NO: 7 and a VL sequence of SEQ ID NO: 11; and (b) a second and optionally a third antigen binding domain that binds to CD19.

In one ~~aspect~~example, the first, the second and/or, where present, the third antigen binding domain is a Fab molecule.

In one ~~aspect~~example, the antibody comprises an Fc domain composed of a first and a second
30 subunit.

In one ~~aspect~~example the first antigen binding domain is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1, particularly the variable domains VL and VH, of the Fab light chain and the Fab heavy chain are replaced by each other.

In one ~~aspect~~example the second and, where present, the third antigen binding domain is a
35 conventional Fab molecule.

In one [aspectexample](#), the second and, where present, the third antigen binding domain is a Fab molecule wherein in the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In one [aspectexample](#), the first and the second antigen binding domain are fused to each other, optionally via a peptide linker.

In one [aspectexample](#), the first and the second antigen binding domain are each a Fab molecule and either (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain.

In one [aspectexample](#), the first, the second and, where present, the third antigen binding domain are each a Fab molecule and the antibody comprises an Fc domain composed of a first and a second subunit; and wherein either (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain and the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain; and the third antigen binding domain, where present, is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

In one [aspectexample](#), the Fc domain is an IgG, particularly an IgG₁, Fc domain. In one [aspectexample](#) the Fc domain is a human Fc domain. In one [aspectexample](#), the Fc comprises a modification promoting the association of the first and the second subunit of the Fc domain. In one [aspectexample](#), the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

In one [aspectexample](#), the second and, where present, the third antigen binding domain comprises (i) a VH comprising a HCDR 1 of SEQ ID NO: 15, a HCDR 2 of SEQ ID NO: 16, and a HCDR 3 of SEQ

ID NO: 17, and a VL comprising a LCDR 1 of SEQ ID NO: 19, a LCDR 2 of SEQ ID NO: 20 and a LCDR 3 of SEQ ID NO: 21; or (ii) a VH comprising a HCDR 1 of SEQ ID NO: 28, a HCDR 2 of SEQ ID NO: 29, and a HCDR 3 of SEQ ID NO: 30, and a VL comprising a LCDR 1 of SEQ ID NO: 32, a LCDR 2 of SEQ ID NO: 33 and a LCDR 3 of SEQ ID NO: 34. In one ~~aspect~~example, the second and, where present, the third antigen binding domain comprises (i) a VH comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 18, and/or a VL comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 22; or (ii) a VH comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 31, and/or a VL comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 35.

~~According to a further aspect of the invention there~~There is provided an isolated polynucleotide encoding an antibody of the ~~invention~~disclosure, and a host cell comprising the isolated polynucleotide of the ~~invention~~disclosure. ~~In another aspect, there is provided an isolated polynucleotide encoding the antibody of the disclosure. In another aspect, there is provided a host cell comprising the isolated polynucleotide of the disclosure.~~

In another aspect, ~~there~~ is provided a method of producing an antibody that binds to CD3 and CD19, comprising the steps of (a) culturing the host cell of the ~~invention~~disclosure under conditions suitable for the expression of the antibody and optionally (b) recovering the antibody.

~~The invention~~In another aspect, the disclosure also encompasses an antibody that binds to CD3 and CD19 produced by the method of the ~~invention~~disclosure.

~~The invention~~In another aspect, the disclosure further provides a pharmaceutical composition comprising the antibody of the ~~invention~~disclosure and a pharmaceutically acceptable carrier.

Also encompassed by the ~~invention~~disclosure are methods of using the antibody and pharmaceutical composition of the ~~invention~~disclosure. In ~~one—another~~ aspect, the ~~invention~~disclosure provides ~~an—the~~ antibody according to the disclosure or the pharmaceutical composition according to the ~~invention~~disclosure for use as a medicament. In ~~one—another~~ aspect, ~~there~~ is provided ~~an—the~~ antibody according to the disclosure or the pharmaceutical composition according to the ~~invention~~disclosure for use in the treatment of a disease.

Also provided is the use of an antibody or pharmaceutical composition according to the ~~invention~~disclosure in the manufacture of a medicament, and the use of an antibody or pharmaceutical composition according to the ~~invention~~disclosure in the manufacture of a medicament for the treatment of a disease. In another aspect, the present disclosure provides use of the antibody of the disclosure or the pharmaceutical composition of the disclosure in the manufacture of a medicament. In another aspect, the present disclosure provides use of the antibody of the disclosure or the pharmaceutical composition of the disclosure in the manufacture of a medicament for the treatment of a disease. The ~~invention~~disclosure also provides a method of treating a disease in an individual, comprising administering to said individual an effective amount of the antibody or pharmaceutical composition according to the ~~invention~~disclosure. In certain ~~aspects-examples~~ the disease is cancer. In other ~~aspects-examples~~ the disease is an autoimmune disease.

