

blind placebo-controlled multicenter trial of Rituximab in patients with primary progressive multiple sclerosis.

SUMMARY

As reported herein is a bispecific antibody comprising

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

10 b) one additional Fab fragment, wherein the additional Fab fragment is fused to any C-terminus of one heavy chain of the (full length) antibody, wherein the binding site of the additional Fab fragment specifically binds to a second antigen,

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

20 wherein each of the (full length) antibody heavy chains comprises in the first constant heavy chain domain (CH1) at position 147 an glutamic acid residue (instead of the wild-type lysine residue; K147E mutation) and at position 213 an glutamic acid residue (instead of the wild-type 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, and

wherein the first antigen is human CD20 and the second antigen is human transferrin receptor.

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
- 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,

10 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 wild-type glutamic acid residue; E123R mutation) and at position 124 the amino acid residue lysine (instead of the wild-type glutamine residue; Q124K mutation) (numbering according to Kabat),

15 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 wild-type lysine residue; K147E mutation) and at position 213 an glutamic acid residue (instead of the wild-type lysine amino acid residue; K213E mutation) (numbering according to Kabat EU index),

20 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, and

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

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

GTASVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC.

As reported herein is a bispecific antibody comprising a (full length) light chain that has the amino acid sequence of SEQ ID NO: 01, a (full length) heavy chain that has the amino acid sequence of SEQ ID NO: 02, a (full length) light chain that has the amino acid sequence of SEQ ID NO: 03, and an antibody Fab fragment comprising the amino acid sequences of SEQ ID NO: 04.

In one embodiment the bispecific antibody is monoclonal.

As reported herein is a bispecific antibody comprising

- a) a first and a second Fab fragment, wherein each binding site of the first and the second Fab fragment specifically bind to a first antigen,
- b) a third Fab fragment, wherein the binding site of the third Fab fragment specifically binds to a second antigen, and wherein the third Fab fragment comprises a domain crossover such that the variable light chain domain (VL) and the variable heavy chain domain (VH) are replaced by each other, and
- c) an Fc-region comprising a first Fc-region polypeptide and a second Fc-region polypeptide,

wherein the first and the second Fab fragment each comprise a heavy chain fragment and a full length light chain,

wherein the C-terminus of the heavy chain fragment of the first Fab fragment is fused to the N-terminus of the first Fc-region polypeptide,

wherein the C-terminus of the heavy chain fragment of the second Fab fragment is fused to the N-terminus of the variable light chain domain of the third Fab fragment and the C-terminus of the heavy chain constant domain 1 of the third Fab fragment is fused to the N-terminus of the second Fc-region polypeptide,

wherein each of the full length light antibody chains of the first and second Fab fragment comprises in the constant light chain domain (CL) at position 123 the amino acid residue arginine (instead of the wild-type glutamic acid

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 antibody as reported herein and a pharmaceutically acceptable carrier.

25 Another aspect as reported herein is the 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 Another aspect 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 Another aspect as reported herein is use of the antibody as reported herein in the manufacture of a medicament.

5 In one embodiment the medicament is for the treatment of a proliferative type disease. In one embodiment the proliferative type disease is a B-cell proliferative disease. In one embodiment the proliferative type disease is B-cell lymphoma. In one preferred embodiment the proliferative type disease is primary central nervous system lymphoma.

In one embodiment the medicament is for the treatment of a tumor.

In one embodiment the medicament is for the treatment of human carcinoma.

As reported herein is a method of treating an individual having a proliferative type disease comprising administering to the individual an effective amount of the bispecific antibody as reported herein. In one embodiment the proliferative type disease is a B-cell proliferative disease.

5 As reported herein is a method of treating an individual having carcinoma comprising administering to the individual an effective amount of the bispecific antibody as reported herein. In one preferred embodiment the carcinoma is carcinoma of/in the brain.

10 As reported herein is a method of treating an individual having lymphoma comprising administering to the individual an effective amount of the bispecific antibody as reported herein. In one preferred embodiment the lymphoma is primary central nervous system lymphoma (PCNSL).

15 As reported herein is a method of treating an individual having an autoimmune disease comprising administering to the individual an effective amount of the bispecific antibody as reported herein. In one embodiment the autoimmune disease is multiple sclerosis. In one preferred embodiment the autoimmune disease is secondary progressive multiple sclerosis.

20 As reported herein is a method for inhibiting growth of tumor cells expressing CD20 in an individual comprising administering to the individual an effective amount of the bispecific antibody as reported herein to inhibit growth of tumor cells expressing CD20. In one embodiment the inhibiting is in the brain.

25 As reported herein is a method for depleting tumor cells expressing CD20 in an individual comprising administering to the individual an effective amount of the bispecific antibody as reported herein to deplete tumor cells expressing CD20. In one embodiment the inhibiting is in the brain.

As reported herein is a method for depleting circulating B-cells expressing CD20 in an individual comprising administering to the individual an effective amount of the bispecific antibody as reported herein to deplete circulating B-cells expressing CD20. In one embodiment the inhibiting is in the brain.

30 As reported herein is a method for depleting brain sequestered B-cells expressing CD20 in an individual comprising administering to the individual an

effective amount of the bispecific antibody as reported herein to deplete brain sequestered B-cells expressing CD20.

As reported herein is a method of treating multiple sclerosis in a human comprising administering to the human a therapeutically effective amount of an antibody as reported herein which binds to human CD20 and depletes B-cells, and wherein the antibody is not conjugated with a cytotoxic agent.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

The knobs into holes dimerization modules and their use in antibody engineering are described in Carter P.; Ridgway J.B.B.; Presta L.G.: Immunotechnology, Volume 2, Number 1, February 1996, pp. 73-73. The additional disulfide bridge in the CH3 domain is reported in Merchant, A.M., et al., Nat. Biotechnol. 16 (1998) 677-681.

General information regarding the nucleotide sequences of human immunoglobulins light and heavy chains is given in: Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991).

As used herein, the amino acid positions of all constant regions and domains of the heavy and light chain are numbered according to the Kabat numbering system described in Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991) and is referred to as "numbering according to Kabat" herein. Specifically, the Kabat numbering system (see pages 647-660) of Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991) is used for the light chain constant domain CL of kappa and lambda isotype, and the Kabat EU index numbering system (see pages 661-723) is used for the constant heavy chain domains (CH1, Hinge, CH2 and CH3, which is herein further clarified by referring to "numbering according to Kabat EU index" in this case).

I. DEFINITIONS

The "blood-brain-barrier" or "BBB" refers to the physiological barrier between the peripheral circulation and the brain and spinal cord which is formed by tight junctions within the brain capillary endothelial plasma membranes, creating a tight

9. The antibody according to any one of claims 1 to 7 for use as a medicament.
10. The antibody according to any one of claims 1 to 7 for use in the treatment of multiple sclerosis.
11. Use of the antibody according to any one of claims 1 to 7 in the manufacture
5 of a medicament.
12. The use of claim 11, wherein the medicament is for treatment of multiple sclerosis.
13. The use of claim 11, wherein the medicament is for depleting brain sequestered B-cells expressing CD20.