

- cultivating a mammalian cell in a cultivation medium (under conditions suitable for the expression of the multispecific antibody), whereby the mammalian cell has been generated by

a) transfecting a mammalian cell (not expressing an antibody) with a first expression vector and one, two or three further expression vectors,

wherein the first expression vector comprises exactly one nucleic acid sequence encoding a polypeptide of the multispecific antibody, and the one, two or three further expression vectors each comprise at least two nucleic acid sequences each encoding different polypeptide chains of the multispecific antibody,

wherein the exactly one nucleic acid sequence of the first expression vector is a nucleic acid sequence encoding a light chain polypeptide of the multispecific antibody,

wherein the transfection with the first expression vector is either concomitant, before or after the transfection with the one, two or three further expression vectors, and

b) selecting a cell (stably) transfected in step a) growing under selective cultivation conditions,

- recovering the multispecific antibody from the cell or the cultivation medium,

and thereby producing the multispecific antibody.

One aspect as disclosed herein is a method for producing a ~~multispecific~~-bispecific antibody, which comprises at least three different polypeptides, comprising the following steps:

- cultivating a mammalian cell in a cultivation medium, whereby the mammalian cell has been generated by

a) transfecting a mammalian cell with a first expression vector and ~~one~~, two or three further expression vectors,

wherein the first expression vector comprises exactly one nucleic acid sequence encoding a polypeptide of the ~~multispecific-bispecific~~ antibody, and the one, two or three further expression vectors each comprise at least two nucleic acid sequences each encoding different polypeptide chains of the ~~multispecific-bispecific~~ antibody,

wherein the exactly one nucleic acid sequence of the first expression vector is a nucleic acid sequence encoding a light chain polypeptide of the ~~multispecific-bispecific~~ antibody, and

wherein the mammalian cell is transfected first with the two or three further expression vectors and transfected thereafter with the first expression vector,

- recovering the ~~multispecific-bispecific~~ antibody from the cell or the cultivation medium,

and thereby producing the ~~multispecific-bispecific~~ antibody; wherein the ~~multispecific-bispecific~~ antibody comprises:

- a) a first light chain,
- b) a first heavy chain comprising a first heteromomeric Fc-region polypeptide,
- c) a second light chain, and
- d) a second heavy chain comprising a second heteromomeric Fc-region polypeptide,

wherein the bispecific antibody is a bivalent bispecific antibody, a trivalent bispecific antibody, or a tetravalent bispecific antibody, and

wherein the bispecific antibody comprises a T366W mutation in the CH3 domain of one heavy chain and T366S, L368A, Y407V mutations in the CH3 domain of the other heavy chain (numbering according to Kabat EU index).

As disclosed herein is a method for generating/producing/obtaining a mammalian cell (capable of (stably)) expressing a multispecific antibody, which comprises/is composed of at least three different polypeptides, comprising the following step:

- 5 a) transfecting a mammalian cell (not expressing an antibody) with a first expression vector and one, two or three further expression vectors,

 wherein the first expression vector comprises exactly one nucleic acid sequence encoding a polypeptide of the multispecific antibody,

Patent Claims

1. A method for producing a ~~multispecific~~bispecific antibody, which comprises at least three different polypeptides, comprising the following steps:

- cultivating a mammalian cell in a cultivation medium, whereby the mammalian cell has been generated by

5 a) transfecting a mammalian cell with a first expression vector and ~~one~~, two or three further expression vectors,

wherein the first expression vector comprises exactly one nucleic acid sequence encoding a polypeptide of the ~~multispecific~~bispecific antibody, and the one, two or three further expression vectors each comprise at least two nucleic acid sequences each encoding different polypeptide chains of the ~~multispecific~~bispecific antibody,

wherein the exactly one nucleic acid sequence of the first expression vector is a nucleic acid sequence encoding a light chain polypeptide of the ~~multispecific~~bispecific antibody, and

15 wherein the mammalian cell is transfected first with the two or three further expression vectors and transfected thereafter with the first expression vector,

- recovering the ~~multispecific~~bispecific antibody from the cell or the cultivation medium,

20 and thereby producing the ~~multispecific~~bispecific antibody; wherein the ~~multispecific~~bispecific antibody comprises:

a) a first light chain,

b) a first heavy chain comprising a first heteromomeric Fc-region polypeptide,

25 c) a second light chain, and

d) a second heavy chain comprising a second heteromomeric Fc-region polypeptide.

wherein the bispecific antibody is a bivalent bispecific antibody, a trivalent bispecific antibody, or a tetravalent bispecific antibody, and

wherein the bispecific antibody comprises a T366W mutation in the CH3 domain of one heavy chain and T366S, L368A, Y407V mutations in the CH3 domain of the other heavy chain (numbering according to Kabat EU index).

2. The method according to claim 1, wherein two of the polypeptide chains of the ~~multispecific~~bispecific antibody comprise a domain exchange.

3. The method according to claim 2, wherein the exactly one nucleic acid of the first expression vector encodes a light chain polypeptide with domain exchange of the ~~multispecific~~bispecific antibody.

~~4. The method according to any one of claims 1 to 3, wherein step a) is co-transfecting the first expression vector and the one, two or three further expression vectors.~~

~~5. The method according to any one of claims 1 to 3, wherein the mammalian cell is transfected first with the one, two or three further expression vectors and transfected thereafter with the first expression vector.~~

~~64.~~ The method according to any one of claims 1 to ~~53~~, wherein the mammalian cell stably expresses the ~~multispecific~~bispecific antibody.

~~75.~~ The method according to any one of claims 1 to ~~64~~, wherein the mammalian cell is a CHO cell.

~~86.~~ The method according to any one of claims 2 to ~~75~~, wherein the domain exchange is a CH1-CL crossover or a VH-VL-crossover.

~~97.~~ The method according to any one of claims 1 to ~~86~~, wherein the ~~multispecific antibody is a~~ bivalent, bispecific antibody ~~comprising~~comprises

a) a first light chain and a first heavy chain of an antibody specifically binding to a first antigen, and

b) a second light chain and a second heavy chain of an antibody specifically binding to a second antigen, wherein the variable domains

VL and VH of the second light chain and the second heavy chain are replaced by each other.

~~108~~. The method according to any one of claims 1 to ~~86~~, wherein the ~~multispecific antibody is a~~ bivalent, bispecific antibody ~~comprising~~comprises

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- a) a first light chain and a first heavy chain of an antibody specifically binding to a first antigen, and
 - b) a second light chain and a second heavy chain of an antibody specifically binding to a second antigen, wherein the constant domains CL and CH1 of the second light chain and the second heavy chain are
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- replaced by each other.

~~119~~. The method according to any one of claims 1 to ~~86~~, wherein the ~~multispecific antibody is a~~ trivalent, bispecific antibody, ~~comprising~~comprises

- a) a first light chain and a first heavy chain of a full length antibody which specifically binds to a first antigen,
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- b) a second heavy chain of a full length antibody which when paired with the first light chain, specifically binds to the first antigen, and
 - c) a Fab fragment, which specifically binds to a second antigen, fused via a peptidic linker to the C-terminus of one of the heavy chains of a) or
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- b), wherein the constant domains CL and CH1 of the second light chain and the second heavy chain are replaced by each other.