CLAIMS

1. An antibody that binds to CD3 and CD19, wherein the antibody comprises
 - (a) a first antigen binding domain that binds to CD3, comprising a heavy chain variable region (VH) comprising a heavy chain ~~complementary~~ complementarity determining region (HCDR) 1 of SEQ ID NO: 2, a HCDR 2 of SEQ ID NO: 3, and a HCDR 3 of SEQ ID NO: 5, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 8, a LCDR 2 of SEQ ID NO: 9 and a LCDR 3 of SEQ ID NO: 10; and
 - (b) a second and optionally a third antigen binding domain that binds to CD19.
2. The antibody of claim 1, wherein the VH of the first antigen binding domain comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 7, and/or the VL of the first antigen binding domain comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 11.
3. An antibody that binds to CD3 and CD19, wherein the antibody comprises
 - (a) a first antigen binding domain that binds to CD3 comprising a VH sequence of SEQ ID NO: 7 and a VL sequence of SEQ ID NO: 11; and
 - (b) a second and optionally a third antigen binding domain that binds to CD19.
4. The antibody of any one of claims 1-3, wherein the first, the second and/or, where present, the third antigen binding domain is a Fab molecule.
5. The antibody of any one of claims 1-4, comprising an Fc domain composed of a first and a second subunit.
6. The antibody of any one of claims 1-5, wherein the first antigen binding domain is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1, particularly the variable domains VL and VH, of the Fab light chain and the Fab heavy chain are replaced by each other.
7. The antibody of any one of claims 1-6, wherein the second and, where present, the third antigen binding domain is a conventional Fab molecule.
8. The antibody of any one of claims 1-7, wherein the second and, where present, the third antigen binding domain is a Fab molecule wherein in the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D)

(numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

9. The antibody of any one of claims 1-8, wherein the first and the second antigen binding domain are fused to each other, optionally via a peptide linker.

10. The antibody of any one of claims 1-9, wherein the first and the second antigen binding domain are each a Fab molecule and either (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain.

11. The antibody of any one of claims 1-10, wherein the first, the second and, where present, the third antigen binding domain are each a Fab molecule and the antibody comprises an Fc domain composed of a first and a second subunit; and wherein either (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain and the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain; and the third antigen binding domain, where present, is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

12. The antibody of claim 11, wherein the antibody consists of the first, the second and the third antigen binding domain, the Fc domain, and optionally one or more peptide linkers, wherein the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and wherein the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

~~12~~13. The antibody of any one of claims 5-~~11~~12, wherein the Fc domain is an IgG, particularly an IgG₁, Fc domain.

~~13~~14. The antibody of any one of claims 5-~~12~~13, wherein the Fc domain is a human Fc domain.

15. The antibody of any one of claims 5-14, wherein the Fc domain is a human IgG₁ Fc domain.

~~14~~16. The antibody of any one of claims 5-~~13~~15, wherein the Fc comprises a modification promoting the association of the first and the second subunit of the Fc domain.

17. The antibody of any one of claims 5-16, wherein in the CH3 domain of the first subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and in the CH3 domain of the second subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable.

18. The antibody of any one of claims 5-17, wherein in the first subunit of the Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the second subunit of the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V); and wherein optionally

(a) in the second subunit of the Fc domain additionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A); and/or

(b) in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C) or the glutamic acid residue at position 356 is replaced with a cysteine residue (E356C), and in the second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C) (numberings according to Kabat EU index).

~~15~~19. The antibody of any one of claims 5-~~14~~18, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

20. The antibody of any one of claims 5-19, wherein the Fc domain comprises an amino acid substitution at a position selected from the group of E233, L234, L235, N297, P331 and P329, particularly a position selected from the group of L234, L235 and P329 (numberings according to Kabat EU index).

21. The antibody of any one of claims 5-20, wherein each subunit of the Fc domain comprises the amino acid substitutions L234A, L235A and P329G (numberings according to Kabat EU index).

~~16~~22. The antibody of any one of claims 1-~~15~~21, wherein the second and, where present, the third antigen binding domain comprises

(i) a VH comprising a HCDR 1 of SEQ ID NO: 15, a HCDR 2 of SEQ ID NO: 16, and a HCDR 3 of SEQ ID NO: 17, and a VL comprising a LCDR 1 of SEQ ID NO: 19, a LCDR 2 of SEQ ID NO: 20 and a LCDR 3 of SEQ ID NO: 21; or

(ii) a VH comprising a HCDR 1 of SEQ ID NO: 28, a HCDR 2 of SEQ ID NO: 29, and a HCDR 3 of SEQ ID NO: 30, and a VL comprising a LCDR 1 of SEQ ID NO: 32, a LCDR 2 of SEQ ID NO: 33 and a LCDR 3 of SEQ ID NO: 34.

