

One aspect as reported herein is a bispecific antibody comprising

- 5 a) one antibody comprising two pairs each of a antibody light chain and a antibody heavy chain, wherein the binding sites formed by each of the pairs of the heavy chain and the light chain specifically bind to a first antigen, and
- 10 b) one additional Fab fragment, wherein the additional Fab fragment is fused to the C-terminus of one of the heavy chains of the antibody, wherein the binding site of the additional Fab fragment specifically binds to a second antigen,
- 15 wherein each of the antibody light chains comprises in the constant light chain domain (CL) at position 123 the amino acid residue arginine (instead of the human IgG glutamic acid residue; E123R mutation) and at position 124 the amino acid residue lysine (instead of the human IgG glutamine residue; Q124K mutation) (numbering according to Kabat),
- 20 wherein each of the antibody heavy chains comprises in the first constant heavy chain domain (CH1) at position 147 an glutamic acid residue (instead of the human IgG lysine residue; K147E mutation) and at position 213 an glutamic acid residue (instead of the human IgG lysine amino acid residue; K213E mutation) (numbering according to Kabat EU index),
- 25 wherein the additional Fab fragment specifically binding to the second antigen comprises a domain crossover such that the constant light chain domain (CL) and the constant heavy chain domain 1 (CH1) are replaced by each other,
- wherein the first antigen is human CD20 protein and the second antigen is human transferrin receptor,
- 30 wherein the human CD20 binding site comprises the VH sequence as in SEQ ID NO: 18, including post-translational modifications thereof and the VL sequence as in SEQ ID NO: 19, including post-translational modifications thereof,
- wherein the human transferrin receptor binding site comprises the VH sequence as in SEQ ID NO: 20, including post-translational modifications

thereof and the VL sequence as in SEQ ID NO: 21, including post-translational modifications thereof.

In one embodiment the additional Fab fragment is fused to the C-terminus of the heavy chain by a peptidic linker.

5 As reported herein is a bispecific antibody comprising two full length light chains that each has the amino acid sequence of SEQ ID NO: 14, a full length heavy chain that has the amino acid sequence of SEQ ID NO: 15, a crossed antibody chain that has the amino acid sequence of SEQ ID NO: 16, and a modified heavy chain that has the amino acid sequence of SEQ ID NO: 17.

In one embodiment the bispecific antibody is monoclonal.

As reported herein is an isolated nucleic acid encoding the bispecific antibody as reported herein.

10 As reported herein is a host cell comprising the nucleic acid as reported herein encoding the bispecific antibody as reported herein.

As reported herein is a method of producing a bispecific antibody as reported herein comprising the following steps:

- a) culturing the host cell as reported herein so that the bispecific antibody is produced, and
- 15 b) recovering the bispecific antibody from the cell or the cultivation medium and thereby producing the bispecific antibody as reported herein.

As reported herein is an immunoconjugate comprising the bispecific antibody as reported herein and a cytotoxic agent.

20 As reported herein is a pharmaceutical formulation comprising the bispecific antibody as reported herein and a pharmaceutically acceptable carrier.

Another aspect as reported herein is a pharmaceutical formulation comprising the bispecific antibody as reported herein and a pharmaceutically acceptable carrier.

25 Another aspect as reported herein is the bispecific antibody as reported herein for use as a medicament.

As reported herein is the antibody as reported herein for the treatment of cancer.

As reported herein is the bispecific antibody as reported herein for use in treating B-cell proliferative disease.

As reported herein is the bispecific antibody as reported herein for use in inhibiting growth of tumor cells expressing CD20. In one embodiment the inhibiting is in the brain.

5 As reported herein is the bispecific antibody as reported herein for use in treating carcinoma. In one preferred embodiment the carcinoma is carcinoma of/in the brain.

As reported herein is the bispecific antibody as reported herein for use in treating lymphoma. In one preferred embodiment the lymphoma is primary central nervous system lymphoma (PCNSL).

10 As reported herein is the bispecific antibody as reported herein for use in treating an autoimmune disease. In one embodiment the autoimmune disease is multiple sclerosis. In one preferred embodiment the autoimmune disease is secondary progressive multiple sclerosis.

15 As reported herein is the antibody as reported herein for use in the treatment of multiple sclerosis.

As reported herein is the bispecific antibody as reported herein for use in depleting tumor cells expressing CD20. In one embodiment the depleting is in the brain.

20 As reported herein is the bispecific antibody as reported herein for use in depleting circulating B-cells expressing CD20. In one embodiment the depleting is in the brain.

As reported herein is the bispecific antibody as reported herein for use in depleting brain sequestered B-cells expressing CD20.

As reported herein is the use of the bispecific antibody as reported herein in the manufacture of a medicament.

25 As reported herein is use of the antibody as reported herein in the manufacture of a medicament for treatment of multiple sclerosis.

Patent Claims

1. A bispecific antibody comprising

a) one antibody comprising two pairs each of an antibody light chain and an antibody heavy chain, wherein the binding sites formed by each of the pairs of the heavy chain and the light chain specifically bind to a first antigen, and

b) one additional Fab fragment, wherein the additional Fab fragment is fused to the C-terminus of one of the heavy chains of the antibody, wherein the binding site of the additional Fab fragment specifically binds to a second antigen,

wherein each of the antibody light chains comprises in the constant light chain domain (CL) at position 123 the amino acid residue arginine (instead of the human IgG glutamic acid residue; E123R mutation) and at position 124 the amino acid residue lysine (instead of the human IgG glutamine residue; Q124K mutation) (numbering according to Kabat),

wherein each of the antibody heavy chains comprises in the first constant heavy chain domain (CH1) at position 147 an glutamic acid residue (instead of the human IgG lysine residue; K147E mutation) and at position 213 an glutamic acid residue (instead of the human IgG lysine amino acid residue; K213E mutation) (numbering according to Kabat EU index),

wherein the additional Fab fragment specifically binding to the second antigen comprises a domain crossover such that the constant light chain domain (CL) and the constant heavy chain domain 1 (CH1) are replaced by each other,

wherein the first antigen is human CD20 protein and the second antigen is human transferrin receptor,

wherein the human CD20 binding site comprises the VH sequence as in SEQ ID NO: 18, including post-translational modifications thereof and the VL sequence as in SEQ ID NO: 19, including post-translational modifications thereof,

wherein the human transferrin receptor binding site comprises the VH sequence as in SEQ ID NO: 20, including post-translational modifications thereof and the VL sequence as in SEQ ID NO: 21, including post-translational modifications thereof.

- 5 2. The bispecific antibody according to claim 1, wherein the additional Fab fragment is fused to the C-terminus of the heavy chain by a peptidic linker.
3. The bispecific antibody according to any one of claims 1 and 2, wherein
 - 10 a) the heavy chain that is fused to the additional Fab fragment has as C-terminal heavy chain amino acid residues the tripeptide LSP wherein the proline thereof is directly fused to the first amino acid residue of the additional Fab fragment or of the peptidic linker via a peptide bond, and
 - 15 b) the heavy chain that is not fused to the additional Fab fragments has as C-terminal heavy chain amino acid residues the tripeptide LSP, or SPG, or PGK.
4. The bispecific antibody according to anyone of claims 1 to 3, wherein the antibody is
 - 20 a) a full length antibody of the human subclass IgG1, or
 - b) a full length antibody of the human subclass IgG4, or
 - 25 c) a full length antibody of the human subclass IgG1 with the mutations L234A, L235A and P329G,
 - d) a full length antibody of the human subclass IgG4 with the mutations S228P, L235E and P329G,
 - e) a full length antibody of the human subclass IgG1 with the mutations L234A, L235A and P329G in both heavy chains and the mutations T366W and S354C in one heavy chain and the mutations T366S, L368A, Y407V and Y349C in the respective other heavy chain,
 - f) a full length antibody of the human subclass IgG4 with the mutations S228P and P329G in both heavy chains and the mutations T366W and

S354C in one heavy chain and the mutations T366S, L368A, Y407V and Y349C in the respective other heavy chain,

- 5 g) a full length antibody of the human subclass IgG1 with the mutations L234A, L235A, P329G, I253A, H310A and H435A in both heavy chains and the mutations T366W and S354C in one heavy chain and the mutations T366S, L368A, Y407V and Y349C in the respective other heavy chain,
- 10 h) a full length antibody of the human subclass IgG1 with the mutations L234A, L235A, P329G, M252Y, S254T and T256E in both heavy chains and the mutations T366W and S354C in one heavy chain and the mutations T366S, L368A, Y407V and Y349C in the respective other heavy chain, or
- 15 i) a full length antibody of the human subclass IgG1 with the mutations L234A, L235A, P329G, H310A, H433A and Y436A in both heavy chains and the mutations i) T366W, and ii) S354C or Y349C, in one heavy chain and the mutations i) T366S, L368A, and Y407V, and ii) Y349C or S354C, in the respective other heavy chain.
- 20 5. The bispecific antibody according to any one of claims 1 to 4, wherein the bispecific antibody comprises
- 25 i) two copies of a light chain that has a sequence identity to SEQ ID NO: 01 of 70 % or more,
- ii) one copy of a heavy chain that has a sequence identity to SEQ ID NO: 02 of 70 % or more,
- iii) one copy of a light chain that has a sequence identity to SEQ ID NO: 03 of 70 % or more, and
- iv) one copy of a heavy chain that has a sequence identity to SEQ ID NO: 10 of 70 % or more.
- 30 6. The bispecific antibody according to any one of claims 1 to 4, wherein in the two pairs of the antibody light chains and the antibody heavy chains, the light chains have the amino acid sequence of SEQ ID NO: 01, one of the heavy chains has the amino acid sequence of SEQ ID NO: 02 and one of SEQ ID

NO: 10, and the light chain in the additional Fab fragment has the amino acid sequence of SEQ ID NO: 03.

7. The bispecific antibody according to any one of claims 1 to 6, wherein the bispecific antibody is monoclonal.
- 5 8. A pharmaceutical formulation comprising the bispecific antibody according to any one of claims 1 to 7 and a pharmaceutically acceptable carrier.
9. The bispecific antibody according to any one of claims 1 to 7 for use as a medicament.