1723. The antibody of any one of claims 1-~~1622~~, wherein the second and, where present, the third antigen binding domain comprises

(i) a VH comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 18, and/or a VL comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 22; or

(ii) a VH comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 31, and/or a VL comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 35.

24. The antibody of any one of claims 1-23, comprising

a) a first antigen binding domain that binds to CD3, wherein the first antigen binding domain is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 11;

b) a second and a third antigen binding domain that bind to CD19, wherein the second and the third antigen binding domain are each a (conventional) Fab molecule, and comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 18 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 22;

c) an Fc domain composed of a first and a second subunit;

wherein

in the constant domain CL of the second and the third antigen binding domain under b) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most preferably by arginine (R)), and wherein in the constant domain CH1 of the second and the third antigen binding domain under b) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index);

and wherein further

the second antigen binding domain under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain under a), and the first antigen binding domain under a) and the third antigen binding domain under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

25. The antibody of claim 24, wherein the Fc domain is a human IgG₁ Fc domain, and wherein in the first subunit of the Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W) and the serine residue at position 354 is replaced with a cysteine residue (S354C), and in the second subunit of the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V), the threonine residue at position 366 is replaced with a serine residue (T366S), the leucine residue at position 368 is replaced with an alanine residue (L368A) and the tyrosine residue at position 349 is replaced with a cysteine residue (Y349C) (numberings according to Kabat EU index), and wherein further in each of the first and the second subunit of the Fc domain the leucine residue at position 234 is replaced with an alanine residue (L234A), the leucine residue at position 235 is replaced with an alanine residue (L235A) and the proline residue at position 329 is replaced by a glycine residue (P329G) (numbering according to Kabat EU index).

26. The antibody of any one of claims 1-25, wherein the antibody comprises a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 23 or SEQ ID NO: 39 (particularly SEQ ID NO: 39), a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 24, a polypeptide (particularly two polypeptides) comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 25, and a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 27.

~~1827~~. An isolated polynucleotide encoding the antibody of any one of claims 1-~~1726~~.

~~1928~~. A host cell comprising the isolated polynucleotide of claim ~~1827~~.

~~2029~~. A method of producing an antibody that binds to CD3 and CD19, comprising the steps of (a) culturing the host cell of claim ~~19-28~~ under conditions suitable for the expression of the antibody and optionally (b) recovering the antibody.

~~2130~~. An antibody that binds to CD3 and CD19 produced by the method of claim ~~2029~~.

~~2231~~. A pharmaceutical composition comprising the antibody of any one of claims 1-~~1726~~ or ~~2130~~ and a pharmaceutically acceptable carrier.

2332. The antibody of any one of claims 1-~~17~~26 or ~~21~~30 or the pharmaceutical composition of claim ~~22~~31 for use as a medicament.

2433. The antibody of any one of claims 1-~~17~~26 or ~~21~~30 or the pharmaceutical composition of claim ~~22~~31 for use in the treatment of a disease.

5 2534. The antibody for use of claim 2433, wherein the disease is cancer or an autoimmune disease.

35. The antibody for use of claim 34, wherein the cancer is (i) a CD19-expressing cancer, and/or (ii) a B-cell cancer, particularly a B-cell lymphoma or a B-cell leukemia, more particularly non-Hodgkin lymphoma or acute lymphoblastic leukemia or chronic lymphocytic leukemia.

10 36. The antibody for use of claim 34, wherein the autoimmune disease is lupus, particularly systemic lupus erythematosus (SLE) or lupus nephritis (LN).

2637. Use of the antibody of any one of claims 1-~~17~~26 or ~~21~~30 or the pharmaceutical composition of claim ~~22~~31 in the manufacture of a medicament.

2738. Use of the antibody of any one of claims 1-~~17~~26 or ~~21~~30 or the pharmaceutical composition of claim ~~22~~31 in the manufacture of a medicament for the treatment of a disease.

15 2839. The use of claim 2738, wherein the disease is cancer or an autoimmune disease.

40. The use of claim 39, wherein the cancer is (i) a CD19-expressing cancer, and/or (ii) a B-cell cancer, particularly a B-cell lymphoma or a B-cell leukemia, more particularly non-Hodgkin lymphoma or acute lymphoblastic leukemia or chronic lymphocytic leukemia.

20 41. The use of claim 39, wherein the autoimmune disease is lupus, particularly systemic lupus erythematosus (SLE) or lupus nephritis (LN).

~~29. A method of treating a disease in an individual, comprising administering to said individual an effective amount of the antibody of any one of claims 1-17 or 21 or the pharmaceutical composition of claim 22.~~

~~30. The method of claim 29, wherein the disease is cancer or an autoimmune disease.~~